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EVALUATION OF POLYPHENOLIC CONTENT AND *In VITRO* ANTI COXSACKIE VIRUS, ANTI INFLAMMATORY, ANTIOXIDANT AND WOUND HEALING ACTIVITIES OF THE MUSHROOMS *Ganoderma* SP. AND *Ganoderma applanatum* EXTRACTS

Waill A. Elkhateeb[1,2], *Walaa S.A. Mettwally*[1], *Ahmed A. F. Soliman*[3], *Mohamed N. Shaheen*[4], *ELmahdy M. Elmahdy*[4] *and Ghoson M. Daba*[*1]

Address(es):
[1] Chemistry of natural and microbial products Department, Pharmaceutical and Drug industries Research institute, National Research Centre, Dokki, Giza, 12622, Egypt.
[2] Department of Biosciences and Bioengineering, Faculty of Agriculture, Graduate School, Kyushu University, Fukuoka, Japan.
[3] Drug Bioassay-Cell Culture Laboratory, Pharmacognosy Department, Pharmaceutical and Drug industries Research institute, National Research Centre, Dokki, Giza, 12622, Egypt.
[4] Environmental Virology Laboratory, Water Pollution Research Department, Environment and Climate Change Research Institute, National Research Centre, Dokki, Giza, Egypt.

*Corresponding author: ghoson.daba@yahoo.com

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ABSTRACT

Ganoderma (immortality mushroom) is one of the key components of ancient Asian remedies used centuries ago for longevity and treatment of diseases. Inspired by its ethnopharmacological importance, *Ganoderma* is currently further studied in order to get maximum nutritional and pharmaceutical benefits. Hence, this study aimed to analyze polyphenolic composition and explore the *in vitro* bioactive capabilities of the ethyl acetate and n-butanol fractions of *Ganoderma* sp. and *Ganoderma applanatum* extracts. Ethyl acetate fractions were having higher phenolic and flavonoid contents, which influenced their antioxidant activity. The highest activity was recorded for *G. applanatum* ethyl acetate fraction. On the contrary, n-butanol fraction of *G. applanatum* exerted the highest nitric acid inhibition as an indication for its anti-inflammatory activity. On the other hand, studying effect of prepared *Ganoderma* fractions on coxsackie virus B3 (CVB3), responsible for viral myocarditis, revealed that during virucidal assay, *Ganoderma sp.* ethyl acetate fraction showed the highest antiviral activity against virus infection with reduction in virus titers (R) equal to 3.0 $\log_{10}$ $TCID_{50}$, while during pre-treatment assay, the highest antiviral activities were recorded for *G. applanatum* ethyl acetate fractions with R=3.0 $\log_{10}$ $TCID_{50}$. During post-treatment assay, the highest antiviral activity was achieved by *G. applanatum* n-butanol fraction with R=3.0 $\log_{10}$ $TCID_{50}$. Concerning wound healing potentials of prepared *Ganoderma* fractions, investigating the migration of fibroblast cells as an indication for wound healing ability of fractions revealed that *G. applanatum* n-butanol fraction had the highest ability among all tested fractions. Further studies are encouraged.

Keywords: *Ganoderma,* mushrooms, Anti-inflammatory, Antioxidant, Coxsackie virus B3, wound healing

INTRODUCTION

Myocarditis is a serious inflammation in the middle muscular layer of the heart wall (myocardium), that can negatively affect heart and weaken its electrical system, which in consequence reduces the ability of heart to pump blood, dilates cardiomyopathy and causes sudden cardiac death (**Ali-Ahmed *et al.*, 2020**). Myocarditis can be caused by viral infection by coxsackie virus B3 (CVB3), a cardiotropic virus belonging to genus Enterovirus and family Picornaviridae (**Qian *et al.*, 2022**). On the other hand, oxidative stress and chronic inflammation are fundamental triggers for many serious diseases such as cancer, neurodegenerative, cardiovascular and age-related diseases (**Altanam *et al.*, 2025**). Hence, targeting inflammation and oxidative stress is considered as one of the key strategies employed to treat many diseases. Wound healing process in diabetic patients is impaired and known to be causing serious complications (**Falanga, 2005; Kim *et al.*, 2026**). Moreover, wound healing is influenced by inflammation among many other factors (**Shukla *et al.*, 2019**). The increase in mortality rates and the seriousness of complications caused by these diseases have oriented researchers to screen for new sources of bioactive compounds. One of the most successful avenues for finding potent bioactive compounds is exploring among microorganisms in general and mushrooms in particular (**Thomas *et al.*, 2019; Thomas *et al.*, 2020; Aboutabl *et al.*, 2022**).

Mushrooms are rich sources of various metabolites that exert potent biological activities (**Elkhateeb *et al.*, 2019; Sun *et al.*, 2024**). The medicinal mushroom *Ganoderma* 'includes over 300 species' has been used from centuries in traditional medicine specifically in Japan, China, and Korea for treatment of gastric ulcer, asthma, bronchitis, hypertension, hepatitis, cancers, and arthritis (**Klaus and Wan, 2024; Hou *et al.*, 2026; Soro *et al.*, 2026**). Reishi, mannentake and lingzhi are oriental old names of *Ganoderma*. Powerful actions of *Ganoderma* have been documented and portrait 25-220 AD in *Shen Nong Ben Cao Jing* book (Shen-nong's Herbal Classics). In 1590 AD, the *Supplement to Classic of Materia Medica* (502-536 AD) and the *Ben Cao Gang Mu* by Li Shin-Zhen, have mentioned mushroom therapeutic properties especially to strengthen body and soul (**Benzie and Wachtel-Galor, 2011**). Inspired by its ethnopharmacological importance, many studies have investigated its biological activities (**Dey, 2026**). However, the whole therapeutic potentials of *Ganoderma* species are not fully studied, due to its richness in known and novel potent bioactive compounds. Hence, continuous researches on *Ganoderma* bioactivity always identify new biological potentials. Understanding its medical and nutritional values, *Ganoderma* is available worldwide in food markets and as a supplement (**Wu *et al.*, 2017**). Moreover, many techniques were developed in order to cultivate different *Ganoderma*, which facilitate large-scale production of its important metabolites (**Karunarathna *et al.*, 2025**).

Different fractions of *Ganoderma* species' extracts are expected to have variation in their polyphenolic contents, which will influence their biological activities. Hence, the objective of our study was to understand the impact of fractionation of mushroom extracts on chemical composition and bioactivity. So, we performed a comparative evaluation of phenolic and flavonoids content of prepared fractions from extracts of two *Ganoderma* species, that were identified as *Ganoderma Sp.* and *G. applanatum.* Also, efficacy (biological potency) of obtained fractions were compared to identify the most promising fraction for potential therapeutic applications.

MATERIAL AND METHODS

Mushroom samples and preparation of mushrooms extracts

Fruiting bodies of *Ganoderma* Sp. (**Fig. 1a**) used in this study were collected from decaying trunk of a Japanese cherry Sakura tree (*Prunus serrulata*) located in

Chihaya, Higashi ward, Fukuok Prefecture, Japan. Using fruiting bodies of this mushroom, it was morphologically identified as *Ganoderma* sp. Similarly, fruiting bodies of the second mushroom (**Fig. 1b**) was collected from bark of dead Sakura tree located in Hakozaki park, Higashi ward, Fukuoka Prefecture, Japan. This mushroom was identified as *Ganoderma applanatum.* Both mushrooms were morphologically identified by Dr. Waill Elkhateeb as described by **Phillips (2013).** Extracts of these mushrooms were prepared separately from their fruiting bodies as described by **Elkhateeb *et al.* (2020)** with some modifications. Briefly, one kilogram of each mushroom fruiting bodies was washed with distilled water, dried, cut into small pieces and placed in an Erlenmeyer flask and extraction was conducted through maceration and sonication for 15 min with gentle heating. The process was repeated till exhaustion, extracts were combined, and evaporated under reduced pressure. After that, obtained crude extracts were suspended in sterilized distilled water and were successively fractionated with elevating polarity using ethyl acetate, and n-butanol to obtain separate fractions. The obtained extracts were stored at 4°C in a clean closed container until further use.

Figure 1 Fruiting bodies of mushrooms used in this study. *Ganoderma* Sp. (a) and *Ganoderma applanatum* (b).

Evaluation of Total phenolic content (TPC) of different fractions of mushrooms extracts

Folin–Ciocalteau method was used to evaluate the total phenolic content in fractions (**Parejo *et al.*, 2002**). Gallic acid standard curve was prepared using different concentrations of gallic acid and the TPC was expressed as mg gallic acid equivalents (GAE) (**Hamdy *et al.*, 2012**).

Evaluation of Total flavonoid content (TFC) of different fractions of mushrooms extracts

The total flavonoid content was evaluated using aluminum chloride assay as described by (**Shraim *et al.*, 2021; Shaffai *et al.*, 2023**) Results were detected colourimetry at λ500nm using spectrophotometer (jasco-V-630, UV/Visible). Quercetin standard curve was prepared using different concentrations of Quercetin and The TFC was expressed as mg of quercetin equivalents (QE).

Quantitative identification of polyphenolic metabolites in different extracts using HPLC analysis

HPLC analysis was carried out according to **Kim et al. (2006)** using Agilent Technologies 1260 Infinity II liquid chromatograph equipped with an auto sampler and a diode-array detector. The analytical column was a Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 ml/min for a total run time of 60 min and the gradient program was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. The injection volume was 50 µl and peaks were monitored simultaneously at 280 and 320 nm for the benzoic acid and cinnamic acid derivatives, respectively as well as 360 nm for flavonoids. All samples were filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards.

DPPH free radical scavenging activity (antioxidant activity) of different fractions of mushrooms extracts

DPPH (1-diphenyl-2-picrylhydrazyl) free radical scavenging potentials of prepared mushrooms extracts were measured as indication for their antioxidant activity (**El-Shazly, *et al.* 2021**). Briefly, equal volumes of ethanolic DPPH solution and each mushroom extract were mixed vigorously then incubated for one hour at 37°C in the dark. Absorbance was measured spectrophotometrically at 517 nm and the free radical scavenging activity was calculated as following:

DPPH free radical scavenging activity (%) $= 1- \frac{As-Ab}{Ac} \text{ X } 100$

Where:
Ab, is the absorbance of the blank (ethanol and mushroom extract),
Ac is the absorbance of the control (DPPH and deionized water),
As is the absorbance of the sample (DPPH and mushroom extract).
Ascorbic acid at the concentration of 0.1% was used as positive control.

Anti-inflammatory activity of different fractions of mushrooms extracts

Macrophage cell line, RAW 264.7 obtained from American Type Culture Collection (ATCC, Rockville, MD, USA) was used for cell culture seeding and treatment. Cells were cultured in RPMI, 1640 medium (Roswell Park Memorial Institute) 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, 1×10^5 cells were seeded per well (in 96 well plates) and incubated for 24 hours for the experiments. The cells were then treated with the mushroom extracts fractions and incubated for 1 hour, then stimulated with 10µg/mL of LPS for another 24 hours. The supernatant was gently transferred to new 96-well plates and used for nitric oxide determination, while the cells 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 (**Bassyouni *et al.*, 2014**). The percentage of change in viability was calculated as following:

Change in viability (%) $=\left(\frac{As}{Ac}-1\right) \text{ X } 100$

Where:
Ac is the absorbance of the negative control,
As is the absorbance of the mushroom extract treated sample.

Nitric oxide (NO) 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 (**Yoon *et al.*, 2009**). After pre-incubation of RAW 264.7 cells (1×10^5cells/mL) with lipopolysaccharide (LPS, 10 µg/mL) for 24 hours, the amount of nitrite, a stable metabolite of NO use as an indicator of NO production, in the culture medium was measured using the Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine 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 for15 min and the absorbance was measured at 540 nm in a micro plate reader. Fresh culture medium was used as a blank in every experiment, and indomethacin was used as a positive anti-inflammatory control. The quantity of nitrite was determined from a sodium nitrite standard curve as expressed in equation:

Nitric Oxide Inhibition (%) $= \frac{Ac - As}{Ac} \text{ X } 100$

Where:
Ac is the absorbance of the control,
As is the absorbance of the mushroom extract treated sample.

Anti-coxsackie virus B3 activity of different fractions of mushrooms extracts

Vero cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% of heat inactivated f*et al* bovine serum(FBS), 100 units/mL penicillin, 100µg/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 ($TCID_{50}$/0.1mL) was estimated as described previously by Kärber method **(Kärber, 1931)**, then stored in small aliquots at – 20°C until used. The cytotoxicity of the tested mushrooms extracts was investigated using the MTT assay after 4 days of cell culture according to the previously described protocol (**Abid *et al.* 2012).** Briefly, 5 X 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 37ºC under a 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:

Viability of treated cells (%) $= \frac{As}{Ac} \text{ X } 100$

Where:
Ac is the absorbance of the control,
As is the absorbance of the mushroom extract treated sample.
The concentrations of tested extract that resulted in a decrease in cell viability by < 10% was regarded as the maximum tolerable concentrations (MTC) and selected for antiviral experiment.

For determination of yield reduction assay, 10-fold dilutions of CVB3 were prepared in FBS free growth medium. One hundred microliter 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 post infection. The virus titer as 50% tissue culture infection dose ($TCID_{50}$) was calculated using Kärber method **(Kärber, 1931)**. 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 ºC then mixture was added to the cell lines in 96-well plates. ii) pre-treatment, 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 ºC, then viral inoculum was removed and the cell lines incubated with fresh medium for 72 h. iii) post-treatment, 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 unbound virus then incubated with test medium containing various non-toxic doses of each extract. The reduction of virus titer was estimated as the difference between the values of the virus with extract and the virus without extract.

In vitro wound scratch assay and evaluation of wound healing activity of different fractions of mushrooms extracts

The migration rates of BJ-1 cells were assessed by the scratch assay method (**Tam *et al*., 2011**). The cell density of 2×10^5 cells was seeded into each well of a 24-well plate and incubated with complete medium at 37ºC and 5% CO_2. After 24h 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 mushroom extracts at a concentration of 100 μg/mL. The cells without treatment were used as negative control. The scratch induced that represented wound was photographed using phase contrast microscopy at ×40 magnification at 0 h, before incubation with the mushrooms extracts. 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 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.

$$\textbf{Wound closure (\%)} = \frac{\text{Measurment at 0h} - \text{Measurment at 24h}}{\text{Measurment at 0h}}\ \textbf{X100}$$

Statistical analyses

Experiments were performed in triplicates and the data were recorded and analyzed statistically using SPSS. Experiments were performed in biological triplicates using independent samples, and results were presented as mean±SD.

RESULTS AND DISCUSSION

Emerging of new diseases and side effects of many drugs have forced scientists to screen for natural sources of bioactive compounds. The potency, richness and variation in mushrooms' metabolites have encouraged for an intensive investigation for their biological activities (**Elkhateeb *et al*., 2025**). Interestingly, there is a notable success in cultivation of many mushroom genera in general and *Ganoderma* species in particular (**Pradhan and Acharya, 2024**), which has removed doubts of rarity of availability of bioactive compounds when needed for large scale production. The genus *Ganoderma* is attracting more attention, thanks to its ability to produce different bioactive compounds belonging to various chemical classes. Hence, we aimed in this study to explore the antiviral, anti-inflammatory, antioxidant and wound healing potentials of two *Ganoderma* samples that where collected from Japan. Successive fractions were prepared from crude extract of *Ganoderma* sp. and G. *applanatum* using ethyl acetate, and n-butanol. Then, the biological activity of each fraction was investigated. Many studies have highlighted the impact of using different solvents in succession on quality (belonging to which chemical class) and quantity of extracted compounds (**Abdel-Aal *et al*., 2015, Palaiogiannis *et al*., 2023**). Phenolic compounds and flavonoids are bioactive secondary metabolites that possess a wide variety of pharmaceutical importance in human health care. They are involved in different forms either as treatment or protective agents in pharmaceutical industry (**Sun and Shahrajabian, 2023**).

Total Phenolic and Flavonoid Contents

The total phenolic and flavonoid contents of the ethyl acetate and *n*-butanol fractions prepared from *Ganoderma* sp. and *G. applanatum* are presented in **Table 1**. The ethyl acetate fraction of *G. applanatum* showed the highest phenolic content (95 mg GAE/g DW), followed closely by the ethyl acetate fraction of *Ganoderma* sp. (93 mg GAE/g DW). In contrast, the *n*-butanol fractions of both species exhibited lower phenolic levels, particularly *Ganoderma* sp. (54 mg GAE/g DW). Similarly, the total flavonoid content was higher in the ethyl acetate fraction of *G. applanatum* (113 mg QE/g DW), with comparable values in the ethyl acetate fraction of *Ganoderma* sp. (110 mg QE/g DW) and the *n*-butanol fraction of *Ganoderma* sp. (108 mg QE/g DW). The lowest flavonoid content was observed in the *n*-butanol fraction of *G. applanatum* (69 mg QE/g DW). Many studies have reported the high phenolic and flavonoid (subclass of phenolic compounds) contents in ethyl acetate fractions of extracts, which can be attributed to the intermediate polarity and better solubility for polyphenols (**Zhao *et al*., 2006; Babbar *et al*., 2014**). This is consistent with our findings, as the ethyl acetate fractions of *G. applanatum* and *Ganoderma* sp. exhibited the highest phenolic and flavonoid contents among the tested fractions. This came also in accordance with results reported by **Park and Kim (2017)** who found that the ethyl acetate fraction of extracts of *Rhynchosia nulubilis* cultivated with *Ganoderma lucidum* mycelium contained the highest phenolic content. Similarly, **Kebaili *et al*. (2021)** reported that ethyl acetate fraction of *Ganoderma lucidum* contained a higher total phenolic content compared to other used solvents.

Table 1 Total phenolic and total flavonoid content in *Ganoderma* sp. and *G. applanatum* fractions

Extracts / **Content** (mg/g Dw)	***Ganoderma* sp.**		***G. applanatum***	
	Ethyl acetate fraction	**n-butanol fraction**	**Ethyl acetate fraction**	**n-butanol fraction**
Total phenolic content (GAE)	**93**	**54**	**95**	**71**
Total flavonoid content (QE)	**110**	**108**	**113**	**69**

GAE: Gallic acid equivalent; QE: Quercetin equivalent.

Quantitative identification of polyphenolic metabolites in different extracts using HPLC analysis

The HPLC chromatographic analysis revealed a diverse profile of phenolic acids and flavonoids across the four fractions, with both qualitative and quantitative variations (Table 2). n-butanol fraction of *G. applanatum* exhibited the highest overall accumulation of detected phenolic compounds, particularly chrysin (571.76 µg/g), gallic acid (295.01 µg/g), epicatechin (213.41 µg/g), and catechin (186.28 µg/g). These compounds are well known for their potent antioxidant, anti-inflammatory, and antiviral properties, which may explain the enhanced biological activity observed for this fraction. Ethyl acetate fraction of *G. applanatum* was also rich in bioactive constituents, with high levels of epicatechin (232.89 µg/g) and p-coumaric acid (156.77 µg/g), along with moderate concentration of Chrysin (121.34 µg/g) and characteristic incidence of Sinapic acid (8.53 µg/g).

This suggests a balanced phenolic composition contributing to its biological effects. Noticing the inimitable detection of p-coumaric and Apigenin in G. applanatum fractions only (Table 2). n-butanol fraction of *Ganoderma* sp demonstrated a distinct composition, characterized by high levels of epicatechin (114.11 µg/g), and vanillic acid (99.70 µg/g), along with unique presence of rosmarinic acid (11.31 µg/g). This variation suggests that different fractions selectively accumulate specific phenolics. In contrast, Ethyl acetate fraction of *Ganoderma* sp showed a relatively limited phytochemical profile, with most compounds either absent (ND) or present at low concentrations, although it demonstrates a high TPC and TFC that may be attributed to the presence of unidentified phenolic compounds.

The observed variation in phenolic composition among the samples highlights the influence of fractionation on phytochemical distribution, which in turn may explain the differences in antioxidant, antiinflammatory, antiviral and wound healing activities reported in this study. The high content of chrysin (571.76 µg/g), detected in the most active fraction, n-butanol of G. applanatum, is known for its potent anti-inflammatory effects via inhibition of NF-κB signaling and pro-inflammatory mediators, in addition to its reported antiviral activity through suppression of viral replication and modulation of host cellular responses (**Del Fabbro et al., 2025**). Likely, epicatechin was reported Monika et al. (2023) as an anti-inflammatory agent.

DPPH free radical scavenging activity (Antioxidant Activity)

The *in vitro* DPPH free radical scavenging assay is widely used as an indication of antioxidant activity (**El-Hagrassi *et al*., 2020).** As summarized in **Table 3**, the antioxidant activities of the fractions varied depending on the solvent used and the species tested.

Generally, there is a strong relation between phenolics and flavonoids secreted by mushrooms and its various biological activities such as antioxidant, antiviral, anti-inflammatory, and wound healing potentials in particular, which is achieved through different mechanisms such as regulation of some inflammatory mediators, modulation of immune signaling, and free radical scavenging (**Sulkowska-Ziaja *et al*., 2023; Saini *et al*., 2026**).

Table 2 Qualitative and Quantitative HPLC analysis of Ganoderma sp. and G. applanatum fractions

Compounds	**RT (min)**	**Concentration (µg/g)**			
		G. applanatum		***Ganoderma* sp.**	
		Ethyl acetate fraction	**n-butanol fraction**	**Ethyl acetate fraction**	**n-butanol fraction**
Gallic acid	4.02	24.34	295.01	ND	60.16
Protocatechuic	7.07	49.36	37.82	10.42	5.19
Gentisic	10.76	29.51	19.90	ND	7.15
p-hydroxybenzoic acid	11.18	22.80	ND	21.26	12.98
Catechin	12.45	ND	186.28	ND	58.94
Chlorogenic acid	13.98	45.36	5.44	ND	5.73
Caffeic	14.80	ND	2.81	ND	48.42
Syringic	15.98	9.07	10.49	3.30	5.04
Epicatechin	16.64	232.89	213.41	21.09	114.11
Vanillic	17.29	29.30	11.71	ND	99.70
Ferulic acid	22.27	10.64	ND	ND	8.49
Sinapic	23.06	8.53	ND	ND	ND
Epicatechin gallate	23.68	9.46	ND	ND	4.84
Rutin	25.27	60.56	ND	7.04	ND
p-coumaric	26.71	156.77	7.21	ND	ND
Rosmarinic	30.85	ND	ND	ND	11.31
Apigenin-7-glucoside	31.49	3.74	9.88	26.71	27.05
Cinnamic	35.55	7.76	7.55	8.04	24.91
Qurecetin	36.70	9.58	ND	6.11	6.42
Apigenin	40.94	11.95	6.88	ND	ND
Kaempferol	41.70	ND	ND	ND	ND
Chrysin	53.43	121.34	571.76	12.66	3.37

ND: Not detected

The highest activity (85%) was recorded for the ethyl acetate fraction of *G. applanatum*, which is suggested to be attributed to its higher phenolic and flavonoid contents. Conversely, the lowest antioxidant activity was observed in the *n*-butanol fraction of *G. applanatum*. Recorded values were higher than those reported by **Elkhateeb *et al.* (2020)** who found that the hydromethanolic extract of *G. applanatum* exhibited 66.24 ± 0.43% DPPH scavenging activity at a concentration of 1.0 mg/mL. **Elhassaneen *et al.* (2025)** have discussed the impact of solvents with different polarities on type and antioxidant activity of compounds extracted from *Ganoderma lucidum.* Studies on other *Ganoderma* species such as *G. pfeifferi* and *G. lucidum* have also linked phenolic and flavonoid richness to antioxidant activity (**Yalcin *et al.*, 2020**), which supported our solvent-dependent results.

Table 3 *In-vitro* antioxidant activity (DPPH scavenging percentage) of *Ganoderma* sp. and *G. applanatum* fractions

Extracts	***Ganoderma* sp. (%)**	***G. applanatum* (%)**
Ethyl acetate	82%	85%
n-butanol	79%	32%

Nitric oxide inhibition (Anti-Inflammatory Activity)

Inflammation is a defense mechanism induced by tissue injury or microbial infection (**Xu and Chenli, 2024**). Among inflammatory mediators, nitric oxide (NO), which is a key signaling molecule involved in vasodilation, blood pressure regulation, neurotransmission, and age-related diseases (**Laroux *et al.*, 2001; Mazuryk *et al.*, 2024).** Therefore, controlling inflammation can be achieved through inhibition of nitric oxide production (**Elkhateeb *et al.*, 2024**). As shown in **Table 4**, fractions obtained from *Ganoderma* spp. and *G. applanatum* exhibit notable nitric oxide (NO) inhibitory, with clear differences depending on both species and solvent fraction. Among all tested samples, the *n*-butanol fraction of *G. applanatum* showed the strongest NO inhibition (38.7%) while maintaining 89.6% cell viability, indicating that its anti-inflammatory effect was not due to cytotoxicity. In contrast, the ethyl acetate and *n*-butanol fractions of *Ganoderma* sp. exhibited only weak inhibition (5.6%), despite similarly high cell viability (>92%), suggesting a lower content of NO-modulating bioactive compounds in these fractions. It should be noted that indomethacin, which was used as a positive control recorded 76% inhibition. Anti-inflammatory effects of *Ganoderma* extracts are widely reported, including attenuation of NO production in LPS-stimulated models. Many *Ganoderma* extracts downregulate inducible nitric oxide synthase (iNOS) and reduce pro-inflammatory mediators (e.g., TNF-α, NF-κB). The synergistic antioxidant and anti-inflammatory effects were reported by **Abu-Serie *et al.* (2018)** for *Ganoderma lucidum* extracts. The correlation between phenolic content, antioxidant potential, and anti-inflammatory activity is also supported by studies showing that phenolic-rich extracts reduce ROS and NO production (**Taofiq *et al.*, 2015; Sánchez, 2017, Chanda *et al.*, 2026**). Previous reports showed that mushroom extracts can suppress LPS-induced NO production in macrophages through modulation of (iNOS) and nuclear factor kappa B (NF-κB) signaling pathways (**Song *et al.*, 2004; Huang *et al.*, 2011**).

Table 4 *In-vitro* nitric oxide inhibition activity (%) of *Ganoderma* sp. and *G. applanatum* fractions

Extract fractions		**NO inhibition (%)**	**Cell viability (%)***
Ganoderma sp.	Ethyl acetate	5.6	93.1
	n-butanol	5.6	92.8
G. applanatum	Ethyl acetate	16.8	96.4
	n-butanol	38.7	89.6
LPS		0	99.6

* Cell viability (%) at 100 µg/mL against RAW cells, LPS: lipopolysaccharide (10 µg/mL) used to induce NO production in macrophages and showed no NO inhibitory effect. Cell viability was assessed by the mitochondrial-dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl.

Antiviral activity against Coxsackievirus B3 (CVB3)

Regarding antiviral activity, the maximum tolerable concentrations (MTCs) were determined as 125 µg/mL for the *n*-butanol fraction and 62.5 µg/mL for the ethyl acetate fraction of *Ganoderma* sp., with cell viabilities of 95% and 94%, respectively. These concentrations were used for antiviral evaluation using TCID$_{50}$ assays. The Kärber method was applied using three approaches: virucidal, pre-treatment, and post-treatment assays, where the effect of fractions on the entry of virus into host cells was studied; by interacting with viral capsid (Virucidal), by blocking the viral receptors located on the host cells (pre-treatment assay), and the effect on virus after entry of into host cells (post-treatment). As presented in **Table 5 and Fig. 2**, the n-butanol and ethyl acetate fractions of *Ganoderma sp.* exhibited a broad range of antiviral activities, with reductions in virus titers (R) ranging from 0.25 to 3.0 $\log_{10}$ TCID$_{50}$.

In the virucidal assay, the ethyl acetate fraction of *Ganoderma* sp. showed the highest activity (R = 3.0 $\log_{10}$ TCID$_{50}$), followed by the *n*-butanol fraction of *G. applanatum* (R = 0.75 $\log_{10}$ TCID$_{50}$), suggests that compounds in this fraction may destabilize the viral envelope or interfere with receptor-binding sites (**Zhang *et al.*, 2014**). The lowest activity (R ≤ 0.25 $\log_{10}$ TCID$_{50}$) was observed for the *n*-butanol fraction of *Ganoderma* sp. and the ethyl acetate fraction of *G. applanatum*. Inhibition rates observed for *Ganoderma sp,* fractions were n-butanol (4.1%), and ethyl acetate (50%), while *G. applanatum* fractions were n-butanol (12.5%), ethyl acetate (4.1%) (**Table 5 and Fig. 2**).

In the pre-treatment assay, ethyl acetate fractions of both *Ganoderma* sp. and *G. applanatum* showed the highest activities (R = 2.5–3.0 $\log_{10}$ $TCID_{50}$), implying that these fractions may block viral attachment or entry by modifying host cell surface receptors (**Ang *et al*., 2021**). *n*-butanol fractions showed weak inhibition (R=0.25 -0.5 $\log_{10}$ $TCID_{50}$). Conversely, in the post-treatment assay, the highest activity was observed for the *n*-butanol fraction of *G. applanatum* (R = 3.0 $\log_{10}$ $TCID_{50}$), followed by the *n*-butanol fraction of *Ganoderma* sp. (R = 1.75 $\log_{10}$ $TCID_{50}$), suggesting inhibition of intracellular viral replication, possibly via interference with viral RNA synthesis or protein processing (**Seo and Choi, 2021**). Inhibition rates percentage of the viral stock in the pre-treatment assay were as follow, *Ganoderma sp.* ethyl acetate (50%), *G. applanatum* n-butanol (12.5%), and 4.1% for both *G. applanatum* ethyl acetate and *Ganoderma sp* n-butanol fractions. Where, in the post-treatment assay were *G. applanatum* ethyl acetate (50%), *Ganoderma sp.* ethyl acetate (41.7%), *Ganoderma sp.* n-butanol (8.3%), and finally *G. applanatum* n-butanol (4.1%) (**Table 5 and Fig. 2**).

The differential antiviral behavior among fractions highlights the presence of multiple bioactive constituents acting at different stages of the viral life cycle. Similar multi-mechanistic antiviral effects have been reported for other *Ganoderma* species, whose triterpenoids, phenolics and polysaccharides are known to inhibit enteroviruses by targeting both viral entry and replication processes (**Zhang *et al*., 2014; Ang *et al*., 2021, Cör Andrejč *et al*., 2022; Rašeta *et al*., 2023**). Importantly, all antiviral effects were observed at non-cytotoxic concentrations, as indicated by high cell viability at the MTC values. This highlights the therapeutic relevance of these fractions and supports their potential as safe, natural antiviral agents. Nevertheless, the complex nature of mushroom extracts necessitates further phytochemical characterization to isolate and identify the specific compounds responsible for the observed bioactivities.

Table 5 Antiviral activity of *Ganoderma* sp. and *G. applanatum* fractions aganist Coxsackie virus B3 (CVB3) determined by Kärber method

Extracts	**Fractions**	**Virucidal**			**Pre-treatment**			**Post-treatment**		
		A	B	R	A	B	R	A	B	R
***Ganoderma* sp.**	**n- butanol**	6.0	5.75	0.25	6.0	6.5	0.5	6.0	4.25	**1.75**
	Ethyl acetate	6.0	2	3.0	6.0	3.5	**2.5**	6.0	5.5	0.5
G. applanatum	**n- butanol**	6.0	5.25	0.75	6.0	5.75	0.25	6.0	3.0	**3.0**
	Ethyl acetate	6.0	5.75	0.25	6.0	3.0	**3.0**	6.0	4.5	1.5

A: virus titer before treatment; **B:** virus titer 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.

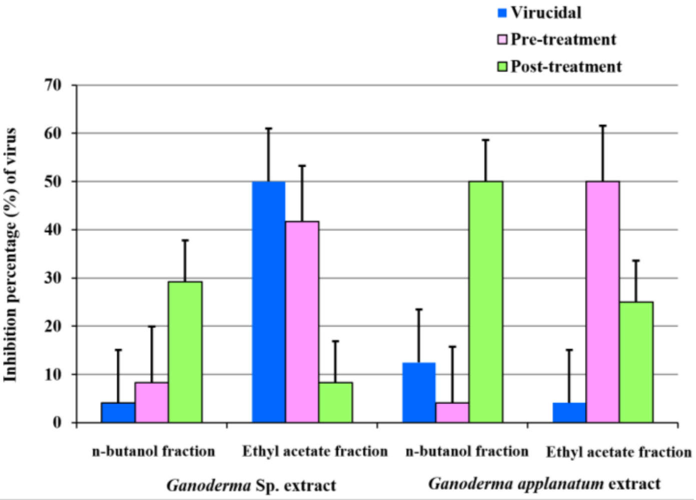
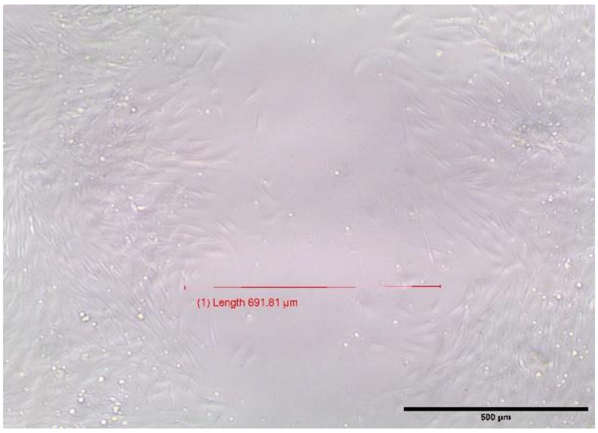


Figure 2 Antiviral activity (%) of different fractions of *Ganoderma* sp. and *G. applanatum* extracts against Coxsackie virus B3 (CVB3)

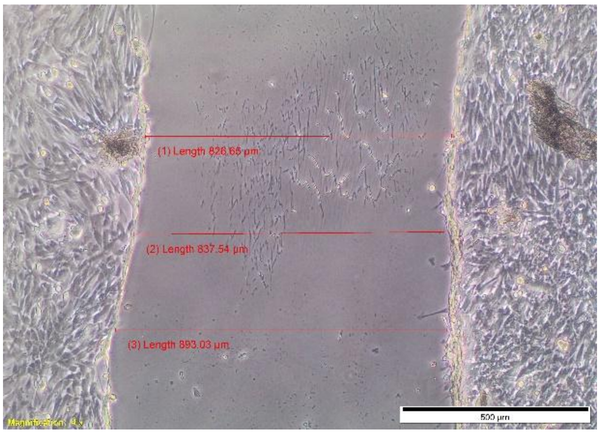
Wound Healing Activity

The wound healing potential of different solvent fractions obtained from the two mushroom extracts was evaluated by monitoring the migration of BJ-1 fibroblast cells following treatment. As illustrated in Fig. 3, all fractions showed variable effects on fibroblast migration into the provisional gap after 24 h. The *n*-butanol fraction of *G. applanatum* exhibited the strongest wound closure activity, promoting 33% migration into the provisional gap. This was followed by the ethyl acetate fraction of *G. applanatum*, which induced 27% wound -healing. In contrast, both fractions of *Ganoderma* sp. showed only minimal effects on fibroblast migration, with wound closure values of 6.6% and 6.8% for the ethyl acetate and *n*-butanol fractions, respectively. The higher wound healing activity observed for *G. applanatum* fractions may be attributed to their higher content of bioactive polyphenolic compounds, which are known to stimulate fibroblast proliferation and migration, enhance extracellular matrix remodeling, and reduce oxidative stress at the wound site (**Utpal *et al*., 2025**). Although direct wound scratch assays on *Ganoderma* extracts are less common, recent work on *G. lucidum* polysaccharide-based hydrogels has shown improved fibroblast proliferation/migration, promoted collagen deposition, and reduced oxidative stress in wound models, supporting your findings **(Li *et al*., 2024).** The wound healing activity observed in BJ-1 fibroblast migration assays is closely aligned with the nitric oxide (NO) inhibitory effects of the same fractions. Notably, the *n*-butanol fraction of *G. applanatum*, which exhibited the highest NO suppression in LPS-stimulated macrophages, also produced the strongest stimulation of fibroblast migration. Excessive NO production is known to impair wound healing by promoting prolonged inflammation and oxidative stress at the injury site. (**Schulz and Stechmiller, 2006**) Therefore, the ability of *G. applanatum* fractions to downregulate NO generation may impact fibroblast tissue repair proliferation and migration processes. Furthermore, suppression of inducible nitric oxide synthase (iNOS) and NF-κB signaling pathways previously reported to be modulated by *Ganoderma* species can accelerate the transition from the inflammatory to the proliferative phase of wound healing (**Hasnat *et al*., 2015; Xu *et al*., 2021**). This mechanistic overlap provides a plausible explanation for the dual anti-inflammatory and pro-healing effects observed in the present study.

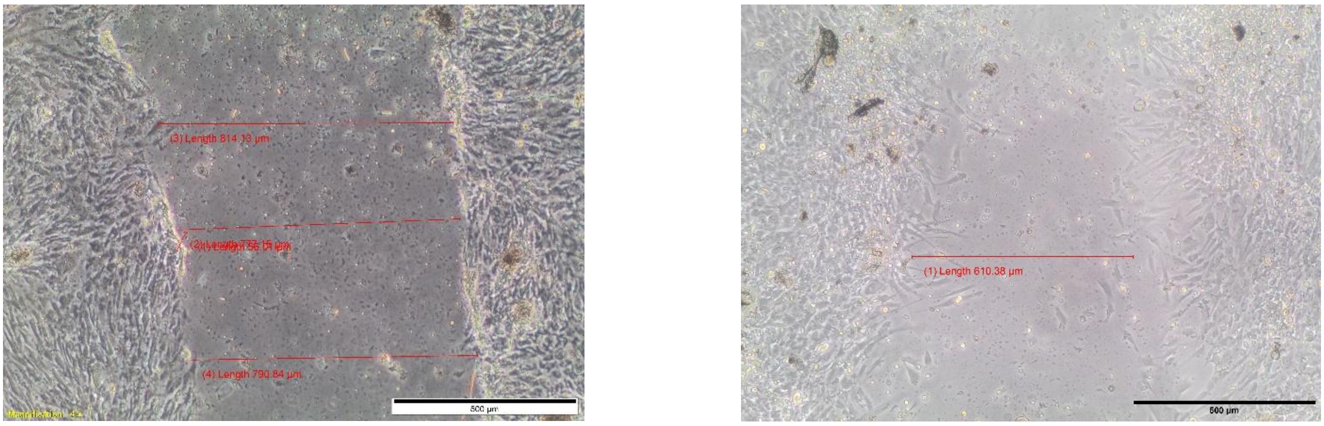
Interestingly, the wound-healing and anti-inflammatory effects correlate with phenolic content rather than flavonoid content alone, suggesting that phenolic-rich fractions provide both antioxidant protection and modulation of cellular signaling pathways involved in tissue repair. This supports previous findings that phenolic compounds from medicinal mushrooms enhance fibroblast migration and extracellular matrix remodeling (**Wasser, 2011**).



-ve 0h

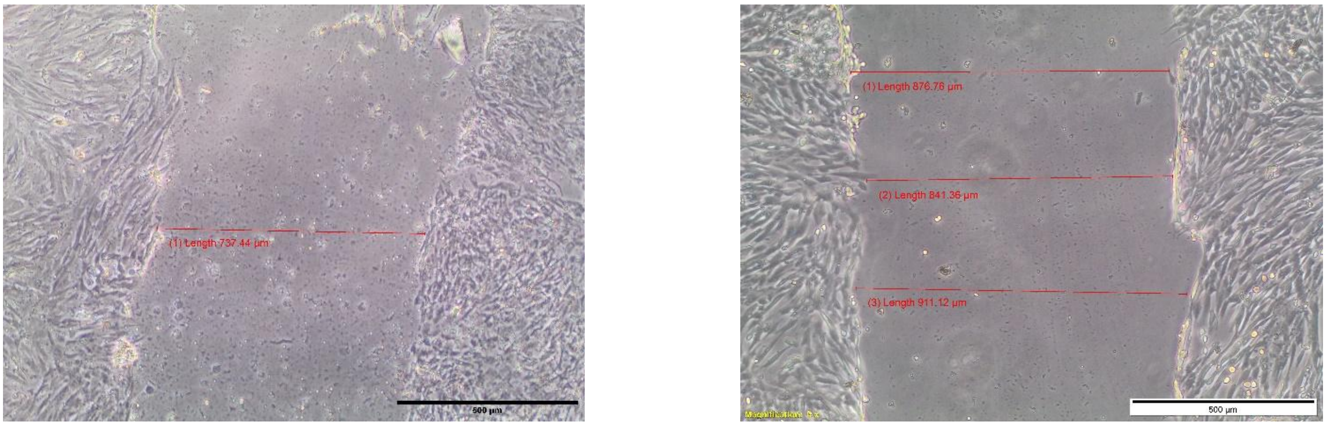


-ve 24h



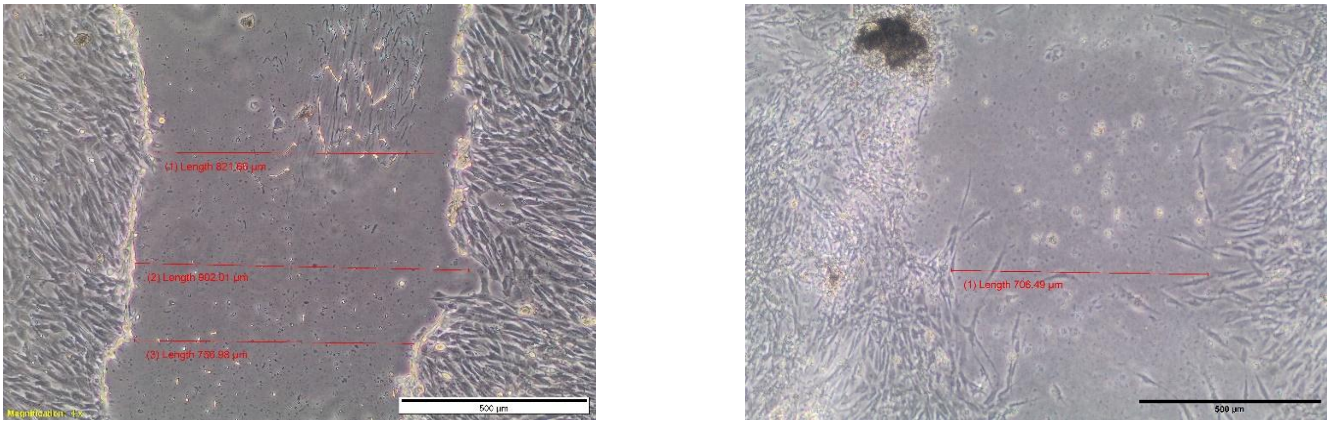
***Ganoderma* sp. n-butanol 0h**

***Ganoderma applanatum* n-butanol 0h**



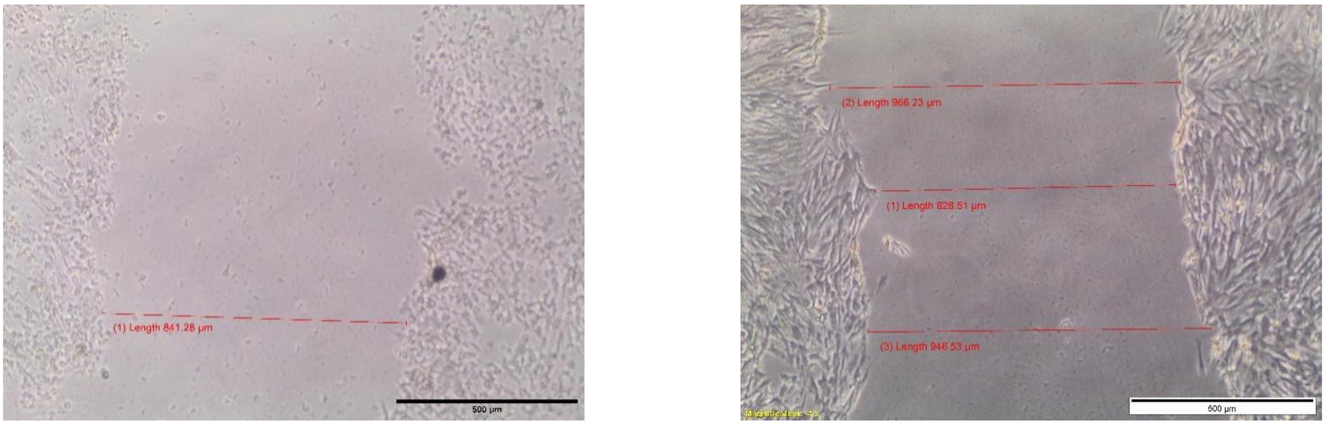
***Ganoderma* sp. n-butanol 24h**

***Ganoderma applanatum* n-butanol 24h**



***Ganoderma* sp. ethyl acetate 0h**

***Ganoderma applanatum* ethyl acetate 0h**



***Ganoderma* sp. ethyl acetate 24h**

***Ganoderma applanatum* ethyl acetate 24h**

Figure 3 Wound healing potential of ethyl acetate and n-butanol extracts of *Ganoderma applanatum* and *Ganoderma* sp. on BJ-1 cells at 0 h and after 24 h of treatment with extract.

CONCLUSION

Exploring potentials of mushrooms inspired by their traditional uses can contribute in understanding the full potency of these mushrooms. This study demonstrates that fractions obtained from *Ganoderma* sp. and *Ganoderma applanatum* exhibit complementary bioactivities, including antioxidant, anti-inflammatory, wound-healing, and anti-Coxsackie virus effects, which appear to be closely associated with their phytochemical profiles. The difference in potency of prepared fractions emphasized the importance of choosing solvent used in extraction process which in return influences the quality and quantity of extracted chemical compounds responsible for biological activities.

Concerning antiviral activity, results obtained in our study suggested potential antiviral activity based on virus titer reduction. Therefore, further studies will focus on conducting a full concentration-dependent antiviral profile through evaluation of IC50, CC50, and SI.

Additionally, further mechanistic studies are needed to verify whether the observed activity is due to interference with viral attachment/entry, intracellular replication, or both. On the other hand, the multi-functional bioactivity of the two tested *Ganoderma* species encourage for their use as natural therapeutic agents. Additional phytochemical characterization and *in vivo* evaluation are needed to support employing of these mushrooms in therapeutic application. Moreover, further studies are encouraged based on bioassay-guided isolation of the active metabolites, and elucidation of their action mechanism. Such efforts can contribute significantly in optimizing the therapeutic benefits obtained from these mushrooms.

Ethical approval: This study was performed according to the guidelines of the Medical Research Ethics Committee of the National Research Centre, Dokki, Egypt (Ethical Approval Number: 04420924).

REFERENCES

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., … & Erlich, H. A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, *239*(4839), 487-491. http://dx.doi.org/10.1088/1367-2630/1/1/006

Abdel-Aal, E.I., Haroon, A.M. and Mofeed, J. (2015). Successive solvent extraction and GC–MS analysis for the evaluation of the phytochemical constituents of the filamentous green alga Spirogyra longata. *The Egyptian journal of aquatic research*, *41*(3), 233-246. https://doi.org/10.1016/j.ejar.2015.06.001

Abid, N.B.S., Rouis, Z., Lassoued, M.A., Sfar, S. and Aouni, M. (2012). Assessment of the cytotoxic effect and in vitro evaluation of the anti-enteroviral activities of plants rich in flavonoids. *Journal of Applied Pharmaceutical Science*, 74-78. https://doi.org/10.7324/japs.2012.2532

Aboutabl, M.E., Elkhateeb, W.A., Masoud, M.A., Daba, G.M., Afifi, A.H. and Hussein, R.A. (2022). 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. *Biomedical Chromatography*, *36*(12), p.e5481. https://doi.org/10.1002/bmc.5481

Abu-Serie, M. M., Habashy, N. H., and Attia, W. E. (2018). In vitro evaluation of the synergistic antioxidant and anti-inflammatory activities of the combined extracts from Malaysian Ganoderma lucidum and Egyptian Chlorella vulgaris. *BMC Complementary and Alternative Medicine*, *18*(1), 154. https://doi.org/10.1186/s12906-018-2218-5

Ali-Ahmed, F., Dalgaard, F. and Al-Khatib, S.M. (2020). Sudden cardiac death in patients with myocarditis: Evaluation, risk stratification, and management. *American heart journal*, *220*, 29-40. https://doi.org/10.1016/j.ahj.2019.08.007

Altanam, S.Y., Darwish, N. and Bakillah, A. (2025). Exploring the Interplay of antioxidants, inflammation, and oxidative stress: Mechanisms, therapeutic potential, and clinical implications. *Diseases*, *13*(9), 309. https://doi.org/10.3390/diseases13090309

Ang, W.X., Sarasvathy, S., Kuppusamy, U.R., Sabaratnam, V., Tan, S.H., Wong, K.T., Perera, D. and Ong, K.C. (2021). In vitro antiviral activity of medicinal mushroom Ganoderma neo-japonicum Imazeki against enteroviruses that caused hand, foot and mouth disease. *Tropical biomedicine*, *38*(3), 239-247. https://doi.org/10.47665/tb.38.3.063

Babbar, N., Oberoi, H.S., Sandhu, S.K. and Bhargav, V.K. (2014). Influence of different solvents in extraction of phenolic compounds from vegetable residues and their evaluation as natural sources of antioxidants. Journal of food science and technology, 51, 2568-2575. https://doi.org/10.1007/s13197-012-0754-4

Bassyouni, F.A., Abu-Baker, S.M., Mahmoud, K., Moharam, M., El-Nakkady, S.S., and Abdel-Rehim, M. (2014). Synthesis and biological evaluation of some new triazolo[1,5-a]quinoline derivatives as anticancer and antimicrobial agents. RSC Advances 4(46): 24131-24141. https://doi.org/10.1039/c3ra46961a

Benzie, I.F. and Wachtel-Galor, S. eds. (2011). Herbal medicine: biomolecular and clinical aspects. https://doi.org/10.1201/b10787

Chanda, A., Ray, H., Das, M., Paul, S., Saha, S., Majumder, R. and Ghosh, S. (2026). Anti-inflammatory Activity of Fungal Extracts in Cosmeceutical Formulation. In Fungal-Based Cosmetics: Formulation and Usage (pp. 87-110). Cham: Springer Nature Switzerland. https://doi.org/10.1007/978-3-032-09291-5_5

Cör Andrejč, D., Knez, Ž. and Knez Marevci, M. (2022). Antioxidant, antibacterial, antitumor, antifungal, antiviral, anti-inflammatory, and nevro-protective activity of Ganoderma lucidum: An overview. *Frontiers in pharmacology*, *13*, p.934982. https://doi.org/10.3389/fphar.2022.934982

Del Fabbro, L., Bortolotto, V.C., Ferreira, L.M., Sari, M.H.M. and Furian, A.F. (2025). Chrysin's anti-inflammatory action in the central nervous system: A scoping review and an evidence-gap mapping of its mechanisms. *European Journal of Pharmacology*, *997*, 177602. https://doi.org/10.1016/j.ejphar.2025.177602

Dey, R. (2026). Edible Mushrooms as Functional Foods: Phytochemical Composition, Biological Activities, and Health Applications-A Comprehensive Review. *Asian Journal of Food Research and Nutrition*, *5*(1), 1-13. https://doi.org/10.9734/ajfrn/2026/v5i1355

El-Hagrassi, A., Daba, G., Elkateeb, W., Ahmed, E., El-Dein, A.N., Fayad, W., Shaheen, M., Shehata, R., El-Manawaty, M. and Wen, T.C. (2020). In vitro bioactive potential and chemical analysis of the n-hexane extract of the medicinal mushroom, Cordyceps militaris. *Malaysian Journal of Microbiology*, *16*(1), 40-48. https://doi.org/10.21161/mjm.180346

El-Shazly, A.I., Gamal, A.A., El-Dein, A.N., Mettwally, W.S. and Farid, M.A. (2021). Production of isoflavones-enriched soy yogurt through soymilk fermentation using probiotic bacteria. *Egyptian Pharmaceutical Journal*, *20*(1), 42-50. https://doi.org/10.4103/epj.epj_46_20

Elhassaneen, Y.E.A. and Mehram, E.B. (2025). Effect of solvents with different polarity on the extraction of bioactive compounds from reishi mushroom (*Ganoderma lucidum*) and their antioxidant and free radicals scavenging activities: application in buffalo meatballs. *Egyptian Journal of Chemistry*, *68*(6), 113-128. https://doi.org/10.21608/ejchem.2024.301308.9945

Elkhateeb W, Soliman GM, Emam M, Daba G. (2025). Nematicidal potency of *Hydnora abyssinca*, *Tuber indicum* and *Agaricus impudicus* extracts against *Meloidogyne incognita* infecting eggplant under screen house conditions. *Research Journal of Pharmacy and Technology*, 18(7), 1-12. https://doi.org/10.52711/0974-360x.2025.00438

Elkhateeb, W.A., Daba, G.M., El-Dein, A.N., Sheir, D.H., Fayad, W., Shaheen, M.N., Elmahdy, E.M. and Wen, T.C. (2020). Insights into the in-vitro hypocholesterolemic, antioxidant, antirotavirus, and anticolon cancer activities of the methanolic extracts of a Japanese lichen, *Candelariella vitellina*, and a Japanese mushroom, *Ganoderma applanatum*. *Egyptian Pharmaceutical Journal*, *19*(1), 67-73. https://doi.org/10.4103/epj.epj_56_19

Elkhateeb, W.A., Daba, G.M., Elnahas, M.O. and Thomas, P.W. (2019). *Fomitopsis officinalis* mushroom: ancient gold mine of functional components and biological activities for modern medicine. *Egyptian Pharmaceutical Journal*, *18*(4), 285-289. https://doi.org/10.4103/epj.epj_46_19

Elkhateeb, W.A., Soliman, A.A., Shaheen, M.N., Elmahdy, E.M. and Daba, G.M. (2024). Bioactive potentials of the truffle mushrooms *Tirmania nivea, Tirmania pinoyi* and *Tuber indicum*. *Egyptian Pharmaceutical Journal*, *23*(1), 94-102. https://doi.org/10.4103/epj.epj_204_23

Falanga, V. (2005). Wound healing and its impairment in the diabetic foot. *The Lancet*, *366*(9498), 1736-1743. https://doi.org/10.1016/s0140-6736(05)67700-8

Hamdy, A.H.A., Mettwally, W.S., Fotouh, M.A.E., Rodriguez, B., El-Dewany, A.I., El-Toumy, S.A. and Hussein, A.A. (2012). Bioactive phenolic compounds from the Egyptian Red Sea seagrass Thalassodendron ciliatum. *Zeitschrift für Naturforschung C*, *67*(5-6), 291-296. https://doi.org/10.5560/znc.2012.67c0291

Hasnat, M.A., Pervin, M., Cha, K.M., Kim, S.K. and Lim, B.O. (2015). Anti-inflammatory activity on mice of extract of *Ganoderma lucidum* grown on rice via modulation of MAPK and NF-κB pathways. *Phytochemistry*, *114*, 125-136. https://doi.org/10.1016/j.phytochem.2014.10.019

Hou, Z., Ji, W., Yang, S., Zhang, Y., Yang, T., Wang, L., Luo, H., Lin, D., Yang, B., Chen, X. and Fang, H. (2026). Protective Effects of Ganoderma lucidum Polysaccharide Peptides Against Cisplatin-Induced Toxicity. *Journal of Ethnopharmacology*, p.121210. https://doi.org/10.1016/j.jep.2026.121210

Huang, M., Mei, X. and Zhang, S. (2011). Mechanism of nitric oxide production in macrophages treated with medicinal mushroom extracts. *International Journal of Medicinal Mushrooms*, *13*(1). https://doi.org/10.1615/intjmedmushr.v13.i1.10

Karunarathna, S.C., Patabendige, N.M., Wijesundara, S., Stephenson, S.L., Jun, H. and Hapuarachchi, K.K. (2025). Cultivation of *Ganoderma*: methodologies and hurdles. *New Zealand Journal of Botany*, *63*(5), 1823-1870. https://doi.org/10.1080/0028825x.2025.2488393

Kebaili, F.F., Tahar, N., Toumi, M.E., Redouane, R., Chawki, B., Pablo, A. and Perduca, M. (2021). Antioxidant activity and phenolic content of extracts of wild Algerian Lingzhi or Reishi Medicinal Mushroom, *Ganoderma lucidum* (Agaricomycetes). *International Journal of Medicinal Mushrooms*, *23*(6). https://doi.org/10.1615/intjmedmushrooms.2021038424

Kim, J.M., Kim, C.H., Kang, S.M., Jung, J.H., Kim, K.C., Ahn, S., Park, T.S. and Park, I.B. (2026). Advanced strategies for the management of patients with diabetic foot ulcers: a comprehensive review. *The Korean Journal of Internal Medicine*, *41*(1), 47. https://doi.org/10.3904/kjim.2024.446

Kim K. H., Tsao R., Yang R., Cui S. W. (2006). Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. Food Chemistry 95, 466–473. https://doi.org/10.1016/j.foodchem.2005.01.032

Klaus, A. and Wan, W.A.A.Q.I. (2024). *Ganoderma* in Traditional Culture. In *Ganoderma* (pp. 35-60). CRC Press. https://doi.org/10.1201/9781003354789-3

Kärber, G. (1931). Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. *Naunyn-Schmiedebergs Archiv für experimentelle pathologie und pharmakologie*, *162*(4), 480-483. https://doi.org/10.1007/bf01863914

Laroux, F.S., Pavlick, K.P., Hines, I.N., Kawachi, S., Harada, H., Bharwani, S., Hoffman, J.M. and Grisham, M.B. (2001). Role of nitric oxide in inflammation. *Acta Physiologica Scandinavica*, *173*(1), 113-118. https://doi.org/10.1046/j.1365-201x.2001.00891.x

Li, F., Liu, T., Liu, X., Han, C., Li, L., Zhang, Q. and Sui, X., 2024. Ganoderma lucidum polysaccharide hydrogel accelerates diabetic wound healing by regulating macrophage polarization. *International journal of biological macromolecules*, *260*, p.129682.

Mazuryk, O., Gurgul, I., Oszajca, M., Polaczek, J., Kieca, K., Bieszczad-Żak, E., Martyka, T. and Stochel, G. (2024). Nitric oxide signaling and sensing in age-related diseases. *Antioxidants*, *13*(10), 1213. https://doi.org/10.3390/antiox13101213

Monika, P., Chandraprabha, M.N. and Murthy, K.C. (2023). Catechin, epicatechin, curcumin, garlic, pomegranate peel and neem extracts of Indian origin showed enhanced anti-inflammatory potential in human primary acute and chronic wound derived fibroblasts by decreasing TGF-β and TNF-α expression. *BMC Complementary Medicine and Therapies*, *23*, 181. https://doi.org/10.1186/s12906-023-03993-y

Palaiogiannis, D., Chatzimitakos, T., Athanasiadis, V., Bozinou, E., Makris, D.P. and Lalas, S.I. (2023). Successive solvent extraction of polyphenols and flavonoids from *Cistus creticus* L. leaves. *Oxygen*, *3*(3), 274-286. https://doi.org/10.3390/oxygen3030018

Parejo, I., F. Viladomat, J. Bastida, A. Rosas-Romero, N. Flerlage, J. Burillo and C. Codina (2002). Comparison between the radical scavenging activity and antioxidant activity of six distilled and no distilled Mediterranean herbs and aromatic plants. *Journal of Agricultural and Food Chemistry* 50(23): 6882-6890. https://doi.org/10.1021/jf020540a

Park, M. and Kim, M. (2017). Analysis of antioxidant and anti-inflammatory activities of solvent fractions from Rhynchosia nulubilis cultivated with *Ganoderma lucidum* mycelium. *Preventive nutrition and food science*, *22*(4), 365. https://doi.org/10.3746/pnf.2017.22.4.365

Phillips R. Mushrooms: a comprehensive guide to mushroom, identification. UK: Pan Macmillan; (2013).

Pradhan, P., De, J. and Acharya, K. (2024). Cultivation Strategies of Ganoderma or the Reishi Mushroom. In *Ganoderma* (pp. 19-34). CRC Press. https://doi.org/10.1201/9781003354789-2

Qian, Y., Yang, Y., Qing, W., Li, C., Kong, M., Kang, Z., Zuo, Y., Wu, J., Yu, M. and Yang, Z. (2022). Coxsackie virus B3 infection induces glycolysis to facilitate viral replication. *Frontiers in Microbiology*, *13*, p.962766. https://doi.org/10.3389/fmicb.2022.962766

Rašeta, M., Mišković, J., Čapelja, E., Zapora, E., Petrović Fabijan, A., Knežević, P. and Karaman, M. (2023). Do Ganoderma species represent novel sources of phenolic based antimicrobial agents?. *Molecules*, *28*(7), p.3264. https://doi.org/10.3390/molecules28073264

Saini, R., Shukla, M., Mishra, A.K., Singh, A. and Tiwari, K.N. (2026). Molecular Mechanism of Action of Polyphenol Nutraceuticals Targeting Human Disease. In *Polyphenol Nutraceuticals and Healthcare* (pp. 123-132). CRC Press. https://doi.org/10.1201/9781003588504-5

Schulz, G. and Stechmiller, J. (2006). Wound healing and nitric oxide production: too little or too much may impair healing and cause chronic wounds. *The international journal of lower extremity wounds*, *5*(1), 6-8. https://doi.org/10.1177/1534734606286633

Seo, D.J. and Choi, C. (2021). Antiviral bioactive compounds of mushrooms and their antiviral mechanisms: a review. Viruses, 13(2), p.350. https://doi.org/10.3390/v13020350

Shaffai, A.E., Mettwally, W.S. and Mohamed, S.I. (2023). A comparative study of the bioavailability of Red Sea seagrass, Enhalus acoroides (Lf) Royle (leaves, roots, and rhizomes) as anticancer and antioxidant with preliminary phytochemical characterization using HPLC, FT-IR, and UPLC-ESI-TOF-MS spectroscopic analysis. *Beni-Suef University Journal of Basic and Applied Sciences*, *12*(1), p.41. https://doi.org/10.1186/s43088-023-00376-7

Shraim, A.M., Ahmed, T.A., Rahman, M.M. and Hijji, Y.M. (2021). Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. *Lwt*, *150*, 111932. https://doi.org/10.1016/j.lwt.2021.111932

Shukla, S.K., Sharma, A.K., Gupta, V. and Yashavarddhan, M.H. (2019). Pharmacological control of inflammation in wound healing. *Journal of tissue viability*, *28*(4), 218-222. https://doi.org/10.1016/j.jtv.2019.09.002

Song, Y.S., Kim, S.H., Sa, J.H., Jin, C., Lim, C.J. and Park, E.H. (2004). Anti-angiogenic and inhibitory activity on inducible nitric oxide production of the mushroom Ganoderma lucidum. *Journal of ethnopharmacology*, *90*(1), 17-20. https://doi.org/10.1016/j.jep.2003.09.006

Soro, B., Kone, N.G.A., Djoue, A.A. and Bakayoko, A. (2026). Traditional knowledge of medicinal macrofungi commonly used by riverine populations of the Taï, Comoé and Marahoué national parks (Côte d'Ivoire). *Ethnobotany Research and Applications*, *33*, 1-12. https://doi.org/10.32859/era.33.19.1-12

Sun, W. and Shahrajabian, M.H. (2023). Therapeutic potential of phenolic compounds in medicinal plants—Natural health products for human health. *Molecules*, *28*(4), p.1845. https://doi.org/10.3390/molecules28041845

Sun, X., Shi, Y., Shi, D., Tu, Y. and Liu, L. (2024). Biological Activities of Secondary Metabolites from the Edible-Medicinal Macrofungi. *Journal of Fungi*, *10*(2), p.144. https://doi.org/10.3390/jof10020144

Sułkowska-Ziaja, K., Trepa, M., Olechowska-Jarząb, A., Nowak, P., Ziaja, M., Kała, K. and Muszyńska, B. (2023). Natural compounds of fungal origin with antimicrobial activity—potential cosmetics applications. *Pharmaceuticals*, *16*(9), p.1200. https://doi.org/10.3390/ph16091200

Sánchez, C. (2017). Reactive oxygen species and antioxidant properties from mushrooms. Synthetic and systems biotechnology, 2(1), 13-22. https://doi.org/10.1016/j.synbio.2016.12.001

Tam, J.C.W., Lau, K.M., Liu, C.L., To, M.H., Kwok, H.F., Lai, K.K., Lau, C.P., Ko, C.H., Leung, P.C., Fung, K.P. and San Lau, C.B. (2011). The in vivo and in vitro diabetic wound healing effects of a 2-herb formula and its mechanisms of action. Journal of Ethnopharmacology, 134(3), 831-838. https://doi.org/10.1016/j.jep.2011.01.032

Taofiq, O., Calhelha, R.C., Heleno, S., Barros, L., Martins, A., Santos-Buelga, C., Queiroz, M.J.R. and Ferreira, I.C. (2015). The contribution of phenolic acids to the anti-inflammatory activity of mushrooms: Screening in phenolic extracts, individual parent molecules and synthesized glucuronated and methylated derivatives. *Food Research International*, *76*, 821-827. https://doi.org/10.1016/j.foodres.2015.07.044

Thomas, P.W., Elkhateeb, W.A. and Daba, G. (2019). Truffle and truffle-like fungi from continental Africa. *Acta mycologica*, *54*(2). https://doi.org/10.5586/am.1132

Thomas, P.W., Elkhateeb, W.A. and Daba, G.M. (2020). Chaga (*Inonotus obliquus*): a medical marvel but a conservation dilemma. *Sydowia*, *72*, 123-130. https://doi.org/10.12905/0380.sydowia72-2020-0123

Utpal, B.K., Sutradhar, B., Zehravi, M., Sweilam, S.H., Panigrahy, U.P., Urs, D., Fatima, A.F., Nallasivan, P.K., Chhabra, G.S., Sayeed, M. and Alshehri, M.A. (2025). Polyphenols in wound healing: Unlocking prospects with clinical applications. *Naunyn-Schmiedeberg's archives of pharmacology*, *398*(3), 2459-2485. https://doi.org/10.1007/s00210-024-03538-1

Wasser, S.P. (2011). Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. *Applied microbiology and biotechnology*, *89*(5), 1323-1332. https://doi.org/10.1007/s00253-010-3067-4

Wu, D.T., Deng, Y., Chen, L.X., Zhao, J., Bzhelyansky, A. and Li, S.P. (2017). Evaluation on quality consistency of Ganoderma lucidum dietary supplements collected in the United States. Scientific reports, 7(1), 7792. https://doi.org/10.1038/s41598-017-06336-3

Xu, J., Xiao, C., Xu, H., Yang, S., Chen, Z., Wang, H., Zheng, B., Mao, B. and Wu, X. (2021). Anti-inflammatory effects of Ganoderma lucidum sterols via attenuation of the p38 MAPK and NF-κB pathways in LPS-induced RAW 264.7 macrophages. *Food and Chemical Toxicology*, *150*, 112073. https://doi.org/10.1016/j.fct.2021.112073

Xu, Z. and Chenli, Z. (2024). Inflammation. In *Textbook of Pathologic Anatomy: For Medical Students* (pp. 75-105). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-99-8445-9_4

Yalcin, O.U., Sarikurkcu, C., Cengiz, M., Gungor, H. and Ćavar Zeljković, S. (2020). Ganoderma carnosum and Ganoderma pfeifferi: M*et al* concentration, phenolic content, and biological activity. *Mycologia*, *112*(1), 1-8. https://doi.org/10.1080/00275514.2019.1689748

Yoon, W. J., Kim, S. S., Oh, T. H., Lee, N. H. and Hyun, C.G. (2009). *Abies koreana* essential oil inhibits drug-resistant skin pathogen growth and LPS-induced inflammatory effects of murine macrophage. *Lipids*, 44, 471 – 476. https://doi.org/10.1007/s11745-009-3297-3

Zhang, W., Tao, J., Yang, X., Yang, Z., Zhang, L., Liu, H., Wu, K. and Wu, J. (2014). Antiviral effects of two *Ganoderma lucidum* triterpenoids against enterovirus 71 infection. *Biochemical and biophysical research communications*, *449*(3), 307-312. https://doi.org/10.1016/j.bbrc.2014.05.019

Zhao, M., Yang, B., Wang, J., Li, B. and Jiang, Y. (2006). Identification of the major flavonoids from pericarp tissues of lychee fruit in relation to their antioxidant activities. Food Chemistry, 98(3), 539-544. https://doi.org/10.1016/j.foodchem.2005.06.028