ESTUDO DE CITOTOXICIDADE, ATIVIDADE ANTIBACTERIANA E COMPOSIÇÃO POR FT-IR E GC-MS PARA O EXTRATO ORGÂNICO DA TRUFA BRANCA *TIRMANIA NIVEA* DO DESERTO DO IRAQUE

STUDY CYTOTOXICITY, ANTIBACTERIAL ACTIVITY, AND COMPOSITIONS BY FT-IR AND GC-MS FOR ORGANIC EXTRACT OF WHITE IRAQI DESERT TRUFFLE *TIRMANIA NIVEA*

دراسة السمية الخلوية، الفعالية ضد بكتيرية، والمركبات بواسطة FT-IR و GC-MS للمستخلص العضوي من الكما الصراوية العراقية البيضاء *TIRMANIA NIVEA*

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RESUMO

O objetivo do presente trabalho foi explorar os potenciais componentes bioquímicos e atividades biológicas de um extrato orgânico da trufa branca *Tirmania nivea* coletada no deserto do Iraque, posteriormente testou-se a citotoxicidade do extrato orgânico contra células de carcinoma da laringe humana e cepas selecionadas de bactérias patogênicas. A espectroscopia no infravermelho com transformada de Fourier (FTIR) e a cromatografia gasosa acoplada a espectrometria de massa (GC/MSS) foram usadas para analisar as composições micoquímicas. A atividade antibacteriana, a concentração inibitória mínima (CIM), e a concentração bactericida mínima (MBC) foram investigadas pelo método do disco-difusão em ágar. O efeito da citotoxicidade do extrato de trufa contra a linha celular da laringe (Hep-2) foi avaliado pelo ensaio MTT (*in vitro*). Os resultados do FTIR domonstraram a presença de fenol, ácido carboxílico e grupo funcional do alceno. A análise GC-MS de *T. nivea* revela a existência de dezenove compostos que podem contribuir para as propriedades farmacêuticas da trufa. Quanto ao resultado da atividade antibacteriana, foi detectada uma atividade de inibição do crescimento do extrato de trufa em (zonas de inibição de 18-40 mm) contra as cepas bacterianas patogênicas testadas, cujos valores mínimos de concentração inibitória variaram de 3,12 a 6,25 mg/mL para *Escherichia coli* (ATCC 25922) e *Staphylococcus aureus* (ATCC 25923), respectivamente. Os resultados da citotoxicidade mostraram que o extrato orgânico de trufa exibiu uma alta taxa inibitória (52.685%) contra a linha celular (Hep-2) a uma concentração de 1,56 µg/mL. Neste trabalho, os resultados mostraram que os extratos orgânicos de *T. nivea* são muito promissores como citotoxicidade do câncer e agente antibacteriano para futuras aplicações médicas.

Palavras-chave: *Tirmania nivea*, Carcinoma da laringe, Teste MTT, Bactérias patogênicas, Propriedades farmacêuticas.

ABSTRACT

The purpose of the present work aimed at exploring the potential biochemical components and biological activities of an organic extract of the white truffle *Tirmania nivea* collected from the Iraqi desert, then test the organic extract against the Cytotoxicity on Human Larynx carcinoma cells and selected strains of pathogenic bacteria. Fourier transform infrared spectroscopy (FTIR) and gas chromatography-mass spectrometry GC/MSS were used to analyze mycochemical compositions. The antibacterial activity and Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was investigated using a disk diffusion agar method. The truffle extract's cytotoxicity effect against the larynx cell line (Hep-2) was assessed by the MTT assay (*in vitro*). FTIR results provided the presence of phenol, carboxylic acid, and

alkane's functional group, The GC-MS analysis of *T. nivea* disclose the existence of nineteen compounds that can contribute to the pharmaceutical properties of the truffle. As for antibacterial activity result, A growth inhibition activity of truffle extract at (18-40 mm inhibition zones) against the tested pathogenic bacterial strains was detected, which minimum inhibitory concentration values ranged from 3.12 to 6.25 mg/mL for *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) Respectively. The results of cytotoxicity shown that the organic truffle extract exhibited a high inhibitory rate (52.685%) against cell line (Hep-2) at a concentration of 1.56 µg/mL. In this work, the results showed that the organic extracts of *T. nivea* are very promising as cancer cytotoxicity and antibacterial agent for future medical applications.

Keywords: Tirmania nivea, Larynx carcinoma, MTT assay, Pathogenic bacteria, Pharmaceutical properties

الملخص

الغرض من العمل الحالي الذي يهدف إلى استكشاف إمكانية المكونات البيوكيميائية والفعاليات الحيوية للمستخلص العضوي من الكمأ الأبيض الذي تم جمعه من الصحراء العراقية، بعدد اختبار المستخلص العضوي ضد السمية الخلوية على خلايا سرطان الحنجرة البشري وسلالات مختارة من البكتيريا المسببة للأمراض. تم استخدام تحويل فورييه الطيفي بالأشعة تحت الحمراء (FTIR) واستشراب غازي-مطياف كتلة GC-MS لتحليل المكونات الكيميائية الفطرية. تم فحص الفعالية ضد بكتيرية والتركيز المثبط الأدنى (MIC) والتركيز القاتل للبكتيريا (MBC) باستخدام طريقة النشر بالأقراص. تم تقييم تأثير السمية الخلوية لمستخلص الكمأ على خط خلايا الحنجرة (Hep-2) بواسطة اختبار MTT (في المختبر). أثبتت نتائج FTIR وجود الفينول، حمض الكربوكسيل والمجموعة الوظيفية للألكان، تحليل GC-MS للكمأ كشف عن وجود تسعة عشر مركباً يمكن أن تساهم في الخصائص الصيدلانية للكمأ. أما بالنسبة لنتيجة الفعالية ضد بكتيرية، فقد تم الكشف عن فعالية تثبيط نمو مستخلص الكمأ عند (18-40 ملم منطقة التثبيط) ضد السلالات البكتيرية المرضية، والتي تراوحت قيم التركيز المثبط الأدنى من 3.12 إلى 6.25 ملغم / مل لـ الإشريكية القولونية سلالة (ATCC 25922) والعنقودية الذهبية سلالة (ATCC 25923) على التوالي. أظهرت نتائج السمية الخلوية أن مستخلص الكمأ العضوي أظهر معدل تثبيط مرتفع (52.685%) ضد خط الخلايا (Hep-2) بتركيز 1.56 ميكروغرام/مل. في هذا العمل، أظهرت النتائج أن المستخلصات العضوية من الكمأ الأبيض واعدة للغاية للتطبيقات الطبية في المستقبل باعتبارها تمتلك سمية للخلايا السرطانية وعامل مضاد للبكتيريا.

الكلمات المفتاحية: الكمأ الأبيض، سرطان الحنجرة، اختبار MTT، البكتيريا المسببة للأمراض، الخصائص الصيدلانية.

1. INTRODUCTION:

Over the past fifty years, many significant advances in medicine have come from the Kingdom of fungi. Fungi are distinct manufacturers of biologically active compounds with antibiotics, growth-regulating, toxic, mutagenic, and immunosuppressive, in addition to other biological properties (Gajos *et al.*, 2014; Manoharachary *et al.*, 2014). The first antibiotic was extracted from fungi, and the drug has been regarded as a wonder for infectious and communicable diseases. New developments in cancer treatment have recently increased in natural chemotherapy, which involves the use of adjuvants of biologically active compounds. In particular, fungi natural products may not only be used as potent immunosorbents, but it can also be used as a source of active metabolites, which penetrate the cell membranes and interfere with a specific pathway for signal transfer in processes like inflammation, cell differentiation, survival, carcinogenesis, and metastases (Liu *et al.*, 2016).

Mushroom is one of the most promising pharmaceutical applications fungi. Mushrooms and truffles are nutrient-dense, versatile foods with putative health benefits associated with fruit and vegetable consumption. China is the world leader in the production of mushrooms, followed

by the United States, Italy, the Netherlands, and Poland. Mushrooms are a rare source of ergothioneine, dietary fiber, selenium, minerals, and vitamins. The mushroom market is expected to rise because of nutritional and potential health benefits, including awareness, oral health, weight control, and reduced cancer risk. Mushrooms and truffles are also alleged to exert an immunomodulatory effect through interaction with the gut microbiota (Saritha *et al.*, 2016). Hall *et al.* (2003) describe mushrooms in a far more realistic portrait as "Every fungus has a Featured fruiting body, large enough to be picked" (Pérez-Moreno and Martínez-Reyes, 2014). The use of mushrooms in ancient eastern treatments is well known. Current researchers have confirmed and documented a significant part of the long time past data. The interdisciplinary research in medicinal mushrooms has been expanded through the last periods and demonstrated further the essential and distinct characteristics of compounds extracted from various species. The field is currently produced in an extremely productive region. Current clinical practice is dependent on mushroom extracts in Japan, China, Korea, and other Asian nations (Murat *et al.*, 2008; Elsayed *et al.*, 2014).

Desert truffle is a hypogene ascomycete obligatory for ectomycorrhizal mushrooms, formed in symbiotic relation to *Helianthemum's*

roots (Kagan-Zur and Roth-Bejerano 2008; Zdrojewicz *et al.*, 2016). It has been documented to be medicinal feeding stuff and was described as a natural miracle in ancient culture as Chinese, Greek, Egyptian and Mesopotamian civilizations (Bahrani 2006; Wang and Marcone, 2011; Badalyan 2012; Patel 2012). Truffles are one of the ancient foods; It was used as a meat replacement and consumed in large quantities because of its delicious flavor and its musky flavor (Al-Shabibi *et al.*, 1982; Mandeel and Al-Laith 2007; Dundar *et al.*, 2012). The scent of the white truffle is often identified as sulfurous or garlicky, and the presence of sulfur-containing VOCs is due to this kind of odor (Vita *et al.*, 2018). Furthermore, It was used in folk medicine for eye treatment. (Janakat and Nassar 2010; Brown *et al.*, 2016). Truffles are among the main ingredients of many favorite Middle East traditional dishes (AL-RUQAIE, 2006). Truffle contains high amounts of P, K, and fair levels of Ca, Mg, Na, Fe, Cu, Zn, and Mn (Hussain and Al-Ruqaie, 1999).

Tirmania nivea, the most known truffles in the world, are white desert truffle common names (zabidee), including Iraq, which is naturally found in the middle, south, and western areas (Gutiérrez *et al.*, 2003; Al-Laith, 2010; Stojković *et al.*, 2013; Vahdani *et al.*, 2017). After a rainy period (February, March, and April), truffles usually arise in the desert. It is periodically and socially and economically indispensable fungi. According to the quantity of rain and other environmental factors, truffle distribution, nutrition value, and therapeutic properties will differ in seasons (Owaid 2016).

Desert truffles include an unlimited but generally untapped source of new pharmaceutical products (Shavit and Shavit 2014). Truffle has a high nutritional value consisting of healthy fatty acids, vitamin supplements, protein, and minerals. There are a few truffle species reported in the genera *Terfezia*, *Tirmania*, and *Tuber* in chemical components as well as in therapeutic characteristics (Villares *et al.*, 2012; Akyüz, 2013), which is a novel exporter of anti-inflammatory, anticancer, and antioxidative compounds (Hannan *et al.*, 1989; Murcia *et al.*, 2002; Dahham *et al.*, 2015). In addition to the antiviral lectin cyanovirin-N (CVN) and fruiting body-specific lectin TBF-1 from *Tuber borchii* Vittad (Percudani *et al.*, 2005; Cerigini *et al.*, 2007).

According to our information, there is little data available on bio-compounds produced from

truffle *Tirmania*, especially Iraqi truffles. Current work has therefore been carried out to investigate the chemical compounds extracted from ascocarps of this fungus. Collected from the desert of Iraq and identified using Fourier transforms infrared spectroscopy (FT-IR) and gas chromatography-mass spectrometry (GC-MSS), tested for cytotoxicity in human larynx carcinoma cells and selected pathogenic bacterial strains.

2. MATERIALS AND METHODS

2.1 Truffles Collection

A sample of truffles locally known as "Alchama" used in the present study was collected from the Al-Salman region in the Iraqi desert during the growing season (February-April) associated with the host roots of *Helianthemum sp.* (Cistaceae), were identified based on the morphological and microscopic characters using standard methods (Alsheikh and Trappe 1983).

2.2 Preparation of Truffles

Ascocarps samples were cleaned from the soil in the laboratory, peeled, sliced, and dried at Shadow (7 days). Crushed the dried slices, and the fine powder was capped in well-labeled airtight containers and stored at room temperature (Al-Laith, 2010).

2.3 Preparation of truffle extract

20 g of truffle powder was soaked for three days in 0.5 L of methanol 400 ml and distilled water (type IV) 100 ml (4:1, v/ v) at room temperature, then filtered through Whitman filter paper No.1, evaporated, redissolved in H₂O, and pH=3 by HCl (2N), separating funnel was used to extract filtrate three times with ethyl acetate (150 mL). The organic composition was obtained and dehydrated in Petri dishes using Na₂SO₄ at room temperature (Berger *et al.*, 2004).

2.4 Antibacterial bioactivity assay

2.4.1 The microorganisms test

Five bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumonia*, and *Salmonella typhi*) were isolated from the clinical cases in Misan, South Iraq and have been used two standard bacterial strains; *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC

25923) for comparison. All samples were cultivated on maintenance media until use (Hateet, 2015).

2.4.2 Discs diffusion agar method

To evaluate the antibacterial activity of the truffle crude extract, disc diffusion agar assay was employed in standard ways (Casals, 1979). Dissolved in 1 mL dimethyl sulfoxide (DMSO) solvent, 2.5 mg of the dried truffle extract from Whitman filter paper, sterilized Discs with a diameter of 0.6 mm was soaked in the fungal extract. Placed on Petri dishes containing medium Muller-Hinton Agar (MHA), inoculated by streaking process of 0.1 mL suspension of bacterial strains. Incubation of bacterial cultures at 37 °C.

2.4.3 Determination of MIC and MBC

A standard test using serial dilutions of truffle extract (100, 50, 25, 12, 6.5, 3.13, 1.56, 0.78, 0.39, 0.2, 0.1, 0.05, 0.025 µg/L) has determined values of MIC. In this assay, the lowest levels indicated that the MIC values inhibit bacterial growth. The low bactericidal (MBC) was registered as the lowest bacterial-killing concentration, and no visible bacterial growth was found (McGinnis 2012).

2.5 Cytotoxicity efficacy of the truffle extract

The potential cytotoxicity of truffle extract using cell line (Hep-2) was tested by MTT (Ibrahim *et al.*, 2011). Briefly, the Hep-2 cell line was cultivated on a specified medium enhance with antibiotics and cells grown in CO₂ and humidity conditions at a temperature of 37 °C. The cell line (Hep-2) was seeded in 96 well tissue plates in quantities of (1 to 105 cell/well). Preparation of different truffle extract concentrations ranging from (0.05-100 µg/mL) and modified to cell lines in the wells and incubated at 37 °C for 24 hours. Non-treated cells were used as control. After the incubation time, the cells were rinsed with PBS, and the viability of the cells was determined. To measurement, the absorption at 550 nm, a spectrometer (APEL PD-303, Japan) was used, and the cell viability percentage was calculated as equation no. 1 (Eq.1). IC₅₀ was identified as the concentration of truffle extract that kills 50% of cells.

2.6 Detection of Chemical Compositions

2.6.1 Fourier transforms infrared spectroscopy (FT-IR) technique

FTIR analytics were performed using the Shimadzu UV-1700 Spectrophotometer system to detect functional groups in organic truffle extracts. For the production of translucent disks, the 5 mg truffle extract was encapsulated into 100 mg of pellet (KBr) potassium bromide, which was then inserted into the sample cup of an accessory for diffuse reflection. The peak values of the FTIR were recorded, and the scanning range was set at 4 cm⁻¹ resolution from 400 - 4000 cm (Zhao *et al.*, 2006).

2.6.2 Gas Chromatography-Mass Spectrometry

The organic extract extracted from truffle *Tirmania nivea* was analyzed using GC SHIMADZU QP2010 Ultra and gas chromatography, interfaced with a Mass Spectrometer (GC-MS) equipped with the DbB5ms capillary column. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adapted to handle mass spectra, and chromatograms were a Turbomass. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST), having more than 62,000 patterns. The frequency of the unknown component was compared with the range of the known elements stored in the NIST library. The Name, Molecular weight, and structure of the Ingredients of the test materials were ascertained (Pacioni *et al.*, 2014).

3. RESULTS AND DISCUSSION

3.1 Truffles Collection, Preparation of the extract

The truffles ordinarily found in Iraq's desert (Ewaze and Al-Naama, 1989; Owaid 2016). Truffle's ascocarps are usually hypogynous, with the basal attachment, such as Tuber. All truffles have been identified as white *Tirmania nivea* (Zabidi) (Pezizaceae), with microscopic characteristics, musk smell, and flavored lightness, as shown in (figure 1). The truffle separate from the root of the host plant *Helianthemum* sp. (Cistaceae), the majority of plant species supporting desert truffle mycorrhizas belong to the genus *Helianthemum* (Cistaceae) (Kagan-Zur and Akyuz, 2014).

3.2 Antibacterial activity of the truffle extract

In the present research, the antibacterial organic extract activity from truffle *T. nivea* was determined using Discs diffusion agar method. The extract of *T. nivea* has antibacterial activity in all the examined pathogenic bacteria in the occurrence or absence of inhibitory zones and zone diameter (Figure 2) (Table 1). The extract of *T. nivea* antibacterial inhibitory activity was measured against *Salmonella typhi* (40 mm), *Klebsiella pneumonia* (35 mm), *Streptococcus pyogenes* (27 mm), *E. coli* (26 mm), *Staph. aureus* (NCTC6571) (25 mm), *E. coli* (ATCC25922) (24 mm), and *Staph. aureus* (18 mm) (Table 1). In contrast to the two standard bacterial strains, *E. coli* (ATCC25922) and *Staph. aureus* (NCTC6571), the values of MIC and MBC were very shallow (Table 2). Previous works confirmed the results of this research, the antimicrobial effects of different extracts from *Terfezia sp.* and *Tirmania sp.* revealed their good antimicrobial activity on different bacterial species (Fortas and Belahouel-Dieb, 2007; Neggaz and Fortas 2013; Neggaz *et al.*, 2018; Hamza *et al.*, 2016). Dib-Bellahouel and Fortas (2011) reported a potent antibacterial activity against *B. subtilis* and *Staph. aureus* with the ethyl acetate extract from *Tirmania pinoyi*. In a further study conducted by Gouzi *et al.* (2011), *Tirmania nivea* aquatic extract has antibacterial efficacy at *Staph. aureus*, *P. aeruginosa*. More recent studies indicate that *Tirmania* truffle methanol extract has higher inhibitory activity than ethyl acetate and aqueous extracts of both gram bacteria (Janakat *et al.*, 2005). However, the results obtained by using organic extract are similar or more effective than this literature. Also, the *Tirmania*-AgNPs had a promising antibacterial activity against bacteria, which consider the main infectious for the eyes *Pseudomonas aeruginosa* bacteria and gram-positive (*Staphylococcus aureus*) bacteria (Owaid *et al.*, 2018).

3.3 Cytotoxicity efficacy of the truffle extract

The *in vitro* cytotoxicity of the organic truffle extract *T. nivea* was tested at various concentrations against the cell line (Hep-2). The results indicated that the truffle *T. nivea* extract inhibition concentration (IC₅₀) of 50% of the mortality of cells was 1.56 µg/mL (Figure 3). Cytotoxicity has, however, been increased at higher truffle extract concentrations. These data suggest that *T. nivea* crude organic compound was toxic onto the cell line Hep-2. Truffle's cytotoxic activity might be clinically significant,

and further anticancer properties must be investigated. The results are similar to those observed in the American National Cancer Institute (NCI) crude extract cytotoxicity activity, which is IC₅₀ < 30 µg/mL⁻¹. Mekawey (2015) shown cytotoxicity of the various *Terfezia boudieri* extracts in Egypt on six tumour cell lines. The study showed that aqueous extract is highly toxic to four cancer lines HEP2, HCT116, MCF7, and CACO2. Dahham *et al.* (2018) reported that *Terfezia claveryi* hexane extract from the Iraq truffle showed IC₅₀ value was 50.3 µg/mL⁻¹, followed by a 72.6 µg/mL⁻¹ ethyl acetate extract from brain cell (U-87) line. Also, the cytotoxicity of ethanol and methanol extracts against U-87 MG was moderate, with IC₅₀ values of 109.25 ± 9.51 and 136.2 ± 7.4 mg/mL, respectively.

3.4 Fourier transforms infrared spectroscopy (FT-IR) technique

The organic extract from truffle *T. nivea* was passed through the FTIR in the range of 400-4000 cm⁻¹. The FT-IR peaks and functional groups in Table 3. The truffle extract showed the presence of broadband at about 3346 cm⁻¹, at 2925 cm⁻¹ and 2854 cm⁻¹ sharp bands; two large groups at about 1660 cm⁻¹ and 1595 cm⁻¹; band at about 1743 cm⁻¹, 1411 cm⁻¹, and 1039 cm⁻¹; at 991 cm⁻¹ and 804 cm⁻¹ reflect the bending vibrations of the hydroxyl bond, fatty acids, amide I and amide II protein, alkyl esters and carbohydrates respectively (Figure 4). The study showed that crude extracts of *T. nivea* have the properties of antimicrobials and antitumor and have phenol, carboxylic acid, and alkanes, functional group.

3.5 Gas Chromatography-Mass Spectrometry

GC - MS analysis showed nineteen chemical components that may relate to and participate in the truffle's pharmaceutical properties. Identify of chemical compounds with their molecular formula, retention time (RT), molecular weight (MW), and peak area value (%) is shown in Table 4 and Figure 5. Results show that methyl ester, (Z) - 13-Docosenoic acid (Erucic Acid) (19.82%), Octadecanoic acid, Stearic acid, methyl ester (15.18%), Oleic acid ester (12.94%), Palmitic acid, methyl ester (11.53%), Methyl 9,10-methylene- octadecanoic (7.04 %), 8-Octadecenoic acid, methyl ester (5.14%) were found as the major components in the methanol extract and the other minor components such as 1,3-Dioxane-2-propanol, 2-methyl-alcohol

(0.87%), isosorbide (Glucitol) (2.95%), N -[(Penta fluorophenyl, Benzeneethanamine Aromatic Compound (0.94%), 4,4'-isopropylidene di-Phenol (Biphenol A) Aromatic alcohol (4.29%), methyl ester, (Z) - 15-Tetracosenoic acid (Methyl neonate) (1.35%), I - (+) - Ascorbic acid 2,6- di hex decanoate (1.72%). Methyl ester has been reported to serve as antibacterial effects, anti-inflammatory, hypocholesterolemic, preventive, hepatoprotective, and antihistaminic properties of palmitic acid, 9,12,15-octadecatrienoic acid, and hexadecanoic acid (Kumar *et al.* 2011). Furthermore, Palmitic acid also had significant cytotoxicity against breast MCF-7, liver cancer WRL-68, colon cancer CaCO₂, Colo-320 DM, and hepatoprotection against galactosamine. Further research by Carrillo and Alonso-Torre (2012) revealed the antitumor and anti-proliferative impact of oleic acid on prostate cancer PC3.

Nevertheless, Hayshi *et al.* (1998) stated that octadecadienoic acid is also antitumoral. Recently, Wang and Marconi (2011) recently concluded in a study that hexadecanoic acid is an essential compound discovered in truffle tuber that has a smell of a truffle. As a result of the above points, the cytotoxicity and antibacterial activity of *T. nivea* can be speculated due to the presence and synergistic effect of these major chemical constituents.

4. CONCLUSIONS

This study revealed the antibacterial properties and cytotoxicity of organic extract *Tirmania nivea*, a wild truffle from the Al-Salman area of the Iraqi desert. Besides, phenolic, fatty acids, triterpenes, and vitamins have been found in truffles, which may be responsible for the antimicrobial and cytotoxic activity reported in this research. The study results showed that the crude extract exhibited inhibitory activity against all pathogenic bacteria and cytotoxicity effects against the cell line (Hep-2), and based on the results of current research, the truffle extract can be a useful source of antibacterial and cytotoxicity drugs. However, it is essential to further investigate the purification and identification of white desert truffle bioactive compounds to explore in more detail potential pharmaceutical effects.

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$$\text{Viability of cells \%} = \frac{\text{absorption of samples}}{\text{absorption of control}} * 100 \quad (\text{Eq.1})$$

Figure 1. Ascocarps of *Tirmania nivea* (Zabidi)

Figure 2. In vitro, the antibacterial activity of organic extracts of desert truffle at concentration 25 µg/discs. (A) *E. coli* (ATCC25922) (B) *Staph. aureus* (NCTC6571) (C) *E. coli* (D) *Staph. aureus* (E) *Streptococcus pyogenes* (F) *Salmonella typhi* (G) *Klebsiella pneumonia*.

Figure 3. In vitro, the cytotoxicity efficacy of the organic truffle extract *Tirmania nivea* against Human larynx carcinoma cell-line (Hep-2).

Figure 4. FTIR analysis of organic extracts of desert truffle *Tirmania nivea*.

Figure 5. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of an organic extract of desert truffle *Tirmania nivea*.

Table 1. The antibacterial activity of organic extract of desert truffle *Tirmania nivea*

Tests bacteria	Inhibition zone
<i>Escherichia coli</i> (ATCC25922)	24*
<i>Staphylococcus aureus</i> (NCTC6571)	25
<i>Escherichia coli</i>	26
<i>Staphylococcus aureus</i>	18**
<i>Streptococcus pyogenes</i>	27
<i>Salmonella typhi</i>	40**
<i>Klebsiella pneumoniae</i>	35**

* Values represent means of four replicates

** Significant difference at $P \leq 0.001$

Table 2. MIC values of the organic extract of desert truffle *Tirmania nivea* ($\mu\text{g/mL}$).

Bacteria	MIC ($\mu\text{g/ml}$)	MBC($\mu\text{g/ml}$)
<i>Escherichia coli</i> (ATCC25922)	3.12	6.25
<i>Staphylococcus aureus</i> (NCTC6571)	6.25	12.5

Table 3. FTIR peak values and functional groups of organic extracts of desert truffle *Tirmania nivea*

Peak Values λ (nm)	Functional group
3346.27	O-H stretch
2925.81	CH ₂ stretch asymmetric
2854.45	CH ₂ stretch symmetric
1743.53	C=O stretch
1660	C=C aliphatic
1595.02	C=C, C=N aromatic
1411.80	C-O, C-N
1149.50	C-N ₃ binding
1039.56	C-F
991.34, 941.20	C-H aromatic
804.26	C-H aliphatic

Table 4. Total ion chromatogram (GC-MS) of crude extracts of desert truffle *Tirmania nivea*

S. No.	RT	Name of the compound	Molecular formula	Molecular weight	Compound nature	Peak area%
1	5.310	1,3-Dioxane-2-propanol, 2 methyl-	C ₈ H ₁₆ O ₃	160	alcohol	0.87
2	6.416	Isosorbide (Glucitol)	C ₆ H ₁₀	146	Diol	2.95
3	7.126	dodecamethyl-Cyclohexasiloxane	C ₁₂ H ₃₆ O ₆ Si ₆	444	Cyclic silyl ether	1.50
4	10.291	N-[(pentafluorophenyl, Benzeneethanamine	C ₂₄ H ₃₄ F ₅ No ₃	563	Aryl amine	0.94
5	11.276	Methyl tetra decanoate (Myristic acid ester)	C ₁₅ H ₃₀ O ₂	242	Saturated fatty acid ester	1.09
6	13.690	Hexadecanoic acid, methyl ester (Palmitic acid, methyl ester)	C ₁₇ H ₃₄ O ₂	270	Saturated fatty acid ester	11.53
7	14.108	L-(+)-Ascorbic acid 2,6-dihexadecanoate	C ₃₈ H ₆₈ O ₈	652	Ascorbic acid ester	1.72
8	15.617	Methyl 10-trans,12-cis octadecadienoate	C ₁₉ H ₃₄ O ₂	294	Unsaturated fatty acid ester	3.55
9	15.700	9-Octadecenoic acid (Z)- (Oleic acid ester)	C ₁₉ H ₃₆ O ₂	296	Unsaturated fatty acid ester	12.94
10	15.777	8-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296	Unsaturated fatty acid ester	5.14
11	16.013	Octadecanoic acid, methyl ester (Stearic acid, methyl ester)	C ₁₉ H ₃₈ O ₂	298	saturated fatty acid ester	15.18
12	16.534	4,4'-isopropylidenedi-Phenol (Biphenol A)	C ₁₅ H ₁₆ O ₂	228	Phenol	4.29
13	17.891	Methyl 11-eicosenoate	C ₂₁ H ₄₀ O ₂	324	Unsaturated fatty acid ester	1.45
14	19.745	hexadecyl- Oxirane (1,2-Epoxyoctadecane)	C ₁₈ H ₃₆ O	268	Epoxy Compound	4.40
15	19.932	methyl ester, (Z)- 13-Docosenoic acid (Erucic acid)	C ₂₃ H ₄₄ O ₂	352	Unsaturated fatty acid	19.82
16	20.002	Methyl 9,10-methylene-octadecanoate	C ₂₀ H ₃₈ O ₂	310	Unsaturated fatty acid ester	7.04
17	20.197	Methyl 20-methyl heneicosanoate(Behenate)	C ₂₃ H ₄₆ O ₂	354	saturated fatty acid ester	1.87
18	21.839	methyl ester, (Z)- 15-Tetracosenoic acid (Methyl nervonate)	C ₂₅ H ₄₈ O ₂	380	Unsaturated fatty acid ester	1.35
19	22.074	Tetracosanoic acid, methyl ester (Lignoceric acid methyl ester)	C ₂₅ H ₅₀ O ₂	382	saturated fatty acid ester	1.15