Bioactive potentials of the truffle mushrooms *Tirmania nivea*, *Tirmania pinoyi* and *Tuber indicum*

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Background
Truffles are ectomycorrhizal wild mushrooms that have ethnomycological importance as a folklore remedy used to treat different skin and eye diseases and they are currently known as expensive food and potential sources of bioactive compounds.
Objective
This study aims to investigate the *in vitro* bioactive potential of the ethyl acetate extracts of three truffle species *Tirmania nivea*, *Tirmania pinoyi*, and *Tuber indicum* collected from Egypt, Saudi Arabia, and China.
Results and discussion
Inhibition of α-glucosidase was investigated as an indication of the antidiabetic potential of extracts. *Tuber indicum* extract caused 21.7% inhibition at a concentration of 100ppm. On the other hand, inhibition of nitric oxide is evaluated as a key way to regulate inflammation. *Tuber indicum* extract at a concentration of 100μg/ml achieved the highest inhibition (50.2%) and had the lowest IC_{50} (86.0±0.09μg/ml) among tested extracts, while *Tirmania pinoyi* extract achieved the lowest inhibition (21.7%) with the highest IC_{50} (104.1±0.12μg/ml). Cell migration was used to evaluate the wound healing activity of extracts. *Tirmania pinoyi* extract at a concentration of 100μg/ml caused 74.71% wound closure followed by 73.43% and 62.38% by *Tuber indicum* and *Tirmania nivea* extracts, respectively. Finally, investigating the antiviral potential of extracts against coxsackie virus B3 revealed that *Tirmania pinoyi* extract showed the highest virucidal effect and pretreatment antiviral activity while *Tuber indicum* extract achieved the highest post-treatment antiviral activity. Results achieved by these truffles encourage further investigations to be used as functional foods or as sources of bioactive compounds.

Keywords:
antidiabetic, anti-inflammatory, coxsackie virus B3, truffles, wound healing

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Introduction

Diabetes mellitus (DM) is a prevalent chronic disease that is considered among the leading causes of microvascular complications and death worldwide [1]. It is also one of the most challenging health conditions that require continuous care and researchers are trying to find solutions to help in its treatment. One of the methods employed for such purpose is using α-glucosidase inhibitors, which is considered one of the most efficient remedies that control type 2 diabetes mellitus (type 2 DM) especially postprandial hyperglycemia and its complications [2]. The α-glucosidase inhibitors are capable of decreasing the release of α-glucose from dietary oligosaccharides [3]. Inflammation, on the other hand, is an organized dynamic mechanism that is initiated to repair tissues and includes a cascade of both cellular and microvascular responses that act together to remove damaged tissues and generate new ones [4]. Inflammation plays a major role in different diseases such as microbial infections, DM, cardiovascular diseases, and asthma [5]. Many natural sources such as plants and microorganisms contain bioactive compounds such as polysaccharides, anthraquinone, terpenoids, alkaloids, coumarin, flavonoid, and polyphenol are reported for having anti-inflammatory agents [6]. Hence, finding potent anti-inflammatory natural compounds can support the currently used ones or substitute them. One of the life-threating viruses especially for children is coxsackie virus B3. It is a cardiotropic enterovirus known as one of the main causes of viral myocarditis which may cause chronic heart failure [7]. Also, coxsackie virus B3 is neurotropic and is the main responsible for non-bacterial aseptic meningoencephalitis which can cause death, especially in

young children [8]. However, there are no specific vaccines or antiviral agents against coxsackie virus B3 infection [9]. Hence, finding anticoxsackie virus B3 agents represents a critical need. Screening nature for potent sources showing activity against such diseases and health conditions is the solution to find potent compounds that could have different action mechanisms or higher efficiency. Out of different natural sources, fungi in general and macrofungi (mushrooms) in particular are attracting extra attention since the discovery of antibiotics.

Native people, Bedouin and habitants of deserts and forests have pointed out the ethnomycological importance of mushrooms centuries ago as a folklore remedy used for curing diseases and health-boosting benefits [10]. Moreover, scientists have confirmed the medical and nutritional importance of mushrooms and many studies have described the generosity of mushroom parts with bioactive compounds [11,12]. Out of different mushroom genera, truffles gain a famous reputation for being expensive, rarely found, releasing pheromones, and being delicious with special gastronomic qualities and having medical benefits [13]. Truffles are ectomycorrhizal wild mushrooms that are characterized by their hypogenous fruiting bodies and are also known as desert truffles [14]. Truffles grow naturally in the Mediterranean area, Middle Eastern countries, and North Africa but some species are currently cultivated in other locations in the Southern Hemisphere [15,16].

In prophetic Islamic medicine truffles were described to heal skin and eye infections. Moreover, in traditional folklore medicine, truffles were used to heal skin diseases and increase fertility [17,18]. Truffles are rich in proteins, minerals, fats, unsaturated fatty acids, carbohydrates, lipids, and fibers which are important for human nutrition. Additionally, truffle produces a wide range of volatile organic compounds such as alkanes, aldehydes, alkenes, phenols, esters, flavonoids, terpenes, ketones, and some Sulphur and aromatic compounds [19,20]. The main truffle genera are *Tirmania*, *Tuber,* and *Terfezia* and the majority of studies published on such truffles are phylogenetic studies or describing their cultivation. However, studies focusing on their bioactive potential are relatively rare and depend on the country of origin where the truffle was collected. Also, the majority of studies highlighting truffles biological activities were investigating their antioxidant, antibacterial, and antifungal activities which don't reflect the actual potential of these promising truffles. We have previously described the promising *in vivo* sedative, anticonvulsant, and antinociceptive effects of *Tirmania* species [21] which have encouraged us to further investigate their bioactive capabilities. Hence, the aim of this work is to study the bioactive potential of three truffle samples collected from different locations (Marsa Matruh governorate, Egypt; desert of Jizan City, Saudi Arabia, and Guiyang city, Guizhou Province, China).

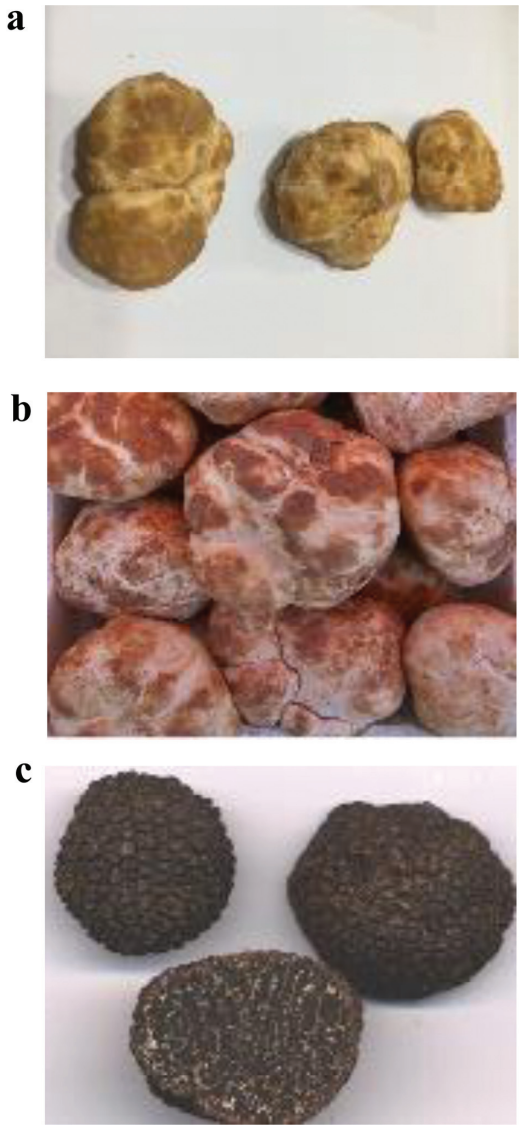
Materials and methods

Truffle samples and preparation of extracts

Truffle samples were collected from Marsa Matruh governorate desert, Egypt, Jizan City desert, Saudi Arabia, and Guiyang city, China in February 2018 (Fig. 1). Samples were identified morphologically and microscopically by Associate Prof. Dr. Waill Elkhateeb, National Research Centre, Egypt, and Prof. Dr. Paul Thomas, Faculty of Natural Sciences, Sterling University, UK. The ascocarps of *Tirmania nivea* (collected from Egypt) and *Tirmania pinoyi* (collected from Saudi Arabia) appeared cracked and subglobose with a basal attachment of mycelia. The difference between the two species appeared in their peridium which was yellowish brown, in the case of *Tirmania pinoyi*, while it was smoother and faint brown in *Tirmania nivea*.

Also, the gleba appeared whitish and veined in *Tirmania pinoyi* but it appeared yellowish in *Tirmania nivea*. The ascospores were eight smooth and globose per asci in *Tirmania pinoyi*, while they were ellipsoid with roughened inner wall sides in *Tirmania nivea*. On the other hand, the third truffle sample collected from China was blackish and was identified as *Tuber indicum.* Gleba of *Tuber indicum* were dark reddish brown with white veins, ascospores appeared ellipsoid about 5 per asci while the peridium appeared with characteristic angular warts and polygonal shape. Ethyl acetate extracts of truffle samples were prepared as described by Daba and colleagues [22] with some modifications. Briefly, 500 g of the fruiting bodies of different truffle samples were separately washed with distilled water, air-dried, then cut into small pieces, and placed at room temperature in an Erlenmeyer flask containing ethyl acetate (Sigma Aldrich, St. Louis, MO, USA). After being left soaked for 48 h, the mixtures were filtrated using Whatman No. 4 paper, then the process of extraction was repeated two times under the same conditions. Obtained filtrates were concentrated using a rotary evaporator (Heidolph, Germany, 40°C) till complete dryness, and ethyl acetate was completely evaporated. The obtained dry extracts were stored at 4°C till further use [23].

Figure 1



Truffles collected from Marsa Matruh governorate desert, Egypt (*Tirmania nivea*, a), Jizan City desert, Saudi Arabia (*Tirmania pinoyi*, b), and Guiyang city, China (*Tuber indicum*, c).

The α-glucosidase inhibitory effect of truffle extracts

The *in vitro* α-glucosidase inhibitory activity (antidiabetic activity) of the prepared extracts of the three different truffles was evaluated as described by Shai and colleagues [24] with minor modification. Briefly, a reaction mixture consisting of 60 µl alpha-glucosidase in phosphate buffer (0.3 U/ml), and 10 µl of varying concentrations of truffles extracts was preincubated in a 96-well plate for 15 min at 37°C. Then, 150 µl P-NPG (1 mM) was added as a substrate and incubated further at 37°C for 40 min The reaction was stopped by adding 150 µl NaOH (50 mM). The absorbance of the released p-nitrophenol was measured at 405 nm using ELISA Reader. Acarbose at various concentrations (0.1–0.5 mg/ml) was included as a standard. The enzyme together with P-NPG was set up in parallel as a control and each experiment was performed in triplicates. The results were expressed as inhibition percentage, which was calculated as follows:

$$\text{Inhibitory activity (\%)} = 1 - \frac{Truffle\ Extract\ Treated\ Sample\ Absorbance}{Control\ Absorbance}\ X\ 100$$

The anti-inflammatory activity of truffle extracts

For cell culture seeding and treatment, the macrophage cell line, RAW 264.7, was obtained from the ATCC (American-type culture collection). The cells were cultured in RPMI,1640 medium (Roswell Park Memorial Institute) and supplemented with 1% pen/strep and 10% heat-inactivated fetal bovine serum. The cells were then incubated in a humidified incubator, in an atmosphere of 5% CO_2 at 37°C and were subcultured twice before each investigation. Under sterile conditions, RAW 264.7 cells were suspended in RPMI medium then after 24 h of seeding 1×10^5 cells/well (in 96 well plates) and incubated for 24 h for the experiments. The cells were then treated with the truffle extracts at concentrations of 100, 50, 25, and 12.5 µg/ml and incubated for 1 h. They were then stimulated with 10 µg/ml of LPS for another 24 h. The supernatant was gently transferred to new 96-well plates and used for NO determination, while the cells that remained in the old plate were used for the MTT assay of cell viability. Samples (stock) were dissolved in DMSO, and the working samples were prepared in the media. Cell viability was assessed by the mitochondrial-dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan [25]. The percentage of change in viability was calculated as following:

$$\left(\frac{Truffle\ Extract\ Treated\ Sample\ Absorbance}{Negative\ Control\ Absorbance} - 1\right)\ X\ 100$$

Nitric oxide assay

Nitric oxide production was assayed by measuring nitrite in the supernatants of cultured RAW 264.7 cells. The assay was carried out as described previously with slight modification [26]. After preincubation of RAW 264.7 cells (1×10^5cells/mL) with LPS (10 µg/ml) for 24 h, the amount of nitrite, a stable metabolite of NO used as an indicator of NO production, in the culture medium was measured using the Griess reagent (1% sulfanilamide and 0.1% naphthyl ethylenediamine dihydrochloride in 2.5% phosphoric acid). A volume of 50 µl of the cell culture medium was mixed with 50 µl of the Griess reagent. Subsequently, the mixture was incubated at room temperature for 15 min and the absorbance was measured at 540 nm in a microplate reader. Fresh culture medium was used as a blank in every experiment. The quantity of nitrite was

determined from a sodium nitrite standard curve as expressed in the equation:

$$\text{Nitric Oxide Inhibition (\%)} = \frac{Control\ Absorbance - Extract\ treated\ Absorbance}{Control\ Absorbance}\ X\ 100$$

In vitro wound scratch assay

The migration rates of BJ-1 cells were assessed by the scratch assay method [27]. The cell density of 2×10^5 cells was seeded into each well of a 24-well plate and incubated with complete medium at 37°C and 5% CO_2. After 24 h of incubation, the monolayer confluent cells were scrapped horizontally with a sterile P200 pipette tips. The debris was removed by washing with PBS. The cells were treated with samples with a concentration 100µg/ml. The cells without treatment were used as a negative control. The scratch induced that represented the wound was photographed at 0 h using phase contrast microscopy at ×40 magnification at 0 h, before incubation with the samples. After 24 h of incubation, the second set of images was photographed. To determine the migration rate, the images were analyzed using 'image J' software, and the percentage of the closed area was measured and compared with the value obtained at 0 h. An increase in the percentage of the closed area indicated the migration of cells. Experiments were performed in the triplicates and the data were recorded and analyzed statistically using SPSS.

$$\textbf{Wound closure (\%)} = \frac{Measurment\ at\ 0\ h - Measurment\ at\ 24\ h}{Measurment\ at\ 0\ h}\ \boldsymbol{X\ 100}$$

Antiviral activity of truffles extracts

Vero cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% of heat-inactivated fetal bovine serum(FBS), 100 units/ml penicillin, 100 µg/ml streptomycin under 5% CO_2 humidified incubator (All purchased from Lonza, Belgium). The diluted ten-fold of coxsackie virus B3 (CVB3) stock was replicated in Vero cells and the cytopathic effect was checked after 72 h of incubation. The 50% tissue culture infectious doses/0.1 ml ($TCID_{50}$/0.1 ml) was estimated as described previously by Karber method [28], then stored in small aliquots at – 20°C until used.

The cytotoxicity of the tested truffle extracts was investigated using the MTT assay after 4 days of cell culture according to the previously described protocol [29]. Briefly, 5×10^3 cells / well were seeded in 96-well plates. After 24 h, the growth medium was removed and the Vero cell monolayers were incubated with various concentrations of the tested extracts. After an additional 48 h at 37°C under humidified 5% CO_2 atmosphere, the tested extract was discarded and 100 µl of MTT solution (5 mg/ml) was added to all wells. After 4 h at 37°C, the MTT was carefully removed from wells and replaced with 50 µl dimethyl sulfoxide (DMSO). The plats were further incubated for 30 min at 37°C. The optical density was read on a multiwell ELISA reader at 540 nm. The viability of treated cells was expressed as the percentage of cell control viability using the following formula:

$$(\%) = \frac{Truffle\ Extract\ Treated\ Sample\ Absorbance}{Control\ Absorbance}\ X\ 100$$

The concentrations of tested extract that resulted in a decrease in cell viability by less than 10% were regarded as the maximum tolerable concentrations (MTC) and selected for antiviral experiment.

For the determination of yield reduction assay, 10-fold dilutions of CVB3 were prepared in FBS free growth medium. One hundred microliters of viral dilutions 10^{-4}–10^{-9} was mixed and incubated with 100 µl of tested extract for 1 h. Microscopic examination for CPE was performed after 72 h postinfection. The virus titer as 50% tissue culture infection dose ($TCID_{50}$) was calculated using Kärber method [28]. The above experiment was performed in three different ways to study the mechanism of action: (i) virucidal, the virus at 10^6 $\log_{10}$ $TCID_{50}$/0.1 ml was mixed with an equal volume of various non-toxic doses of the extract and incubated for 1 h at 37°C then the mixture was added to the cell lines in 96-well plates. (ii) pretreatment, the cell monolayers were treated with the extracts for 24 h at 37°C in 5% CO_2 atmosphere then after discarding the extracts, the cell culture was infected with the virus for 1 h at 37°C, then viral inoculum was removed and the cell lines incubated with fresh medium for 72 h. (iii) posttreatment, Confluent cell monolayers were infected with the CVB3 for 1 h. After removing the viral inoculum, the cells were rinsed twice with PBS to remove the unbound virus and then incubated with a test medium containing various nontoxic doses of each extract. The reduction of virus titer was estimated as the difference between the values of the virus with extract against the virus without extract.

Results and discussion

Mushrooms are known as promising sources of bioactive compounds. Edible species are attracting further attention as they were consumed by human and animals safely. Truffles are those ectomycorrhizal wild mushrooms belonging to Orders: Tuberales and Pezizales and are characterized by their hypogeous fruiting bodies and are found living in symbiosis

with the roots of specific trees Daba and colleagues, Elkhateeb and colleagues [13,30]. Truffles are found in forests and deserts but are difficult to harvest as they are season dependent and sometimes require digging in the soil to collect them. However, they are currently cultivated for their nutritional value, delicious taste, and bioactive potential. Studies published on truffles species were focusing on their chemical composition, cultivation, phylogenetic relation between species Strojnik and colleagues, Fan and colleagues [31,32]. On the other hand, majority of studies discussing their biological activities were focusing only on their antibacterial, antifungal and antioxidant effects Dib-Bellahouel and Fortas, Sallam and colleagues [33,34]. Similarly, traditional use of truffles by native people was also focusing on their antimicrobial activity or their use as aphrodisiacs Mustafa and colleagues [35]. Nowadays, there is a serious focus on truffles in order to reveal their pharmaceutical and medical potential and scientists started to evaluate their anticancer and immunomodulatory Elsyed and colleagues, Sawaya and colleagues [36–38]. Hence, we aimed in the current study to collect different desert truffle samples from different countries over the world (Egypt, Saudi Arabia and China) and compare the bioactive potential of their ethyl acetate extracts in order to reach a further understanding of the actual potential of these truffles. Morphological and microscopic identification of truffle samples reveal that they are *Tirmania nivea* (collected from Egypt), *Tirmania pinoyi* (collected from Saudi Arabia) and *Tuber indicum* (collected from China).

Evaluating the antidiabetic effect of prepared ethyl acetate extracts of the three truffles through their ability to inhibit α-glucosidase revealed that *Tuber indicum* extract has achieved the highest inhibition effect among tested truffles extracts recording 21.7% α-glucosidase inhibition (using concentration of 100 ppm), while *Tirmania nivea* and *Tirmania pinoyi* extracts achieved only 2.5 and 1.2%, respectively using the same concentration. It should be noted that the positive control (Acarbose) caused 40% inhibition at 23 ppm.

The enzyme α-glucosidase is the main enzyme responsible for catalyzing the last step in carbohydrate digestion. Therefore, inhibitors of α-glucosidase are capable of retarding the breakdown of carbohydrates into d-glucose hence delaying glucose absorption which has a hyperglycemic effect Hossain and colleagues, Kumar and colleagues [2,3]. Natural sources of α-glucosidase inhibitors can be used to produce physiologic functional food especially if they are edible as in the case of truffles.

Nitric oxide inhibition activity of truffles extracts

Inflammation is a pathological and physiological depending mechanism of defense that is induced by pathogens attacks or injury of tissues Zhou and colleagues [39]. The activation of immune cells such as macrophages and monocytes produces inflammation mediators such as nitric oxide (NO) and others Wojdasiewicz and colleagues [40]. Additionally, NO is a main signaling biological molecule that is involved in the regulation of blood pressure, vasodilation, host immune defense system, and neurotransmission Lundberg and Weitzberg [41]. Hence, inhibition of NO is one of the significant ways to regulate inflammation Meng and colleagues [42]. So we investigated the inhibiting effect of ethyl acetate extracts of the three truffles samples at different concentrations (12.5, 25, 50, and 100 μg/ml) on nitric oxide. As shown in Table 1, nitric oxide inhibition % is directly proportional to truffle extract which means that increasing the extract concentration was accompanied by an increase in the inhibition %. The highest inhibition activity was recorded by *Tuber indicum* extract (50.2%) using a concentration of 100 μg/ml, while the lowest inhibition activity was recorded by *Tirmania pinoyi* extract (21.7%) at a concentration of 12.5 μg/ml. On the other hand, *Tuber indicum* extract has recorded the lowest IC_{50} (86.0±0.09 μg/ml), while *Tirmania pinoyi* extract has recorded the highest IC_{50} (104.1±0.12 μg/ml). The highest recorded cell viability% at 100 μg/ml against raw cells was achieved by *Tirmania pinoyi* (99.1%) which is close to that achieved by the negative control, LPS (99.6%).

Table 1 Nitric oxide inhibiting activity of truffles extracts

	Nitric oxide inhibition (%)					
Truffle extracts	100 μg/ml	50 μg/ml	25 μg/ml	12.5 μg/ml	IC_{50} (μg/ml)	Cell viability% at 100 μg/ml against Raw cells
Tuber indicum	50.2	41.3	36.9	32.6	86.0±0.09	92.6%
Tirmania nivea	45.6	39.1	34.2	30.6	96.9±0.10	97.4%
Tirmania pinoyi	43.4	36.9	26.0	21.7	104.1±0.12	99.1%
LPS(-ve control)	0					99.6%

IC_{50} is the concentration required to inhibit 50% of Nitric oxide.

Wound healing activity of truffles ethyl acetate extracts

The influence of different truffles extracts at concentration of 100 µg/mL on the migration of fibroblast cells (BJ-1) was investigated as an indication of their wound healing potentials. As shown in Fig. 2, cells migration towards the provisional gap was slightly induced after 24 h of exposure to *Tirmania pinoyi* extract with 74.71% wound closure followed by 73.43% and 62.38% by *Tuber indicum* and *Tirmania nivea* extracts, respectively. Cell migration has a major role in wound healing and repairing process and as shown in Fig. 2 *Tirmania pinoyi* extract exerted the highest wound closure %. Such promising activity nominates this extract for further investigations in order to optimize and specify compounds responsible for it especially that this extract is rich in polyphenols and flavonoids as gallic acid, catechin, ethyl gallate, rutin, ferulic acid, kaempferol, and hesperetin Abuotabi and colleagues [21] and many studies have demonstrated.

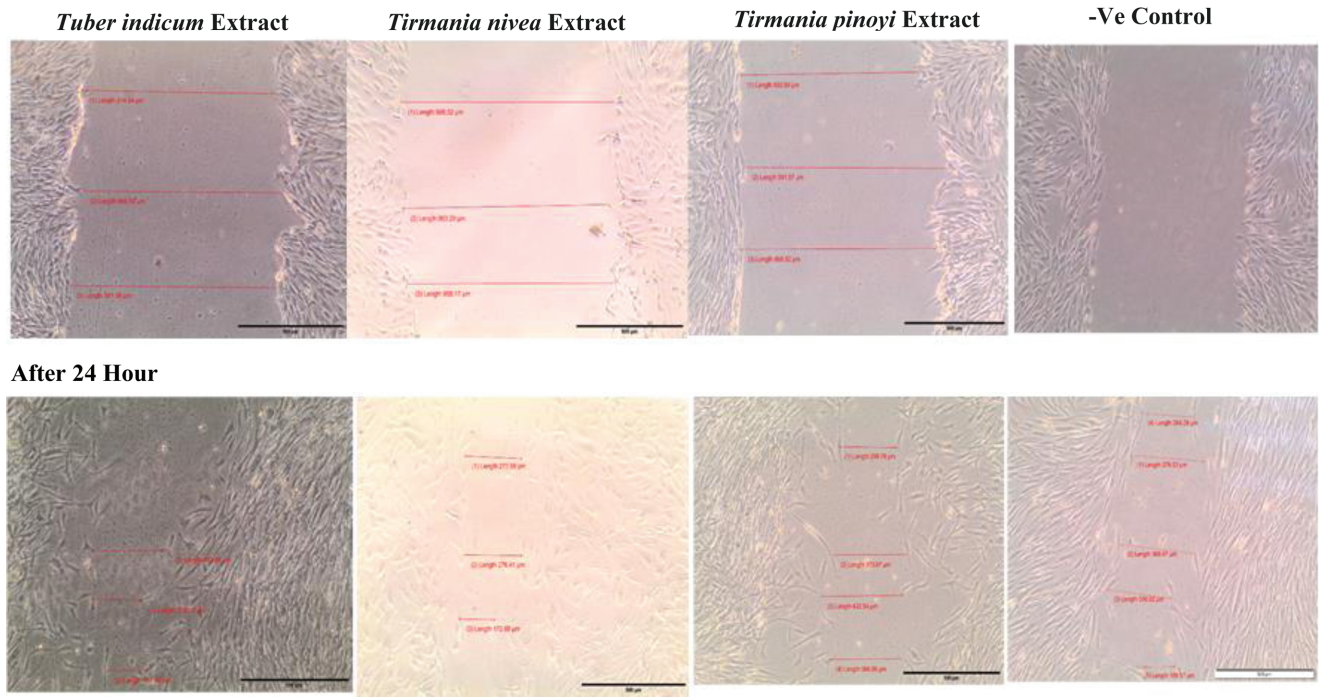
That flavonoids have promising wound-healing activities Asao and Asaduzzaman, Zulkefli and colleagues [43,44]. As far as we know, this is the first study that describes the in vitro wound healing potential of truffle species. Previous studies have described this activity in mushroom extracts Alkolaibe and colleagues, Mapoung and colleagues [45,46].

Antiviral potential of truffles extracts

The maximum tolerable concentrations (MTC) were assumed to be 500 µg/ml for *Tirmania pinoyi*, 125 µg/ml for *Tirmania nivea*, and 125 µg/ml for *Tuber indicum* with cell viability of 96%, 93%, and 92%, respectively, thus these concentrations were selected to test the anti-CVB3 activity by $TCID_{50}$ measurements. In order to characterize the mechanism of antiviral activity of these extracts against the virus infections, Karber methods were applied to investigate of $TCID_{50}$ in three different strategies in order to elucidate whether the tested extracts affected the viral capsid (virucidal) or viral receptors (pre-treatment), preventing the virus entry into the host cells, or affected the virus inside the host cells (posttreatment), preventing their replication. Our results demonstrated that *Tirmania pinoyi*, *Tirmania nivea*, and *Tuber indicum* ethyl acetate extracts showed antiviral activities against CVB3 infections via different mechanisms with the reduction in virus titers ranging from 0. 5 to 3.0 $\log_{10}$ $TCID_{50}$.

During virucidal assay, *Tirmania pinoyi* extract showed the highest antiviral activity against virus infection with a reduction in virus titers (R) equal to 3.0 then the lowest antiviral activities were shown from *Tirmania nivea* (R=0.5 $\log_{10}$ $TCID_{50}$) and *Tuber indicum* (R=0.5 $\log_{10}$ $TCID_{50}$).

Figure 2



Wound healing potential of ethyl acetate truffles extracts on BJ-1 cells at 0 h and after 24 h of treatment with extract.

Table 2 The antiviral activity of tested compounds on CVB3 determined by Karber methods

	Virucidal			Pre-treatment			Post-treatment		
Compound	A	B	R	A	B	R	A	B	R
Tirmania pinoyi	6.0	3.0	3.0	6.0	4.5	1.5	6.0	5.25	0.75
Tirmania nivea	6.0	5.5	0.5	6.0	5.0	1.0	6.0	0.4	2.0
Tuber indicum	6.0	5.5	0.5	6.0	5.25	0.75	6.0	3.75	2.25

Inhibition rates observed from *Tirmania pinoyi*, *Tirmania nivea*, and *Tuber indicum* were 50%, 8.3%, and 8.3% of the viral stock, respectively. During pretreatment assay, the highest antiviral activities were shown from *Tirmania pinoyi* and *Tirmania nivea*, with a reduction in virus titers equal to 1.5 $\log_{10}$ $TCID_{50}$ and 1.0 $\log_{10}$ $TCID_{50,}$ respectively. The lowest antiviral activity was shown from *Tuber indicum* with R=0.75 $\log_{10}$ $TCID_{50}$. Inhibition rates observed from *Tirmania pinoyi*, *Tirmania nivea*, and *Tuber indicum* were 25%, 16.6%, 12.5% of the viral stock, respectively.

During post-treatment assay, the highest antiviral activities were shown from *Tuber indicum* and *Tirmania nivea* extracts with R=2.25 $\log_{10}$ $TCID_{50}$ and R=2.0 $\log_{10}$ $TCID_{50}$, respectively. The lowest antiviral activity against virus infection with R=0.75 $\log_{10}$ $TCID_{50}$ was produced from *Tirmania pinoyi*. Inhibition rates observed from *Tirmania pinoyi*, *Tirmania nivea*, and *Tuber indicum* were 12.5%, 33.3%, 37.5% of the viral stock, respectively (Table 2 and Fig. 3).

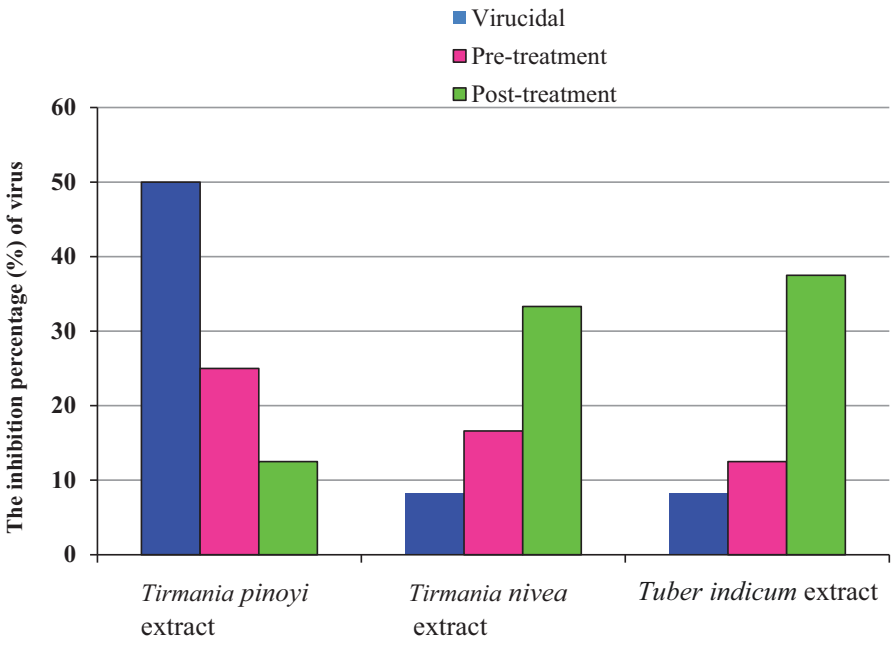
This is the first study describing the antiviral activity of *Tirmania pinoyi*, *Tirmania nivea*, and *Tuber indicum* ethyl acetate extracts against coxsackie virus B3. However, several studies have discussed the antiviral potentials of different mushrooms such as *Phellinus pini*; *Morchella conica, Pleurotus ostreatus, Terfezia boudieri, Tricholoma anatolicum, Fomes fomentarius, Phellinus igniarius, Laetiporus sulphureus,* and *Pyrofomes demidoffii* against coxsackie virus B3 [47,48].

A: the virus titers before treatment; B: virus titers after treatment; R: Reduction in virus titer calculated as the difference between treated and untreated virus and expressed in $\log_{10}$ $TCID_{50}$/0.1 ml.

Conclusion

The emergence of new diseases and the slowness in the discovery of new drugs have directed scientists to screen nature in general and microorganisms in particular in order to discover potent compounds to support and/or substitutes of the currently used drugs. Desert truffles are edible, known centuries ago as potent remedy for skin and eye infections and as nutritional food. Also,

Figure 3



Inhibition percentage produced from the tested truffles extracts against coxsackie virus B3 infections.

researchers have identified them as potential sources rich in bioactive compounds which nominate them for intensive investigations.

The promising anti-inflammatory, wound healing and antiviral activities achieved by these truffles (*Tirmania pinoyi*, *Tirmania nivea*, and *Tuber indicum*) encourage further studies on them to be used as functional foods or as sources of bioactive compounds.

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Conflicts of interest

Authors declare that there are no conflicts of interest.

References

1 Tamayo T, Rosenbauer J, Wild SH, Spijkerman AM, Baan C, Forouhi NG, *et al.* Diabetes in Europe: an update. Diabetes Res Clin Pract 2014; 103:206–217.

2 Hossain U, Das AK, Ghosh S, Sil PC. An overview on the role of bioactive α-glucosidase inhibitors in ameliorating diabetic complications. Food and chemical toxicology 2020; 145:111738.

3 Kumar S, Narwal S, Kumar V, Prakash O. α-glucosidase inhibitors from plants: A natural approach to treat diabetes. Pharmacogn Rev 2011; 5:19.

4 Schmid-Schönbein GW. Analysis of inflammation. Annu Rev Biomed Eng 2006; 8:93–151.

5 Maleki SJ, Crespo JF, Cabanillas B. Anti-inflammatory effects of flavonoids. Food Chem 2019; 299:125124.

6 Hou C, Chen L, Yang L, Ji X. An insight into anti-inflammatory effects of natural polysaccharides. Int J Biol Macromol 2020; 153:248–255.

7 Garmaroudi FS, Marchant D, Hendry R, Luo H, Yang D, Ye X, *et al.* Coxsackievirus B3 replication and pathogenesis. Future Microbiol 2015; 10:629–653.

8 Mamana J, Humber GM, Espinal ER, Seo S, Vollmuth N, Sin J, Kim BJ. Coxsackievirus B3 infects and disrupts human induced-pluripotent stem cell derived brain-like endothelial cells. Front Cell Infect Microbiol 2023; 13:1171275.

9 Shaheen M, Borsanyiova M, Mostafa S, Bopegamage S, El-Esnawy N. In Vitro and In Vivo Evaluation of *Bauhinia variegata* extracts to Prevent Coxsackievirus B3 Infection. J Proteomics Bioinform 2017; 10:73–77.

10 Stephenson S, Semwal KC. Distribution, Ecology, and Taxonomy of Medicinal Mushrooms. In Wild Mushrooms and Health. Boca Raton, Florida, United States: CRC Press; 2023. 11–22.

11 Patel S, Rauf A, Khan H. The relevance of folkloric usage of plant galls as medicines: Finding the scientific rationale. Biomedicine & Pharmacotherapy 2018; 97:240–247.

12 Alzand K, Bofaris M, Ugis A. Chemical composition and nutritional value of edible wild growing mushrooms: a review. World J Pharm Res 2019; 8:31–46.

13 Daba GM, Elkhateeb WA, Wen TC, Thomas PW. The continuous story of truffle-plant interaction. Microbiome in Plant Health and Disease: Challenges and Opportunities. Singapore: Springer 2019. 375–383

14 Veeraraghavan VP, Hussain S, Papayya Balakrishna J, Dhawale L, Kullappan M, Mallavarapu Ambrose J, *et al.* A comprehensive and critical review on ethnopharmacological importance of desert truffles: *Terfezia claveryi*, *Terfezia boudieri*, and *Tirmania nivea*. Food Reviews International 2022; 38(sup1):846–865.

15 Hall IR, Haslam W. Truffle cultivation in the Southern Hemisphere. In Edible Ectomycorrhizal Mushrooms: Current Knowledge and Future Prospects. Berlin, Heidelberg: Springer. 2013. 191–208. Berlin Heidelberg

16 Khan F, Hussain I, Akram M, Owaid MN. Truffles: The Cultivation and Health Benefits. In Biology, Cultivation and Applications of Mushrooms, 2022; (pp. 285–300). Singapore: Springer Singapore

17 El Enshasy H, Elsayed E, Aziz R, Wadaan MA. Mushrooms and truffles: historical biofactories for complementary medicine in Africa and in the middle East. Evidence-Based Complementary and Alternative Medicine 2013; 620451:1–11. Article ID

18 Shavit E. The history of desert truffle use. In *Desert Truffles:* Phylogeny, Physiology, Distribution and Domestication, 2013; (pp. 217–241). Berlin, Heidelberg: Springer Berlin Heidelberg

19 Khalifa SA, Farag MA, Yosri N, Sabir JS, Saeed A, Al-Mousawi SM, *et al.* Truffles: From Islamic culture to chemistry, pharmacology, and food trends in recent times. Trends Food Sci Technol 2019; 91:193–218.

20 Thomas PW, Elkhateeb WA, Daba G. Truffle and truffle-like fungi from continental Africa. Acta mycologica 2019; 54:1–10.

21 Aboutabl ME, Elkhateeb WA, Masoud MA, Daba GM, Afifi AH, Hussein RA. HPLC and GC-MS-based metabolic profiles and in vivo anticonvulsant, sedative, and antinociceptive potentials of truffles *Tirmania nivea* and *Tirmania pinoyi* hydromethanolic extracts in mice. Biomed Chromatogr 2020; 36:e5481.

22 Daba GM, Elkhateeb WA, El-Dein AN, Ahmed EF, EL Hagrassi AM, Fayad W, Wen TC. Therapeutic potentials of n-hexane extracts of the three medicinal mushrooms regarding their anti-colon cancer, antioxidant, and hypocholesterolemic capabilities. Biodiversitas 2020; 21:2437–2445.

23 El-Hagrassi A, Daba G, Elkateeb W, Ahmed E, El-Dein AN, Fayad W, *et al.* In vitro bioactive potential and chemical analysis of the n-hexane extract of the medicinal mushroom, Cordyceps militaris. Malaysian Journal of Microbiology 2020; 16:1–9.

24 Shai LJ, Magano SR, Lebelo SL, Mogale AM. Inhibitory effects of five medicinal plants on rat alpha-glucosidase: Comparison with their effects on yeast alpha-glucosidase. Journal of Medicinal Plants Research 2011; 5:2863–2867.

25 Bassyouni FA, Abu-Baker SM, Mahmoud K, Moharam M, El-Nakkady SS, Rehim MA. Synthesis and biological evaluation of some new triazolo [1, 5-a] quinoline derivatives as anticancer and antimicrobial agents. RSC Advances 2014; 4:24131–24141.

26 Yoon WJ, Kim SS, Oh TH, Lee NH, Hyun CG. *Abies koreana* essential oil inhibits drug-resistant skin pathogen growth and LPS-induced inflammatory effects of murine macrophage. Lipids 2009; 44:471–476.

27 Tam JC, Lau KM, Liu CL, To MH, Kwok HF, Lai KK, *et al.* The in vivo and in vitro diabetic wound healing effects of a 2-herb formula and its mechanisms of action. J Ethnopharmacol 2011; 134:831–838.

28 Karber G. Archives of Experimental Pathology and Pharmacology. 50% end-point calculation 1931; 162:480–483.

29 Nabil B, Zyed R, Mohamed AL, Souad S, Mahjoub A. Assessment of the cytotoxic effect and in vitro evaluation of the anti-enteroviral activities of plants rich in flavonoids. J of Applied Pharmaceutical Science 2012; 2:74–78.

30 Elkhateeb WA, Somasekhar T, Thomas PW, Wen TC, Daba GM. Mycorrhiza and lichens as two models of fungal symbiosis. Journal of microbiology, biotechnology and food sciences 2021; 11:1–10.

31 Strojnik L, Grebenc T, Ogrinc N. Species and geographic variability in truffle aromas. Food and Chemical Toxicology 2020; 142:111434.

32 Fan L, Li T, Xu YY, Yan XY. Species diversity, phylogeny, endemism and geography of the truffle genus Tuber in China based on morphological and molecular data. Persoonia-Molecular Phylogeny and Evolution of Fungi 2022; 48:175–202.

33 Dib-Bellahouel S, Fortas Z. Antimicrobial Activities of Desert Truffle Extracts and Their Chemical Identification. Journal of Pharmacy and Pharmacology 2019; 7:593–597.

34 Sallam FE, El-mokadem MT, Mekawey AA, Saker EA. Egyptian truffles as a source of antimicrobial and antioxidant agents. Journal of Scientific Research in Science 2022; 39:49–61.

35 Mustafa AM, Angeloni S, Nzekoue FK, Abouelenein D, Sagratini G, Caprioli G, Torregiani E. An overview on truffle aroma and main volatile compounds. Molecules 2020; 25:5948.

36 Elsayed EA, Alsahli FD, Amr AE, El Enshasy HA, Wadaan MA. In vitro anti-proliferative potentials of *Phaeangium lefebvrei* desert truffle towards MCF-7, HepG2 and L929 cell lines. Journal of Scientific & Industrial Research 2019; 78:858–862.

37 Al Obaydi M, Hamed WM, Al Kury LT, Talib WH. Terfezia boudieri: a desert truffle with anticancer and immunomodulatory activities. Front Nutr 2020; 7:38.

38 Sawaya K, Abou Najem S, Khawaja G, Khalil M. Proapoptotic and Antiproliferative Effects of the Desert Truffle Terfezia boudieri on Colon Cancer Cell Lines. Evidence-Based Complementary and Alternative Medicine 2023; 1693332:1–13.

39 Zhou HY, Shin EM, Guo LY, Youn UJ, Bae K, Kang SS, *et al.* Anti-inflammatory activity of 4-methoxyhonokiol is a function of the inhibition of iNOS and COX-2 expression in RAW 264.7 macrophages via NF-κB, JNK and p38 MAPK inactivation. Eur J Pharmacol 2008; 586:340–349.

40 Wojdasiewicz P, Poniatowski ŁA, Szukiewicz D. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. Mediators Inflamm 2014; 561459:1–19.

41 Lundberg JO, Weitzberg E. Nitric oxide signaling in health and disease. Cell 2022; 185:2853–2878.

42 Meng T, Xiao D, Muhammed A, Deng J, Chen L, He J. Anti-inflammatory action and mechanisms of resveratrol. Molecules 2021; 26:229.

43 Asao T, Asaduzzaman M. Phytochemicals: Source of Antioxidants and Role in Disease Prevention. In techOpen: London, eds. 2018; (pp. 25–47)

44 Zulkefli N, Che Zahari CN, Sayuti NH, Kamarudin AA, Saad N, Hamezah HS, *et al.* Flavonoids as potential wound-healing molecules: Emphasis on pathways perspective. Int J Mol Sci 2023; 24:4607.

45 ALKolaibe AG, Elkhateeb WA, Elnahas MO, El-Manawaty M, Deng C, Ting-Chi W, Daba GM. Wound healing, anti-pancreatic cancer, and α-amylase inhibitory potentials of the edible mushroom, *Metacordyceps neogunnii*. Research Journal of Pharmacy and Technology 2021; 14:5249–5253.

46 Mapoung S, Umsumarng S, Semmarath W, Arjsri P, Thippraphan P, Yodkeeree S, Limtrakul P. Skin wound-healing potential of polysaccharides from medicinal mushroom *Auricularia auricula-judae* (Bull.). Journal of Fungi 2021; 7:247.

47 Lee SM, Kim SM, Lee YH, Kim WJ, Park JK, Park YI, *et al.* Macromolecules isolated from Phellinus pini fruiting body: chemical characterization and antiviral activity. Macromolecular Research 2010; 18:602–609.

48 Doğan HH, Karagöz S, Duman R. In vitro evaluation of the antiviral activity of some mushrooms from Turkey. International journal of medicinal mushrooms 2018; 20:201–212.