The use of mycology as a tool to investigate and identify fungi collected in Algeria and highlight their toxins

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***Abstract*— This study explores the potential contributions of mycology to forensic science and criminal investigations, with a focus on toxicology and psychoactive compounds. A collection of 28 macromycete species was assembled, including 12 freshly collected from three peri-urban forests in Algiers. The collection comprises 13 known toxic species, two lethal species, and one hallucinogenic species. The identification was based on morphological and microscopic characteristics. This work provides a foundational resource for understanding toxic and psychoactive fungi in forensic contexts.**

The Meixner test was utilized for detecting amatoxins, and the Weber test was applied to identify psilocybin/psilocin in *Gymnopilus junonius*. Then Gas Chromatography-Mass Spectrometry (GC-MS) was employed as a robust technique to analyze the constituents of *Gymnopilus junonius,* focusing on the reliable identification of psilocybin.

***Keywords*— Fungal material, weber test, Meixner test, Gas Chromatography-Mass Spectrometry, Psilocybin.**

I. INTRODUCTION

In recent years, Algeria has seen a growing interest in wild mushroom foraging, particularly in peri-urban forests, alongside the development of several edible mushroom production units. However, numerous cases of poisoningand even fatalitiesare reported annually due to the consumption of wild mushrooms containing amatoxins [1].

Some edible species can become toxic or deadly due to acquired toxicity, as demonstrated by several cases worldwide. A notable example is Pleurocybella porrigens, once considered an excellent edible mushroom, which was implicated in 59 poisoning cases, including 19 fatalities in China in 2004 [2].

Fungi are not only consumed for their culinary value; some species are sought after for their hallucinogenic properties and are commonly referred to as "magic fungi" [3-4]. In Algeria, the cultivation, commercialization, consumption, and possession of biological material containing psychotropic substances are prohibited under Law No. 04-18 of December 25, 2004. The enforcement of this law necessitates forensic services—particularly forensic analysts—to closely examine these organisms to better understand and identify them in various forms and their substances in different fluids and organs.

Major toxins present in fungal species, such as orellanine, α- and β-amanitin, muscarine, ibotenic acid, muscimol, and gyromitrin, have been identified using various analytical techniques. Research highlights challenges in correlating clinical symptoms with specific toxins due to the vast diversity of fungal species and their associated toxic compounds [5] . Sulah Lee et al. successfully isolated three sesquiterpenes from *Gymnopilus junonius* and evaluated their cytotoxic activity against human lung and prostate cancer cell lines. The findings suggest that these isolated compounds may possess potential anti-cancer properties [6].

Detection of fungal toxins (mycotoxins) and psychoactive compounds plays a crucial role in forensic science, food safety, and clinical toxicology. Gas Chromatography-Mass Spectrometry (GC-MS) is a powerful tool for analyzing psilocybin and psilocin in forensic contexts. Since these compounds are non-volatile, they typically require derivatization to improve volatility before GC-MS analysis. Liquid Chromatography-Mass Spectrometry (LC-MS) and LC-MS/MS offer high sensitivity without the need for derivatization, making it ideal for accurately detecting psilocybin and psilocin in biological samples [7].

The Weber test is a simpler colorimetric method used for preliminary identification of these compounds; it produces a characteristic blue or purple color when mushrooms or extracts are treated with specific reagents. Although less reliable than chromatographic methods, the Weber test is useful for initial screenings in forensic cases. The Meixner test, also known as the Meixner-Schulze test, is a spot test specifically for amatoxins found in highly toxic mushrooms like Amanita phalloides [7].

This study addresses the critical need to tackle forensic challenges associated with fungal poisonings—whether accidental or intentionalthat can result in fatalities. Forensic analysts play a vital role in identifying implicated fungi and/or toxins to resolve cases of suspicious death involving poisoning, suicide or consumption of large doses of hallucinogenic fungi. Accurate identification of both species and substances involved is essential [2, 8].

The study aims to introduce mycology [9] as a forensic investigative tool by focusing on two key aspects: first, identifying fungi through a workflow that distinguishes toxic, lethal, and hallucinogenic mushroom species based on their morphological and microscopic traits; second, identifying mycotoxins and psychotropic substances through methods such as the Meixner test for amatoxins detection and the Weber test for screening psilocybin/psilocin, and confirming these substances by GC-MS.

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II. EXPERIMENTAL

For this study, a collection of fungal material was compiled from freshly harvested fruiting bodies in the field and dried specimens provided by the Laboratory of Forest Pathology and Mycology at INRF (LPMF-INRF). Fresh specimens were collected from accessible peri-urban forests—specifically Bainem Forest, Bouchaoui Forest, and Zeralda Forest.

The entire collection was used for staining tests and GC-MS analysis. Remaining fungal material was preserved as reference specimens by drying them at a stable temperature of 30°C. Each dried specimen was placed in a clean plastic bag labeled with its species name along with the date and location of collection.

A. Description and identification of the collected fungal material

The description of the fungal material was conducted at the Laboratory of Forest Pathology and Mycology using methods proposed by Courtecuisse and Duhem (1994). This widely used method relies on studying morphological and microscopic characteristics along with ecological data collected in the field. Identification utilized determination keys from Eyssartier et al. 2011 [10].

1) Morphological study of the collected specimens

The morphological study focused on all parts of the mushroom, including:

Spore print: The color of the spore mass, which is commonly used to define major groups and assign a species to a family or genus.

Cap: The shape, margin, surface, color, and size of the cap were observed on all specimens of each species to note changes during the mushroom's development.

Hymenophore: This is the fertile part of the mushroom where spores are produced. It can consist of gills, pores, spines, or teeth.

Stipe (or stem): Characteristics to note include attachment, consistency, size, shape, color, ornamentation, presence of partial and universal veils, and surface texture.

Flesh: thickness, color, odor, and taste were recorded.

2) Microscopic study

Microscopy is recommended for confirming a particular species, so a study of the microscopic characteristics was conducted for some species. This study primarily focused on Spores, Hymenium and Caulocystidia.

B. Determination of species toxicity

After identifying the species, their toxicity was investigated in the literature using two guides: the 3rd edition of the Guide des Champignons, France et Europe by Eyssartier & Roux (2010) and the Edible and Poisonous Mushrooms of the World by Hall et al. (2003). To determine the POISINDEX toxicity classes and groups of the species, Hallen et al were referred [11].

C. Staining tests

1) Meixner test

To perform the Meixner test, a fragment of fresh or rehydrated mushroom (using distilled water heated to 40°C) is crushed onto newspaper so that the juice is absorbed. It is important to use paper with a high lignin content. The outline of the resulting stain should be circled with a pencil (without touching the stain itself) to prevent losing the spot after drying. The spot is then allowed to dry at room temperature. Afterward, 5 µL of concentrated hydrochloric acid (37.4%) is applied to the spot, and any color change is observed and noted.

For each sample, two spots were prepared, with one control spot where only concentrated hydrochloric acid was applied. The time required for color development generally ranges from 1 to 20 minutes, depending on the concentration of amatoxins in the sample. The appearance of a blue to blue-green color after the application of concentrated hydrochloric acid indicates the presence of amatoxins.

2) Weber test for the detection of psilocybin and psilocin

The Weber test was developed by Weber State College in 1984. Since then, it has been regarded as the reference screening test for detecting the presence of psilocybin and psilocin in mushrooms.

To perform this test, 1 to 2 mg of mushroom powder is placed in a clean watch glass. One drop of the first reagent, Fast Blue B, is added, and the resulting color change is observed and recorded. Following this, one drop of the second reagent, concentrated hydrochloric acid (37.4%), is introduced, and any subsequent color change is noted.

The development of a red to reddish-orange color after the application of the first reagent, followed by a blue to blue-green color change after the application of the second reagent, indicates the presence of either psilocybin or psilocin.

D. Analysis of Psilocybin by GC-MS

1) Sample Preparation for Extraction

Parts of the fungal fruit (cap and stem) were freeze-dried, cut into small pieces, and ground into a fine powder using a mortar. A weight of 2 g of the powdered sample was extracted with 200 mL of methanol and vortexed until the fungal material was suspended. The mixture was then placed in an ultrasonic bath for 1 hour, as ultrasonic extraction provides a high yield in a very short extraction time. The obtained extract was centrifuged at 14,000 rpm for 15 minutes according to Suzuki et al. (2005). This centrifugation resulted in two phases: a clear supernatant and a pellet. The supernatant was collected and evaporated to dryness under a gentle stream of nitrogen. Trimethylsilyl derivatives were prepared by dissolving the dry sample in 50 µL of TMS, followed by heating at 70°C for 20 minutes. The evaporated sample dissolved in TMS was then pipetted into a GC-MS vial and injected[8].

2) Conditions for the GC Method

Injector temperature: 250°C, Helium flow rate: 1 mL/min, Mode: Split. Column: Capillary column S8 30 (5% diphenyl / 95% dimethylpolysiloxane) 30 m x 0.25 mm x 0.25 µm stationary phase thickness. Programming: Initial temperature: 80°C for 1 minute, increased to 220°C at a rate of 20°C/min, then to 250°C at a rate of 2°C/min, followed by an increase to 300°C at a rate of 20°C/min, and finally maintained at 300°C for 3 minutes [12].

3) Conditions for the SM Method

Ionization mode: Electron impact, Analyzer: Quadrupole, Source temperature: 230°C, Transfer line temperature: 250°C, Ionization energy: 70 eV. Full scan mode: 20 to 600 Daltons. Solvent delay: 5.90 min. Scan time: 6 to 44 min [13].

III. RESULTS AND DISCUSSION

A. Identification and Determination of Toxicity of the Studied Fungal Material:

Based on bibliographic research combined with macroscopic/microscopic studies conducted on collected species, these are distributed across eleven genera within twenty-three families (see Table 1), our collection includes thirteen recognized toxic

species along with two highly toxic/l lethal ones plus one hallucinogenic species.

1) Amanita phalloides

Known as the deadliest mushrooms, it is responsible for over 90% of fatal poisonings [14].According to POISINDEX's taxonomic classification, this species is in Group I [11]. It contains three main groups of toxins: amatoxins, phallotoxins, and virotoxins. Among these, amatoxins, particularly α-amanitin, are the primary cause of toxic effects in humans. α-amanitin inhibits RNA polymerase II, leading to a protein deficiency that causes cell death, though other mechanisms may also be involved. The liver is the primary target organ, with symptoms typically appearing after a latency period, including gastrointestinal disturbances followed by jaundice, seizures, coma, and eventually death. Treatment involves gastric decontamination, pharmacotherapy, and in severe cases, liver transplantation if the clinical condition worsens [14].

Cases of *Amanita phalloides* poisoning in Algeria were first reported by Trabut in 1907, and later by Sartory in 1912 in Collo, with 8 victims, 6 of whom died. Other cases were reported by the same author in Béjaïa and Constantine in 1913, with 2 and 6 deaths respectively [15].



Fig. 1. *Amanita phalloides*, under cork oak.

2) Omphalotus olearius

According to POISINDEX's taxonomic classification, this species is in Group IV, with early onset of muscarinic symptoms, usually within an hour of ingestion [16]. Omphalotus species are characterized by the presence of terphenylquinones (atromentin, thelephoric acid) and pulvinic acid derivatives [17]. Grayson et al. reported that all Omphalotus species are toxic, causing severe gastroenteritis. The toxic substances have been identified as sesquiterpenes, particularly illudin S [18].



Fig. 2.*Omphalotusolearius*, on an olive tree stump.

3) Gymnopilus junonius

A hallucinogenic species containing psilocybin, known as the "Laughing Gym" (Ohwaraitake in Japanese) because it induces excessive laughter in those who consume it [19]. Subjective effects range from intentional feelings of relaxation, dizziness, uncontrollable laughter, energy, joy, euphoria, enhanced visual perception, visual disturbances, to mostly unintentional delusions, altered perceptions of real events, images, and faces, or true hallucinations. Other symptoms include dizziness, nausea, weakness, muscle pain, chills, abdominal pain, and pupil dilation (mydriasis) [20]. According to POISINDEX's taxonomic classification, this species is in Group IV [11].

In a series of studies on the acute subjective, psychological, and perceptual effects of psilocybin, it was shown that psilocybin induces a psychotic state that mimics some aspects of the acute and early stages of schizophrenia [21]. Although these reports do not establish a causal link between psilocybin and psychiatric illness, the potential role of hallucinogens in triggering or exacerbating persistent psychosis, other psychiatric disorders, and long-lasting visual perceptual disturbances should be more closely evaluated [22].

No official reports of recent poisonings caused by these toxic and lethal species in Algeria was found. Based on our inquiries with Poison Control Centers (CAP) and the Pasteur Institute, there is significant difficulty in identifying the mushroom species responsible for the recorded or treated poisoning cases. Patients are often treated based on the observed syndrome.

It is important to mention here that it is impossible to determine the toxicity or edibility of a species based on morphological characteristics such as color or size. It is always necessary to first identify the species and then research its toxicity or edibility through published literature. Moreover, even some species recognized as edible can become toxic or lethal due to the phenomenon of acquired toxicity when exposed to agricultural or industrial pollutants. Additionally, some edible species produce toxins as they age and thus become toxic, as is the case with the poplar mushroom Cyclocybe aegerita.



Fig. 3. *Gymnopilus junonius*, on a Eucalyptus gomphocephala stump and spores (magnification × 40).

B. Staining Tests

1) Meixner Test

As described in the literature, this test is used to detect amatoxins. However, it can yield false positives with psilocybin and psilocin. Our results, presented in Table 1, indicate the presence of mycotoxins such as amanitin and psilocybin.

The development of a blue coloration with Agaricus moelleri, *Amanita phalloides*, and *Lepiota subincarnata* highlights the presence of amatoxins in these species. Our findings are consistent with the works of Beutler et al and Fiedziukiewicz et al [23-24]. According to the latter author, amatoxins (alpha, beta, and gamma-amanitin) all contain a 6-hydroxy-substituted indole group. The observed blue coloration results from the reaction between the aromatic aldehyde in lignin and the indole ring in amatoxin. These results confirm that the test was

properly performed, as an excess of hydrochloric acid inhibits the reaction [23].

The formation of a light blue-green color, observed with *Gymnopilus junonius* and Psathyrella candolleana, suggests the presence of psilocin. This color is characteristic of tryptamines substituted at the 6-position, such as psilocin. Psilocin and psilocybin are considered "false positives" because they do not contain amatoxins [25-26].

The negative reactions obtained with the other species suggest the absence of both amatoxins and psilocybin/psilocin. Our results are in line with those of other researchers, particularly the negative reaction of Amanita citrina, which according to Beutler et al, does not contain amatoxins [23]. The two authors report that the red-blue coloration (Fig.4) observed in the case of Amanita citrina is due to bufotenine, with concentrations ranging from 0.4 to 7.5 mg/g of the mushroom's dry weight.

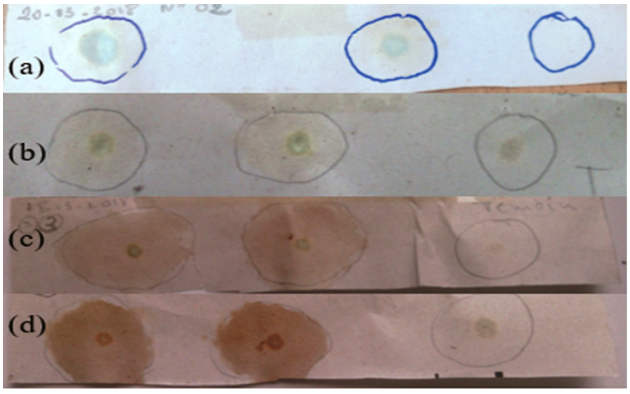


Fig. 4. Meixner test of (a) *Amanita phalloides*, (b) Psathyrella candolleana, (c) *Gymnopilus junonius* and (d) *Amanita citrina*.

2) Weber Test

The Weber test is the standard screening test for detecting psilocybin and psilocin in mushrooms, this test was performed on the hallucinogenic species *Gymnopilusjunonius*, which tested positive with the Meixner test (though considered a false positive since this species does not contain amatoxins) to confirm the presence of psilocybin and psilocin.

During the Weber test, an immediate red coloration appeared in the first phase (after the addition of Fast Blue B reagent), followed by a blue-green coloration in the second phase after the addition of concentrated hydrochloric acid (Fig.5).

The development of the blue-green color in the second phase clearly indicates the presence of psilocybin or psilocin in this species. *Gymnopilus* junonius is consumed in various regions of the world for its psychotropic effects and has been implicated in several cases of intoxication involving hallucinogenic experiences [27-28].

The presence of psilocybin in this species has been demonstrated by several authors, notably Hatfield et al, using thin-layer chromatography (TLC) on American specimens [27].

Confirmation of psilocybin presence via gas chromatography-mass spectrometry GCMS or TLC is a procedure adopted by the majority of forensic laboratories after an initial screening with the Weber test [29]. The same procedure was followed and submitted for GCMS analysis.

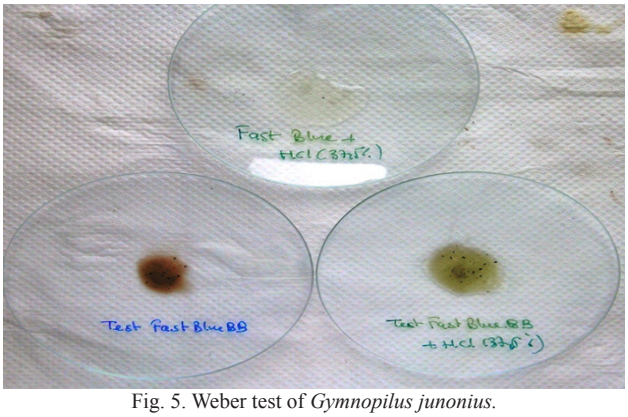


Fig. 5. Weber test of *Gymnopilus junonius*.

TableI.
Collected fungal material, their families and Meixner test results.

N°	Species	Family	Meixner Test Result
1	*Agaricus moelleri*	Agaricaceae	Positive: blue
2	*Agaricus xanthodermus*	Agaricaceae	Negative
3	*Clathrus rubber*	Phallaceae	Negative
4	*Conocybesp*	Bolbitiaceae	Negative
5	*Crinipellisscabella*	Marasmiaceae	Negative
6	*Cyclocybeaegerita*	Strophariaceae	Negative
7	*Gymnopilusjunonius*	Strophariaceae	False positive: blue-green
8	*Inocybe geophylla*	Inocybaceae	Negative
9	*Melanoleuca sp*	Tricholomataceae	False positive: blue-green
10	*Mycena pura*	Mycenaceae	Negative
11	*Psathyrellacandolleana*	Psathyrellaceae	Negative
12	*Schizophyllum commune*	Schizophyllaceae	Positive: blue
13	*Amanita citrina*	Amanitaceae	Negative
14	*Amanita phalloides*	Amanitaceae	Negative
15	*Armillaria ostoyae*	Physalacriaceae	Negative
16	*Cortinariuscasimiri*	Cortinariaceae	Negative
17	*Helvella acetabulum*	Helvellaceae	Negative
18	*Helvellaleucomelaena*	Helvellaceae	Negative
19	*Hygrocybeconica*	Hygrophoraceae	Negative
20	*Inocybe tenebrosa*	Inocybaceae	Positive: blue
21	*Lactarius atlanticus*	Russulaceae	Negative
22	*Lepiotasubincarnata*	Agaricaceae	Negative
23	*Macrolepiota mastoidea*	Agaricaceae	Negative
24	*Omphalotusolearius*	Omphalotaceae	Negative
25	*Pholiotahighlandensis*	Strophariaceae	Negative
26	*Psathyrellaconopilus*	Psathyrellaceae	Negative
27	*Suillellusluridus*	Boletaceae	Positive: blue
28	*Tapinellapanuoides*	Tapinellaceae	Negative

C. Analysis of Psilocybin by Gas Chromatography-Mass Spectrometry (GC-MS)

The identification of psilocybin, the active compound in the species *Gymnopilus junonius*, was carried out using gas chromatography coupled with mass spectrometry (GC-MS).

The chromatogram of the mushroom *Gymnopilus junonius* in SIR mode presented in Fig.6 (a) is characterized by the presence of a peak at a retention time of 8.73 minutes, corresponding to psilocybin according to Smith et al [30].

The interpretation of its mass spectrum reveals a base peak at (m/z) = 204, as reported by Smith, this is the most intense peak in the spectrum with an intensity of 99.9, corresponding to the most abundant ion in the spectrum in the Fig.7 (a). The presence of the parent peak (m/z) = 284.3, although at a very low abundance which corresponds to the molecular mass of the substance, and the fragment peak appears at m/z = 58 [30].

The chromatogram of the *Gymnopilus junonius* mushroom in scan mode shown in Fig.6 (b) is characterized by the presence of peaks corresponding to some carbohydrates, which are part of the mushroom's chemical composition. It shows also a peak at a retention time of 9.290 min corresponds to 1,5 Anhydro-D-sorbitol, tetrakis (trimethylsilyl) ether, its mass spectra is presented in Fig.7 (b) which shows a molecular peak at (m/z) = 452.

A peak at a retention time of tr = 9.779 min is observed in the chromatogram of *Gymnopilus junonius* corresponds to D-mannitol,1,2,3,4,5,6-hexakis-O-(trimethylsilyl). Its mass spectrum in Fig.7 (c) reveals the presence of a base peak at (m/z) = 73, the most intense peak in the spectrum, and corresponds to the most abundant ion. The presence of the molecular peak (m/z) = 612.

A peak at a retention time of tr = 9.928 min corresponds to Myo-inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-ss inositol hexa is observed in the chromatogram. Fig.7 (d) shows its mass spectrum which reveals the presence of a base peak at (m/z) = 73 with an intensity of 99.9, it corresponds to the most abundant ion in the spectrum. The presence of the parent peak (m/z) = 612 with a low abundance.

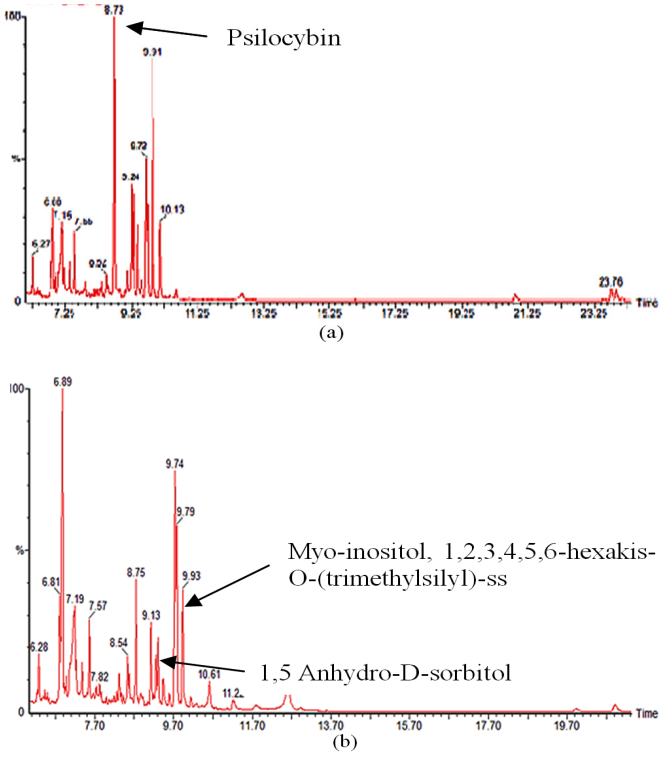


Fig. 6. Chromatographs of *Gymnopilus junonius* in (a) SIR mode and (b) scan mode.

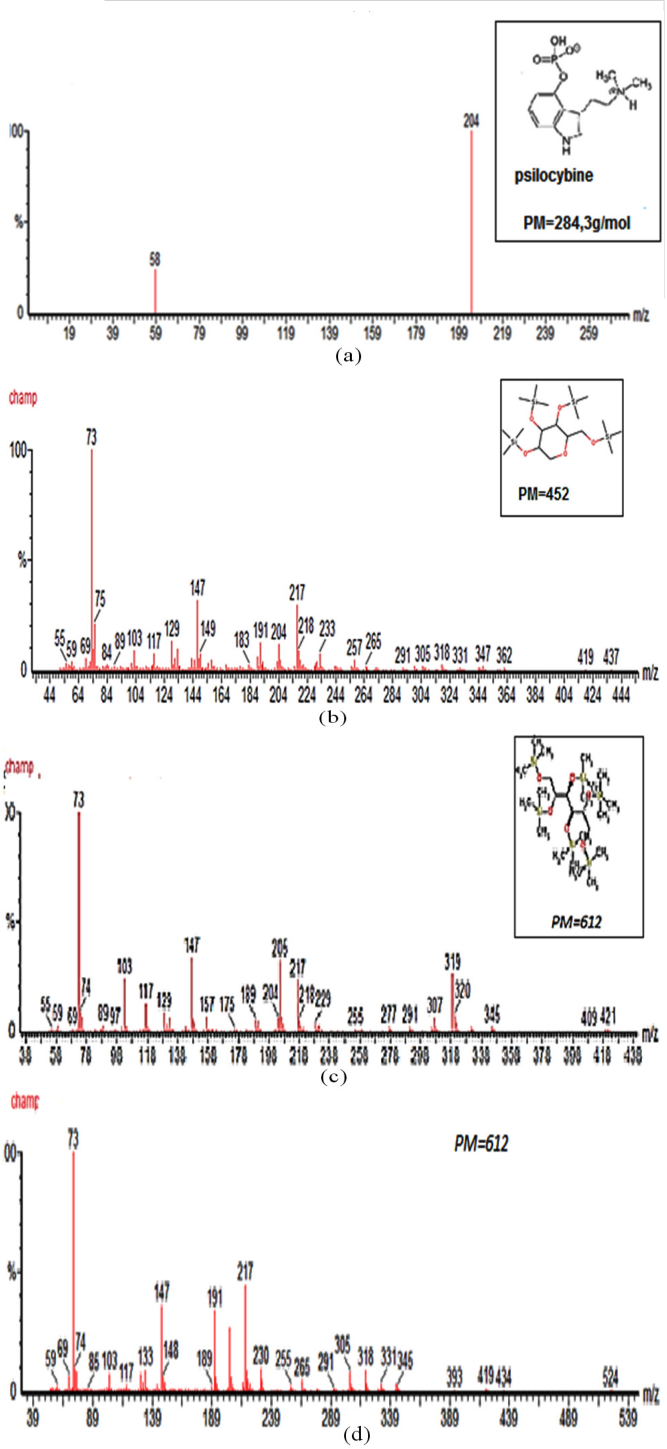


Fig. 7. Mass spectrum at retention time (tr), (a) 8.689 min for psilocybin, (b) 9.290 min for 1,5-anhydro-d-sorbitol, tetrakis(trimethyl silyl) ether, (c) 9.779 min for D-mannitol, 1, 2, 3, 4, 5, 6-hexakis-o-(trimethylsilyl), and (d) 9.928 min for Myo-inositol, 1, 2, 3, 4, 5, 6-hexakis-o-(trimethylsilyl)-ss inositol hexa.

IV. CONCLUSION

Our study involved collecting 28 species of macromycete, the research was focused on mycotoxins and psychotropic substances identification through Meixner test and Weber test alongside GC-MS to confirm the presence of hallucinogenic substances in mushrooms.

To screen for the presence of amatoxins in the fungal material, the Meixner test was conducted. The results indicate the presence of amatoxins in three species: *Agaricus moelleri*, *Lepiota subincarnata*, and the deadly *Amanita phalloides*, all of which are known to cause phalloides syndrome. However,

the Meixner test also yielded two false-positive results for *Gymnopilus junonius* and *Psathyrella candolleana*. The blue-green coloration observed in these species suggested the presence of psilocin or psilocybin.

To verify this hypothesis, the Weber test was performed, confirming the presence of psilocybin in *Gymnopilus junonius*. This finding aligns with previous studies that have reported similar results.

GC-MS analysis, renowned for its high sensitivity, enabled the detection of psilocybin in *Gymnopilus junonius* through direct analysis of the mushroom's methanolic extract. The precise identification of psilocybin and other constituents was achieved through computerized searches in reference spectral libraries.

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