Journal of Applied and Natural Science
17(3), 1333 - 1344 (2025)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Molecular diagnosis of the *Ganoderma* isolated from Mosul city, Iraq and isolation of some of its active compounds

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Article Info
https://doi.org/10.31018/jans.v17i3.6825
Received: May 13, 2025
Revised: August 29, 2025
Accepted: September 07, 2025

How to Cite
Mula Abed, F. N. *et al.* (2025). Molecular diagnosis of the *Ganoderma* isolated from Mosul city, Iraq and isolation of some of its active compounds. *Journal of Applied and Natural Science*, 17(3), 1333 - 1344. https://doi.org/10.31018/jans.v17i3.6825

Abstract
Macrofungi are extremely important organisms in our lives because they produce numerous biologically active compounds, which have been utilized in various industrial applications, as well as in the medical field. They also produce antibiotics and anticancer pharmaceutical compounds, making them promising therapeutic agents.The present study aimed to discover the presence of macrofungi in different areas of Mosul city / Iraq, and identify bioactive secondary compounds from them. The survey trips lasted four months in the forests of Mosul and in separate areas of the left coast of Mosul city. These surveys demonstrated that 10 fruiting bodies belonged to the genus Agaricus sp. (Mo1-Mo2-Mo3) and Ganoderma sp. (Mo4), *Polyporus* sp. (Mo5), and *Coprinellus* sp. (Mo6). The isolate Mo7-Mo8 belongs to the genus *Pluteus* sp., and M10 belongs to the genus Ascomycetes *Nigrospora sphaerica*. As for the *Ganoderma* sp., its molecular diagnosis was confirmed by polymerase chain reaction (PCR). Band separation was observed, with a size of 650 base pairs. When studying the sequence of the nitrogenous bases of the DNA, no sites of genetic variation were found compared to the ITS gene on the National Centre for Biotechnology Information (NCBI), given the code PP534170.1. Gas chromatography-mass spectrometry (GC-MS) was used to separate the active bioactive secondary compounds, such as Benzene, 1-isocyano-2-methyl-, 4-Formylbenzeneboronic acid, 9-Octadecenamide, (z)- and Bis(2-ethylhexyl) phthalate, based on the retention time for each compound. The present study is the first of its kind to combine genetic and chemical analysis of *Ganoderma* in Mosul city . This leads to the development of environmentally friendly compounds in the treatment of many diseases.

Keywords: Ascomycetes, Basidiomycetes, *Ganoderma* fungi, Gas chromatography-mass spectrometry (GC-MS analysis), Molecular diagnosis

INTRODUCTION

Fungi play an indispensable role in our lives, with an impact on human life that is commensurate with their role in maintaining the environmental balance necessary for the continuation of life on Earth. They are considered among the most important organisms in the world, containing a huge diversity of species and phenotypic characteristics. This diversity ranges from amoeba-like organisms to Ascomycetes and Basidiomycetes fungi (Qassim *et al*., 2024). Survey studies on fungi contribute to increasing our knowledge of their numbers, as well as their distribution and extent in various types of environments around the world. These changes often occur in response to environmental disturbances, whether natural or caused by human intervention, such as global warming, air pollution, and forest fires (Angelini *et al*., 2015; Taha *et al*., 2021).

Although fungi have long had a bad reputation among the general public, who associate them with food spoilage and disease, their ability to produce metabolic compounds especially macrofungi, which produce many biologically active compounds such as organic acids, enzymes, vitamins, alcohols, and polysaccharides, which have been used in many industrial applications, as well as in the medical field. They also produce antibiotics and anticancer pharmaceutical compounds, making them promising therapeutic agents (Singh *et al*., 2019 ; Qassim *et al*., 2024).

It is known that the fungal metabolic compounds are not necessary for growth, but are formed under special conditions as a result of secondary metabolic path-

ways. They are important due to their antifungal and antibacterial properties (Sultan and Abed, 2023). Therefore, some fungal products can be considered competitive tools used by fungi against surrounding organisms, as they act as defensive weapons that inhibit the growth of neighbouring organisms and may even kill them (Taha *et al*., 2020).

Global interest in macrofungi with therapeutic uses has increased since the discovery of antibiotics such as penicillin, a drug that revolutionized medicine. With the continuation of scientific research, it was found that there are about 60 species of Basidiomycete fungi that have an inhibitory effect on the growth of microbes and cancer cells. Other types have been used in folk medicine since ancient times to treat various diseases, such as the use of the fruiting bodies of Armillaria tabescens in China to treat cholecystitis and acute and chronic hepatitis due to their content of the substances Armillaria A and B (Aytar *et al*., 2020).Macrofungi represent a significant food source, containing carbohydrates, fats, proteins, and vitamins, and many of them hold promise for the biomedical and pharmaceutical sectors, particularly in disease treatment (De Silva, 2012; De Silva *et al.,* 2013; Vishwakarma *et al*., 2017).

The *Ganoderma* mushroom is considered one of the most important fungi in the Basidiomycota family. Its name is derived from two Greek words (ganos) and (derma), which means (bright skin). This genus is also referred to as "resinous," referring to a hard, sticky liquid that emerges from damaged fruit bodies (Mawar *et al*.,2020). The yellow resin solidifies quickly at the edges of its surface as the fruiting body advances. When the solid support is broken or cut, a thick, yellow resin seeps from the mushroom and quickly hardens to form a bright, shiny solid surface, and its name reflects this characteristic. The bracket fungi are considered very hard and inedible (Teke *et al*., 2018) Some describe it as a woody mushroom with a bitter taste. Over the years, Ganoderma has played a significant role in alternative medicine. It has been used to treat many diseases and is also available commercially in powder form. It is widely used in China to lower blood pressure, protect the liver, and serve as an anti-ageing agent. Its antioxidant and anti-inflammatory properties, support the functioning of the nervous system and supports blood circulation. It also helps regulate blood cholesterol levels by lowering triglyceride content and increasing HDL cholesterol. These benefits are attributed to its content of saponins and certain sugars, which may inhibit the cellular development of certain types of cancer (Ndeh et al., 2024).

The purpose of the present study was to confirm the diagnosis of *Ganoderma* after its isolation from various areas in Mosul/Iraq, and to identify the bioactive secondary compounds from it, to exploit them as environmentally friendly compounds in the treatment of various diseases.

MATERIALS AND METHODS

The study was completed in the Fungal Laboratory of the Department of Biology/College of Science/ University of Mosul/Iraq

Research plan

as shown in Fig.1. Large fungi were isolated from different areas in Mosul city/Iraq (Forests of Mosul and from other sites in the city of Mosul, which included Al-Kindi Farms, Al-Rashidiyah Farms, Al-Atibaa region, Al-Mithaq region, and Dumiz region) . The isolates were identified morphologically and microscopically. Then, the Ganoderma fungus was identified by using Plymerase Chain Reaction. Then, the active compounds were separated from the Ganoderma extract by using the Gas Chromatography –Mass analysis.

Collection of samples

The samples were collected randomly during the period from October 30, 2023, to March 17, 2024. Several sites were chosen, and it was natural for the journey to begin towards the forests of Mosul, which cover an area of 10 dunams and constitute a rich environment for fungal diversity due to the abundance of fallen leaves that cover its floor. Additional samples were collected from other sites in the city of Mosul, Iraq, including Al-Kindi Farms, Al-Rashidiyah Farms, the Al-Atibaa region, the Al-Mithaq region, and the Dumiz region.

Preparation of the Potato Dextrose Agar (PDA) medium

This medium was prepared according to the instructions of its manufacturer (United Kingdom Lab M.Limited), by dissolving 39 g/L in distilled water. Using a magnetic stirrer, the components were dissolved, and the pH was adjusted to 6.5 using a pH meter. The medium was sterilized in an autoclave at a temperature of 121 °C and a pressure of 1 kg/cm² for 15 minutes. The medium was left in the laboratory atmosphere to cool and 250 mg/L of chloramphenicol was added to prevent bacterial growth. The chloramphenicol was sterilized using a Millipore filter with a 0.45-micron diameter. The medium was poured into sterile 9 cm diameter Petri dishes with a capacity of 15 ml per dish. The dishes were left in the refrigerator at 4°C until use. The medium was used to isolate, purify and diagnose the fungal isolates (Atlas, 1998).

Isolation and development of fungal hyphae

Fruiting bodies were collected at different stages from their natural habitats (trunks and cavities of living, dead and felled trees, forests) using hands and scalpels. Several pictures were taken with an indicative marker

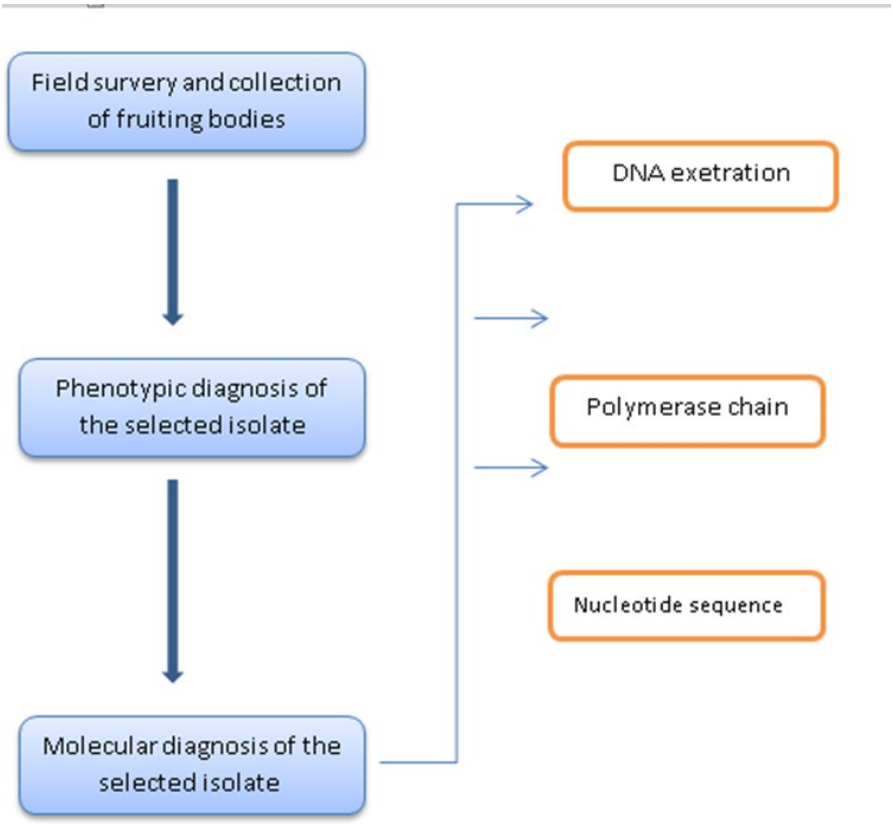

Fig. 1. *A detailed outline of the research plan*

(pen, ruler)and preserved in polyethylene bags and plastic boxes labelled with the herbarium information (collection site, type and description of the tree, date of collection, and suitability for eating). The samples were transferred to the laboratory after each survey trip and washed several times with running water to remove dust and impurities that had adhered to them. To obtain pure fungal colonies the culture cabinet was sterilized with 70% ethanol. Small pieces were taken from the core of the body using a sterile scalpel and immersed in 70% alcohol for 5 minutes. They were then transferred using sterilized forceps into a sterile bottle containing a 2% sodium hypochlorite solution for 2 minutes. The pieces were then transferred to a bottle containing sterile distilled water to remove sterilizing agents. The pieces were left to dry on sterile Whatman No. 1 filter papers. The pieces were distributed, one or two per dish, on the surface of sterile Petri dishes containing PDA medium. The dishes were closed with aluminium foil and left in the incubator at a temperature of 27±2°C for 7-14 days until the fungal colonies appeared (Kala *et al*., 2020).

Preservation of fungal isolates

The fungal isolates were inoculated on slant PDA medium (Valls *et al*., 1999) and then placed in the incubator at a temperature of 27 ± 2 °C for 7 days. Once the growth on the tubes was finished, they were simply put in the fridge until ready to use them. To maintain fungal isolates for an extended period, the growth surface was covered with 60% sterile glycerol, and the tubes were stored in the refrigerator at 4°C.

Solution preservation of fruiting body

The preservation solution was prepared by adding 90 mL of 70% ethanol to 5 mL of glacial acetic acid and then completing the volume to 100 mL by adding 5 mL of formalin, followed by thorough mixing (Al-Khazraji, 1990).

Identification of fungal Isolates

The fungi were isolated from different sources and identified to the genus level using a compound light microscope at 40X magnification. Staining was done with lactophenol Cotton Blue. Identification was based on the phenotypic characteristics of the fruiting body (shape of the fruiting body, its colour, texture) using the taxonomic keys available in well-known scientific literature (Song *et al*., 2014).

Molecular Identification of Ganoderma

Identification was further supported by molecular diagnostics, based on the laboratory equipment and materials listed in Table 1.

Extracting DNA from fungi

The extraction kit was used according to the manufacturer's instructions (ZYMO Cat.No.D6005, USA). Ap-

Table 1. Laboratory materials used in the study.

Materials	Company
Acarose gel	USA
Red dye solution	Korea
Dye 6X Loading	Korea
Ladder1000 plus bp	Korea
Pre mix per	Korea
TBE buffer 10X	USA

proximately 50-100 mg of wet weight fungal spore were suspended in 200 microliters of water, and transferred to a ZR lysis tube with a capacity of 0.5 mm, containing 750 microliters of lysis solution. The tubes were placed in a 2 mL tube holder, and the samples were mixed thoroughly using a vortex mixer at high speed for 5 minutes. The lysis tubes were centrifuged at 10.000 revolutions for one minute. Approximately 400 microliters of filtrate was transferred to the spin filter in the Zymo Spin filter collection tube and centrifuged at 7000 revolutions per minute for 1 minute. 1.200 µL of DNA binding solution was added to the filtrate from step 4. And 800 microliters of the mixture from step 5 were transferred to the Zymo Spin IIC Column 3 in the collection tube and centrifuged at 10,000 revolutions for one minute. Step 6 was repeated.Next, 200 microliters of DNA Pre-wash Buffer were added to the collection tube and centrifuged at 10,000 revolutions per minute for one minute.Add 500 microliters of DNA wash solution to the collection tube and centrifuged at 10,000 revolutions per minute for one minute. The column was transferred to a clean 1.5 mL microcentrifuge tube, and 100 µL of DNA elution solution was added. The mixture was then centrifuged at 10,000 revolutions for 30 seconds to extract the DNA.

Primers used in the study

Specific primers for the *ITS* gene were chosen (Zarrin *et al*., 2016), as shown in Table 2.

Preparation of agarose gel

Agarose gel was prepared by dissolving 1.5 g of agarose in 100 ml of the previously prepared Tris-Borate-EDTA solution, heating the agarose until boiling and leaving it to cool at (45-50) C˚. Then gently pour the gel so as not to leave air bubbles in the pouring plate after installing the comb to make holes through which the samples, and leave to cool for 30 minutes. Then, gently remove the comb from the agarose after it has hardened. The plate was placed on its stand in the horizontal electrophoresis unit, represented by the tank used in the migration process, and then filled with TBE buffer that covers the gel (Sambrook *et al*., 1989).

Electrophoresis on agarose gel

Electrophoresis was performed to determine the size of the DNA bands and to confirm the purity and concentration of the DNA after the extraction process.

Mixed 3 µL of the processor loading solution (Intron/ Korea) with 5 µL of DNA. The extract, which was supposed to be bound to the loading dye and mixture field directly into the holes of the gel and exposed to an electric current at 7 V/cm for 1-2 hours until the DNA sample reacheed the other side of the gel, then the gel is exposed to a U.V. Transilluminater source. At a wavelength of 336 nm, after placing the gel in a water bath containing 30 microliters of Red Safe dye solution and 500 ml of distilled water, the gel was photographed using a digital camera to show the bands.

Optimal conditions for Polymerase Chain Reaction (PCR) technology

The polymerase chain reaction technique was performed according to the conditions mentioned in Table 3.

Gas Chromatography –Mass Spectrometry (GC-MS) analysis

GC-MS Analysis Instrument- Agilent 7890B GC system coupled with a 5977A MSD (Mass Selective Detector) was used for analysis. A DB-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness) was employed for separation. Carrier gas, Helium (purity >99.99%), was used at a flow rate of 1 mL/min. Injection 1 µL of the concentrated extract was injected, in the splitless mode, into an injector at 250°C. The temperature of the GC oven was programmed to hold initially at 50°C for 3 minutes, then to increase at 10°C per minute up to 280° C, and finally to hold at this temperature for 5 minutes. The mass spectra were obtained within the range of 30 – 550 m/z under electron ionization at 70 eV. The sample material was collected , and then mixed at room temperature to remove the moisture. Solvent use: Ethyl acetate (analytical grade) was chosen because it is efficient in isolating bioactive compounds. The procedures for extraction involved mixing 250 mL of liquid with 50 mL of ethyl acetate and leaving the mixture for three days to enhance the extraction of active ingredients, allowing for the separation of water and solvent. Filtration of the extract to remove solid residues was performed using Whatman No. 150 filter paper (Chiara *et al.*, 2025).

RESULTS AND DISCUSSION

Field survey of local fungal fruiting bodies

Survey trips began to collect and discover the biodiversity of macrofungi and as a diverse number of fruiting bodies was recorded, including two fruiting bodies of different sizes, belonging to the type of basidiomycete fungi, which were given the scientific symbols Mo1-Mo2, and which grew at the base of the stem of *Eucalyptus camaldulensis* trees and *Pinus sylvestris*. The

scope of the search expanded to include farms in the Al -Kindi region. During an early morning trip, a fruiting body belonging to the basidiomycete fungi, which has the scientific symbol Mo3, was obtained. On the same day, the survey trip continued to the farms near the Domiz area, during which two fruiting bodies belonging to the same type of basidiomycete fungus, specifically the genus Ganoderma, were obtained and assigned the scientific symbols Mo4 and Mo5. The olive trees provided a suitable host for them.

The present work resumed research after a brief hiatus, and it was possible to obtain fruiting bodies belonging to one genus of basidiomycete fungi, which cover the floor of an area in the Al-Atibaa region and are located among the grasses; they were given the scientific code Mo6. The next trip took place on a rainy day at the farm near the Al-Mithaq region. It was crowned with success, as various fruiting bodies were obtained, which were given scientific symbols Mo7 and Mo8. The survey trip also extended to the forest area, where a single fruiting body was obtained that belongs to the Basidiomycete and is associated with the genus of Ascomycete fungi and was given the scientific code Mo9. The last survey trip was to the Rashidiyya region, and after two weeks of heavy rains, a single fruiting body belonging to the Basidiomycete was obtained on the farm floor, which is covered with dense plant leaves and given the scientific symbol Mo10 (Table 4, Fig. 2).

A research team led by Unekwu et al. (2014) collected macrofungi from a Nigerian forest, including eight fruiting bodies of different basidiomycete genera, some of which were observed parasitizing trees, while others decomposed saprotrophically in the soil. Further research published in 2015 by Professor Dr M. Hakki ALMA documented 43 fruiting bodies of Basidiomycetes and Ascomycetes fungi collected during a field trip in Dohuk, northern Iraq. Fungi in general, including basidiomycetes, are widespread in all parts of the earth, but they increase in prevalence in areas with dense, shading trees and high humidity. Seventeen of them are edible, twenty-four are inedible, and two of them are poisonous. This historical wealth must be preserved, developed and benefited from in all ways that benefit the city itself and students of science. It is necessary to pay attention to and preserve the forests of Mosul due to their diverse species, including fungal species, and not to consider them as mere general forests lacking scientific character.

Phenotypic diagnosis of selected fungal isolates

Most of these fungi are considered common edible species. They are saprophytic, growing on the trunks of living and dead trees in forests, in small or large groups, and in grasslands and dung soils rich in organic matter. Phenotypic and microscopic characteristics serve as a starting point for classifying selected fungi to the genus level, using taxonomic keys contained in approved sources (Song et al., 2018; Almashhadani and Qassim, 2025). However, full identification requires molecular methods to determine the complete identity of the fungus.

Ganoderma resinaceum

The head of the fruiting body has a light-yellow edge and a reddish-brown top with an orange tinge. This mushroom sometimes forms layers of brackets that may merge together. Individual brackets are 15 to 35 cm wide and 4 to 8 cm thick. When fully developed, the spores are spaced 3 to 4 per mm, and are initially white or often very pale yellow when the fruiting body is small, turning light brown at maturity. The spores are oval, cut off at one end. It has a spicy smell and a bitter taste. It basically lives off and feeds on the trunks of living trees, especially those with broad leaves like oaks, as shown in (Fig. 3).

Coprinellus domesticus

Cap size is approximately 7 cm, oval when young, expanding to convex or conical. Its appearance is yellow and white towards the margin, greyish with a brown centre, covered with white to brown fragments in the form of small scales or granules, finely grooved or lined almost from margin to centre. The gills are attached to the stem or free of it, white at first, but soon become grey, then black, eventually dissolving (turns into black "ink"). The fruiting body is very thin, fragile, with indistinct odour and taste. Spores are ovoid and smooth, as shown in (Fig. 4).

Nigrospora sphaerica

Colonies grow rapidly and appear hairy or woolly . The conidiophores are short and clustered on the surface of the mycelium; they appear transparent in colour and have an average diameter of 8-11 µm. The conidiophores are often straight or slightly curved stems. The conidia grow from the tips of the transparent conidiophores. Conidia are black-brown, flattened spheroids,

Table 2. Sequence of nitrogenous bases for primers ITS4-ITS1 (Source: Zarrin *et al.,* 2016)

The name and type of primer	Sequence of nitrogenous bases
ITS1(Forword)	5'-TCCGTAGGTGAACCTGCGG-3'
ITS4(Reverse)	5'TCCTCCGCTTATTGATATGC-3'

Fig. 2. *Fruiting bodies obtained during survey trips from different areas of the city of Mosul*

and unicellular, with an average diameter of 16-18 µm. The white colony of *N. sphaerica* turns to brown/black due to extensive sporulation of conidia from conidiophores. *N. sphaerica* is often considered pathogenic and recent studies have identified metabolites isolated from *N. sphaerica*. Some of these act as phytotoxins, such as fumalactone produced by *N. sphaerica,* which inhibits the growth of phytopathogenic fungi, including *Phytophthora infestans* (Fig. 5).

Molecular identification of *Ganoderma*

A simulation of DNA replication inside living systems can be performed externally using polymerase chain reaction (PCR) technology, with modifications to certain conditions to achieve the amplification of a specific piece of genetic material in quantities sufficient for analysis. The amplification of a specific segment of a single -stranded DNA involves designing highly specialised primers (*ITS1* and *ITS4*), which bind to sequences that match or complement them precisely. The PCR process required 30 cycles to produce a well-defined, bright, sharp band with horizontal homogeneous surfaces on an agarose gel (Al-Nuaimy and Mulla Abed,2021). This band appeared within the amplification range corresponding to the marker scale with a known weight 1500 bp, confirming the correct binding of the primers. The amplified DNA product reached a size of 650 base pairs, confirmed by comparison with the marker scale. It was photographed with a digital camera and multiple images were taken, and the one with the highest clarity was selected, as shown in (Fig. 6). This result was consistent with the data from the primer design available in the free program on the multi -purpose website of the National Center for Bioinformatic Information (NCBI) (Sultan *et al*., 2024).

Abdulla *et al.* (2025) isolated and identification mushroom fungi from Basra Governorate, southern Iraq, based on the morphological and molecular characterization,found five genera belonging to the basidiomycetes Agaricales, which are (*Agaricus bitorquis, Conocybe velutipes, Coprinellus radians, Gymnopilus purpureosquamulosus,* and *Psathyrella trinitatensis*) and compared those with genes in the genebank

One of the important pillars for the success of the process was the quality and purity of the extracted DNA, as well as the absence of DNA polymerase inhibitors. These factors confirm that the polymerase chain reaction effectively targets a specific piece of DNA for amplification. Other essential indicators include the reaction temperature, duration, and component concentrations. The reaction mixture was maintained within the recommended ranges, allowing optimal amplification conditions for the desired target. This also confirms that the workplace and tools used were free from contamination.

Nucleotide sequence of the *Ganoderma resinaceum*

To determine the nucleotide sequence, the amplified product was obtained as a single band from a local fungal isolate phenotypically classified within the genus

Table 3. Optimal conditions for polymerase chain reaction technology

No.	Stage	Temperature C	Time	Number of Cycles
1.	Initial denaturation	95	5 min.	1
2.	Denaturation	95	1 min.	
3.	Annealing	58	1 min.	35
4.	Extension	72	1 min.	
5.	Final extension	72	5 min.	1
6.	Stop reaction	4	5 min	1

Table 4. Survey trips, fungal sample collection sites, and collection dates

Number of fruiting bodies	Host status	Host type	Collection date	Collection site	Scientific symbol
2	Lively	Eucalyptus	2023/11/30	Mosul forests	Mo1
1	Dead	Pine			Mo2
4	----	Soil	2023/12/16	Canadian	Mo3
2	Lively	Olive	2023/12/29	Domiz neighborhood	Mo4
3	Burnt	Anonymous			Mo5
1	---	Soil	2024/1/22	Al-Atibaa district	Mo6
5	Cut off	Eucalyptus	2024/2/15	Al Mithaq District	Mo7
2	Good	The garden			Mo8
2	Lively	Pine	2024/3/1	Mosul forests	Mo9
4	Good	The garden	2024/3/17	Errachidia	Mo10

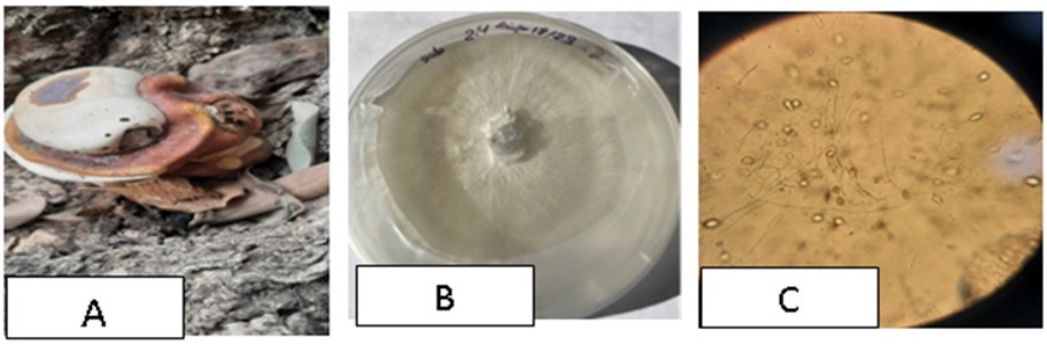

Fig. 3. *Phenotypic diagnosis of the fungal isolate Ganoderma resinaceum, A: fruiting body,B: Fungal colony on PDA medium, C: Fungal hyphae and basidiomycete spores at 40 X magnification and stained with lactophenol dye*

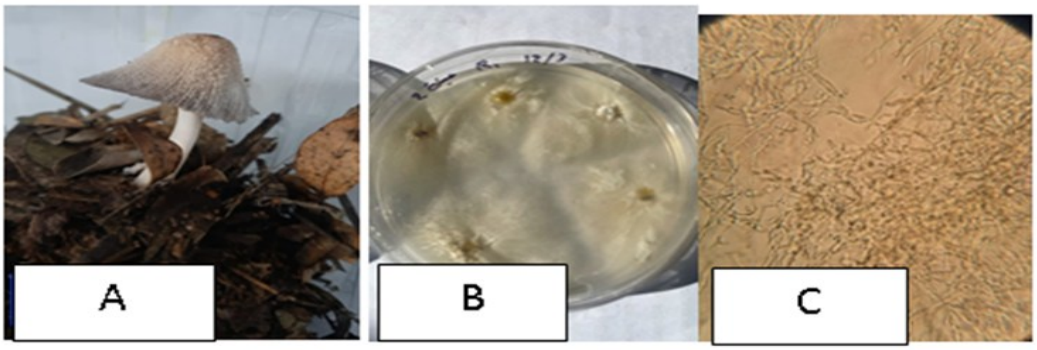

Fig. 4. *Phenotypic diagnosis of the fungal isolate Coprinellus domesticus, A: fruiting body,B: Fungal colony on PDA medium, C: Fungal hyphae and basidiomycete spores at 40 X magnification and stained with lactophenol dye*

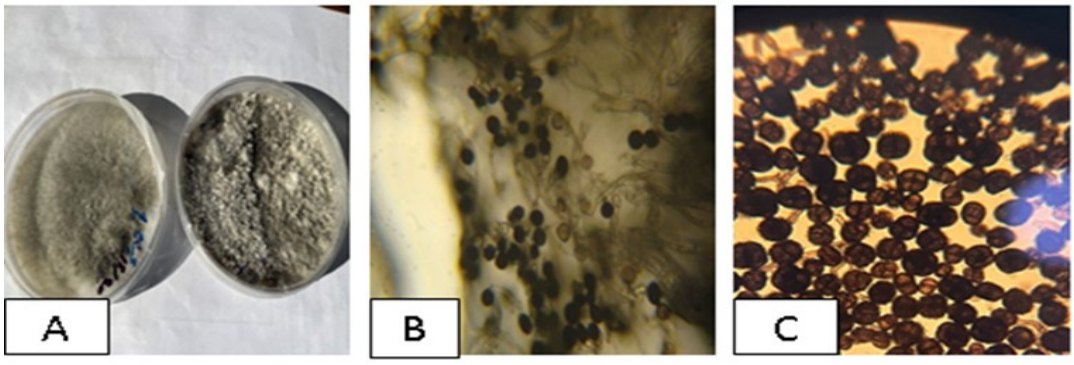

Fig. 5. *Phenotypic diagnosis of the fungal isolate Nigrospora sphaerica. A: Fungal colony on PDA medium. B: Fungal hyphae and cystic spores at 40 X magnification and stained with lactophenol dye. C: Reproductive bodies*

Table 5. Active chemicals compounds isolated from the *Ganoderma resinaceum* by Gas Chromatography

Cpd	Name	Formula	K1	CAS	ID Source	Score	Score (Lib)
1	Cyclohexane, 1-butenylidene-	C10 H16	5.160	36144-40-8	LibSearch	71.04	71.04
2	Benzene, 1-isocyano-2-methyl-	C8 H7 N	7.233	10468-64-1	LibSearch	87.58	87.58
3	2-Isobutoxy-4-methyl-[1,3,2]dioxaborinane	C8 H17 B O3	9.731	161616-29-1	LibSearch	65.66	65.66
4	Benzene, (1-butylheptyl)-	C17 H28	11.104	4537-15-9	LibSearch	60.34	60.34
5	Ribitol, 1,3:4,5-di-O-(ethylboranediyl)-2-deoxy-	C9 H18 B2 O4	12.475	1000149-52-8	LibSearch	62.36	62.36
6	Benzene, (1-pentylheptyl)-	C18 H30	13.041	2719-62-2	LibSearch	63.19	63.19
7			13.139				
8	Octadecane, 1-	C19 H37 N O	14.430	112-96-9	LibSearch	64.54	64.54
9	trans-2-methyl-4-n-	C11 H22 O2 S	15.030	1000215-75-3	LibSearch	62.85	62.85
10	1H-S-Triazolo[1,5-hydroxy-1-methyl-,	C7 H7 N3 O	15.443	13980-64-8	LibSearch	57.45	57.45
11	Clorophene	C13 H11 Cl O	15.919	120-32-1	LibSearch	71.95	71.95
12	n-Hexadecanoic acid	C16 H32 O2	16.685	57-10-3	LibSearch	64.76	64.76
13	4-Formylbenzeneboronic	C7 H7 B O3	16.761	87199-17-5	LibSearch	60.00	60.00
14	Decane, 3,8-dimethyl-	C12 H26	18.088	17312-55-9	LibSearch	60.61	60.61
15			18.762				
16	Octadecanoic acid	C18 H36 O2	19.027	57-11-4	LibSearch	62.41	62.41
17			19.168				
18	1-Methylsilacyclohexane	C6 H14 Si	19.225	765-62-8	LibSearch	51.89	51.89
19	2-(4-isothiocyanate	C14 H11 N S2	19.384	81431-97-2	LibSearch	54.97	54.97
20	2-Isopropyl-5-methyl-1-	C11 H24 O	19.406	91337-07-4	LibSearch	69.32	69.32
21	Allopregnane	C21 H36	19.766	641-85-0	LibSearch	50.90	50.90
22	Trihexadecyl borate	C48 H99 B O3	20.020	2665-11-4	LibSearch	58.98	58.98
23			20.449				
24	Pyrrolidine, 1-(9-yl)-	C12 H22 B N	20.566	22516-41-2	LibSearch	63.91	63.91
25	9-Octadecenamide, (Z)-	C18 H35 N O	20.983	301-02-0	LibSearch	53.06	53.06
26	Cyclodecasiloxane,	C20 H60 O10 Si10	21.008	18772-36-6	LibSearch	53.63	53.63
27			21.033				
28	Pyrrolidine, 1-(9-yl)-	C12 H22 B N	21.060	22516-41-2	LibSearch	60.08	60.08
29			21.190				
30	Phenol, 2,2'-dimethylethyl)-4-methyl-	C23 H32 O2	21.549	119-47-1	LibSearch	66.35	66.35
31	Ethyl 3-(dimethylamino)-2-isothiocyanatoprop-2-Enoate	C8 H12 N2 O2 S	21.881	1000387-41-0	LibSearch	50.00	50.00
32			21.946				
33	2-{[1-(3-Methoxypropyl)-1H-1,2,3-triazol-4-yl]methyl}-4-[methyl(propyl)amino]isothiazolidine 1,1-dioxide	C14 H27 N5 O3 S	22.106	1333142-62-3	LibSearch	51.33	51.33
34	Cyclononasiloxane, octadeca-methyl	Cyclononasiloxane, octadecamethyl	22.158	556-71-8	LibSearch	50.95	50.95
35	Boron, [3-imino-2-(1-iminoethyl)butanenitrilato-N2,N3]dipropyl-, (t-4)-	C12 H22 B N3	22.445	136704-96-6	LibSearch	53.34	53.34
36	,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15,15-Octadecamethyloctasilox Ane	C18 H54 O7 Si8	22.526	556-69-4	LibSearch	50.30	50.30
37	Bis(2-ethylhexyl) Phthalate	C24 H38 O4	22.571	117-81-7	LibSearch	52.87	52.87
38	Pyrrolidine, 1-(9-borabicyclo[3.3.1]non-9-yl)-	C12 H22 B N	22.785	22516-41-2	LibSearch	56.64	56.64
39	Trihexadecyl borate	C48 H99 B O3	22.901	2665-11-4	LibSearch	55.48	55.48

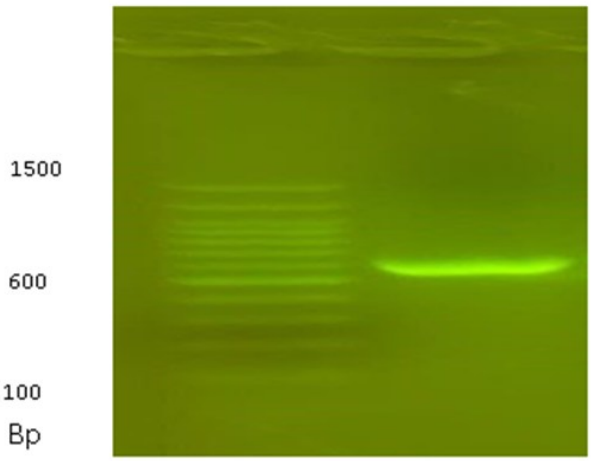

Fig. 6. *DNA amplification products of the selected fungal isolate on an agarose gel, the nucleic acid amplification products of the Ganoderma*

Ganoderma, after being excised from an agarose gel. It was then purified using a specialized extraction kit and added to a reaction mixture containing one of the primers and non-ionic water in a 1.5 ml Eppendorf tube. The sample was subsequently sent to the Korean company Macrogen for sequencing. Using the published nucleotide sequences deposited in the GenBank databases, the alignment process was carried out using the Blast program available on the NCBI website and using the copy and paste feature of the nucleotide sequences received from Macrogen. For the local isolate, the alignment results showed that the nucleotide sequence belongs to the species *Ganoderma resinaceum*, which showed 100% complete identity with the nucleotide sequences of the reference strain *G. resinaceum* (Reference registration number pp534170; Accession number), as shown in (Fig. 7). The isolate was recorded in the GenBank under with name *Ganoderma resinaceum* strain FHM4, as shown in Fig. 8.

After reviewing the isolates deposited in the GenBank, it becomes clear that the local isolate was discovered for the first time in the city of Mosul, Iraq, and it was recorded in the GenBank database under the serial number MG706250.1 the urgent need for further work by fungi specialists to discover and describe previously unknown species that may possess novel characteristics and capabilities at the fundamental and applied scientific levels.

Identification of the active compounds in the *Ganoderma resinaceum*

To achieve the objective of identifying the active compounds secreted by Ganoderma resinaceum (local isolate), Gas Chromatography-Mass Spectrometry (GC-MS) was employed. The results, shown in Table 5, indicate that the mushroom extract contained many chemical compounds depending on the number of peaks that appeared in the gas chromatogram. The retention time ranged between 5.669 and 22.570, as shown in (Fig. 9), following injection of the sample into the GC device. Four main peaks were identified, and the correspond-

Ganoderma resinaceum voucher LGAM 462 - ACAM A990 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: MG706250.1 Length: 642 Number of Matches: 1

Range 1: 6 to 586 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1074 bits(581)	0.0	581/581(100%)	0/581(0%)	Plus/Minus

```
Query  1    TAAAGCTGTCTCACAAACGAGACGGTTAGAAGCTCGCCAAAACGCTTCACGGTCGCGGCG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  586  TAAAGCTGTCTCACAAACGAGACGGTTAGAAGCTCGCCAAAACGCTTCACGGTCGCGGCG  527

Query  61   TAGACATTATCACACCGAGAGCCGATCCGCAAGGAATCAAGCTAATACATTTAAGAGGAG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  526  TAGACATTATCACACCGAGAGCCGATCCGCAAGGAATCAAGCTAATACATTTAAGAGGAG  467

Query  121  CCGACCAAAACACGGCCGACAAGCCTCCAAAGTCCAAGCCTACAAACCCGCAAAGGTCTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  466  CCGACCAAAACACGGCCGACAAGCCTCCAAAGTCCAAGCCTACAAACCCGCAAAGGTCTG  407

Query  181  TAAGTTGAAGATTTCATGACACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  406  TAAGTTGAAGATTTCATGACACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAA  347

Query  241  GGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  346  GGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTT  287

Query  301  CGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGCTGAAAGTTGTATATAGAT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  286  CGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGCTGAAAGTTGTATATAGAT  227

Query  361  GCGTTACATCGCAATACACATTCTAATACTTTATAGAGTTTGTGATAAACGCAGGCACAG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  226  GCGTTACATCGCAATACACATTCTAATACTTTATAGAGTTTGTGATAAACGCAGGCACAG  167

Query  421  GCACGCCGCTTTACAAGCTCCGTAAAGAGCCCGCTTCACAACGTCTGGAACCCACAGTAA  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  166  GCACGCCGCTTTACAAGCTCCGTAAAGAGCCCGCTTCACAACGTCTGGAACCCACAGTAA  107

Query  481  GTGCACAGGTGTAGAGTGGATGAGCAGGGTGTGCACATGCCTCGGAAGGCCAGCTACAAC  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  106  GTGCACAGGTGTAGAGTGGATGAGCAGGGTGTGCACATGCCTCGGAAGGCCAGCTACAAC  47

Query  541  CCAGTCAAAACTCGATAATGATCCTTCCGCAGGTTCACCTA  581
            |||||||||||||||||||||||||||||||||||||||||
Sbjct  46   CCAGTCAAAACTCGATAATGATCCTTCCGCAGGTTCACCTA  6
```

Fig. 7. *Nucleotide sequences of the reference isolate deposited in GenBank with serial number MG706250.1.*

Fig. 8. *Recording of the Ganoderma resinaceum at the the National Center for Biotechnology Information (NCBI) site*

ing mass spectra revealed four major compounds with the highest concentration, as indicated by the highest peak heights compared to other compounds.

The compound Bis(2-ethylhexyl) phthalate had a retention time of 22.571, the compound 9-Octadecenamide, (z) had a retention time of 20.983, and finally, the compound 4-Formylbenzeneboronic acid had a retention time of 16.761. The highest peak for the compound was Benzene,1-isocyano-2-methyl, with a retention time of 7.233, as shown in Fig. 9.

Secondary metabolite compounds found in fungal extracts have multiple therapeutic benefits for humans, animals, and plants. The extraction of these compounds depends on several factors, including time, temperature, and the type of solvent used (Orole, 2016). In this study, several types of compounds were identified in the extract of *G. resinaceum*. Among them, Benzene, 1-isocyano-2-methyl-, which belongs to volatile oils, exhibits antagonistic activity against Gram-negative bacteria. This was followed by the compound 4-formylbenzeneboronic acid, a boronic acid-containing compound with antagonistic activities against bacteria, viruses, and pathogenic fungi. It also has antioxidant, anti-inflammatory, anticancer, and anti-tumour properties (Silva *et al*., 2020). Next, 9-Octadecenamide (Z) is recognized as an antibacterial compound. Octadecene, a related compound, was previously isolated from *Ganoderma lucidum* and belongs to the group of un-

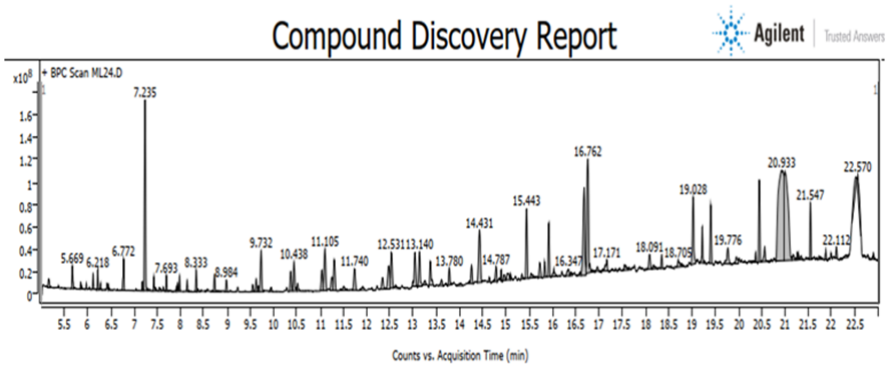

Fig. 9. *Active compounds of Ganoderma resinaceum*

saturated fatty acids. It also functions as an oxygen receptor, and possesses antioxidant, anticancer, and antibacterial properties (Lee *et al.,* 2007; Mishra and Sree, 2007). Finally, the compound Bis (2-ethylhexyl) phthalate, which is one of the secondary metabolites secreted by many types of bacteria, fungi, and algae, is considered antimicrobial. This compound, isolated from the bacterium *Lactiplantibacillus plantarum* BCH-1, led to the inhibition of several types of bacteria, including *E. coli and S. aureus* (Javed *et al*., 2022). In addition to killing mosquito larvae, which are carriers of parasitic and viral diseases. Given that the continuous use of insecticides contributes to mosquito resistance, alternative, environmentally friendly and effective compounds are necessary to control both pathogenic organisms and disease-carrying larvae (Chen *et al.,* 2013). Karunarathna *et al.* (2024) isolated secondary metabolites from *Ganoderma*, such as triterpenoids, polysaccharides, and glycoproteins, which hold significant potential for developing novel antiviral drugs. Similarly, Fogarasi et al. (2024) isolated bioactive compounds, such as polyphenols, from mushrooms. These polyphenols not only confer antioxidant effects but also offer preventive and therapeutic benefits.

Conclusion

The isolate of the fungal *G. resinaceum* was first diagnosed phenotypically and then confirmed by molecular diagnosis for the first time in Mosul, Iraq, according to data obtained from NCBI. It was possible to obtain several isolates from the isolated areas, including those belonging to Basidiomycete fungi and Ascomycete fungi, through the primary screening process. The search focused on fungi that form fruiting bodies, which serve as a vital component for preserving natural life and biodiversity, particularly in response to environmental changes. Secondary metabolites were extracted from local isolated *G. resinaceum*, revealing several active compounds including: Bis (2-ethylhexyl) phthalate, 9-Octadecenamide, (z), 4-Formylbenzeneboronic acid and Benzene,1-isocyano-2-methyl-, as identified by their retention time and the height of the peaks using the Gas chromatography method. The originality of the present work lies in the molecular diagnosis of the *Ganoderma* isolated locally from Mosul, Iraq, coupled with the isolation of some of its active compounds. To present knowledge, this study is the first of its kind to combine genetic and chemical analysis of this fungus in this region. Unlike previous studies that often focused on morphological characteristics or compounds extracted from known global strains, this study reveals new insights into strain diversity and its potential therapeutic applications.

ACKNOWLEDGEMENTS

The authors greatly thank to staff of the Department of Biology in the College of Science, University of Mosul.

Conflict of interest

The authors declare that they have no conflict of interest.

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