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Antioxidant activity, metal content, and essential oil composition of two desert truffles species: *Terfezia boudieri* and *Terfezia claveryi*

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Abstract
Terfezia species have been shown to be nutrient-dense and can be used as functional meals for human nutrition. They outperformed other diets in terms of iron, potassium, copper, and zinc. The physical features, chemical composition, and mineral content of two Terfezia species were studied. The antioxidant activity of the aqueous extract was further evaluated by assessing its radical scavenging capacity against 2, 2'-diphenyl-1-picrylhydrazyl. Terfezia samples were chemically evaluated using GC-MS to establish if there was a link between antioxidant activity and polyphenol content and quantity. *Terfezia claveryi* and *Terfezia boudieri* samples were shown to have similar polyphenolic compositions and, as a result, virtually identical antioxidant activity. The results were compared to Terfezia species found in other places. *Terfezia claveryi* essential oil had more than fifty volatiles, whereas *Terfezia boudieri* essential oil contained more than eighty. The scientific findings of this study may be useful for marketing, the food industry, human nutrition, and maximum usage, particularly in Terfezia-growing areas.

Keywords: Wild edible truffles, *Terfezia claveryi*, *Terfezia boudieri*, radical scavenging, metal content, essential Oil, GC-MS analysis

1. Introduction
Terfezia truffles are hypogeous fruits produced by macrofungi of the Ascomycetes class [1]. Mycorrhizal linkages between truffles and the roots of numerous Cistaceae plants, including Helianthemum, have been demonstrated [2, 3]. This link may play an important role in the maintenance of Mediterranean shrub lands and xerophytic grasslands, as well as in preventing erosion and desertification [4]. Terfezia truffles are edible foods that grow naturally following the rainy season in the Mediterranean region, the Arabian Peninsula, and North Africa, as well as, some areas of South Africa and China [5, 6]. The edible component of the truffle is made up of an ascoma, which grows underground and gradually emerges above ground, causing significant bulging and eventually cracking of the surface soil. The mature ascoma may be easily separated from the basal section (nombril), which is made up of several branching hyphae that surrounds the host root [7].
Terfezia truffles have traditionally been used in folk medicine for the prevention and treatment of various eye disorders, infections, inflammations, and allergies [8]. They are, nevertheless, abundant sources of unexploited active compounds with antioxidant, anticancer, antibacterial, anti-inflammatory, and other therapeutic characteristics, comparable to other fungus [9, 10]. Desert truffle extracts have been used by Bedouins in the desert to treat diseases of the face (alopecia), eyes, diabetes, and as an aphrodisiac [11]. In the 11th century, Ibn Sina (Avicenna), a great Persian scientist, recommended truffles for weakness, vomiting, and wound healing [12]. In Islamic literature, the prophet Muhammed attests to the therapeutic efficacy of desert truffle extracts in the treatment of ocular diseases, citing in one of his sayings (hadiths): “Truffles are a form of manna, and its liquid is a cure for the eye” [13].
Wild edible truffles not only give taste to dishes, but they are also foods in their own right. Due to its nutritious and volatile composition, it might be an ideal product to employ in the context of the "plant based meat" concept if manufactured on a bigger scale and at cheaper prices. Truffles are well recognized in the Middle East for their nutritional value and are used as a meat alternative in diets [6, 14]. This might be explained by their high fiber, carbohydrate, fat, protein, vitamin, and mineral content. The lipids of desert truffles have a high concentration of essential, saturated, and unsaturated fatty acids. Linoleic acid (C 18:2) is prevalent in *T.claveryi* and *Picoa juniper* [15].

The fatty acids linoleic (C 18:2), oleic (C 18:1), stearic (C 18:0), and palmitic (C 16:0) have been identified in truffles from Europe and Africa [16]. Brassicasterol is the most abundant sterol in Terfezia (85 % of total sterols), while ergosterol is the most prevalent in Tuber [17, 18, 19] detected comparable components in distinct truffle species. Truffles are valued for their high protein content; they contain 20% protein by dry weight, and humans can digest 85% of it. This content is substantially greater in comparison to other edible mushrooms. About 250g of Terfezia contains 23-27% of the daily protein requirements and 16-22% of the daily fiber requirements [20]. Protein quality is determined by the quantity of essential amino acids present. Desert truffles contain both essential (lysine, threonine, leucine, phenylalanine, tryptophan, isoleucine, methionine, and valine) and non-essential (serine, cystine, arginine, proline, histidine, etc.) amino acids [21].

According to mineral analysis, truffles have a high potassium, iron, and phosphorus content (especially in *Tuber melanosporum* and *T. uncinatum*). Calcium, magnesium, and sodium levels are all low. *T. boudieri* contains a high concentration of iron and zinc, two microelements involved in the synthesis of metalloenzymes [22].

The underground truffles would have stayed hidden if not for the scents they emit when they reach maturity, firmly impregnating the area around them. The exquisite aromas of particular truffle species, such as *T. magnatum*, *T. melanosporum*, and *T. uncinatum*, have long captivated gourmets. Aromatic chemicals emerge in the fruiting body when the fungus development ends, during spore maturation [23]. Various writers have extensively researched the volatile components of tubers [24-26]. The scent of the *Périogord* black truffle, *Tuber melanosporum*, comprises more than fifty chemicals (aldehydes, alcohols, esters, sulfur compounds), aromatic volatile molecules, particularly methyl-2-butanol and dimethyl-sulphide, which truffle dogs identify. Caryophyllene, aldehydes, ketones, esters, acids, alkanes, ethers, heterocycles, amides, and unsaturated fatty acids were among the many volatile chemicals discovered [27].

The generation of free radicals is a well-known consequence of unregulated metabolic oxygen synthesis. Excessive synthesis damages several active molecules such as proteins, lipids, and carbohydrates. In addition, DNA and membranes would be badly damaged [28]. Oxidative enzymes and many antioxidant substances, on the other hand, can cause protection against free radicals. Antioxidants include phenols, flavonoids, sesquiterpenes, ascorbic acid, tocopherols, and a wide range of other naturally occurring compounds [29].

Antioxidant foods or dietary antioxidants are thought to keep the body healthy and guard against illnesses by lowering oxidative stress and free radical damage. Antioxidant-based formulations are increasingly in demand for the prevention and treatment of complicated illnesses such as atherosclerosis, stroke, diabetes, Alzheimer's disease, Parkinson's disease, cancer, and others. *Terfezia claveryi* and *Picoa lefebvrei*, according to [9], can block the oxidation of food lipids more than the antioxidants used in industry for food preservation such as α-tocophenol; BHA (E-320), BHT (E-321) and propyl gallate (E-310). This feature is preserved even after industrial treatments (cooling, canning).

T. nivea, which is endemic to various Middle Eastern nations, also has antioxidant/radical scavenging potential [30]. These two antioxidant/antiradical qualities would be connected because lipid oxidation produces free radicals, which interact with vitamins, particularly vitamin E, and lower the nutritional value of desert truffles [31].

The study of antioxidant effect of Terfezia truffles, and phytochemical investigation of their active ingredients in addition to their mineral contents will be of great interest to attest their protective role in reducing oxidative damage. Among the many edible desert truffle varieties, our research focuses on two species of dark brown color truffles from the genus Terfezia, locally known as Ikhlasi (*Terfezia claveryi* and *Terfezia boudieri*) grown in Syria, and their chemical contents, particularly metals and volatile oils that may have biological activities. In addition, the antioxidant activity of both species aqueous extracts was investigated in vitro.

2. Materials and Methods

2.1. Truffles Source and Identification

Terfezia claveryi and *Terfezia boudieri* fruiting bodies were selected for analysis. Syrian ones were purchased for analysis in February 2017 and frozen at -3 °C. The two species were identified taxonomically by Prof. George Tohme, the professor of Taxonomy, and the previous president of the National Council for Scientific Research (CNRS). Dried voucher specimens were deposited at the Faculty herbarium museum.

2.1.1. Chemicals

Products of Sigma Aldrich Company-India were used. All chemicals used were of high analytical grade.

2.2. Methods

2.2.1. Preparation of Extracts

Approximately 500 g of each Truffle's fruiting bodies were washed and homogenized with water (1:3 w/v). After using a household blender, the homogenate was kept in the refrigerator. Filtration through cotton cloth was used to remove the main debris, and the filtrates of both species were centrifuged at 5,000 rpm for 15 minutes. The "aqueous extracts" from the supernatants were concentrated using a rotary evaporator at 50°C and low pressure. These extracts were kept at 4°C in the refrigerator. After extracting 100 g of each residue with acetone and butanol in a 50:50 ratio, the semi-solid extract was filtered and the filtrate was kept at 4°C for further processing [8, 32].

2.2.2. Chemical Analysis

2.2.2.1. Scavenging of DPPH radicals

In our study, the manifestation of the in vitro antioxidant activity of the phenolic compounds in *Terfezia claveryi* and *boudieri* was carried out by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical trapping method. DPPH theis a stable free radical, purple in solution and exhibiting characteristic absorbance at 517 nm. This color quickly disappears when DPPH is reduced to diphenyl picryl hydrazine in the presence of an antioxidant which gives hydrogen or an electron, thus causing discoloration due to formation of the non-radical form DPPH-H (Fig. 1), [29, 33].

A 300 µl of polyphenolic aqueous extracts (0.02, 0.08, 0.16, 0.3 mg/ml) were added to 2700 µl of a methanolic DPPH (0.002% w/v) solution. The absorbance was measured at 517 nm against a blank after 30 minutes of incubation in the dark at room temperature. A solution of a standard antioxidant, Quercetin, was used as a control positive, and its absorbance was measured in the same way as the sample. The following formula was used to compute the percent inhibition (% I) of the DPPH radical:

(%I)= [(Acontrol-Asample)/Acontrol]x100
Acontrol: Absorbance of the DPPH solution alone
Asample: Absorbance of the sample

2.2.2.2. Metal content
The samples were cleaned without washing, cut into slices, air-dried for several days, dried in an oven at 105˚C overnight, then grinded and stored at room temperature. Metal concentrations were calculated in mg/100g dry weight. All the glassware was kept overnight in 10% nitric acid solution, rinsed with deionized water and air dried before use.
A sample containing 0.5 g of the crushed material were transferred to 150 ml beakers, and digested. In the fume hood, 10 ml of conc. HNO_3 and 2 ml 30% H_2O_2 were added to each beaker, mixed by swirling, covered with a watch glass and kept overnight. Each beaker was placed on a hot plate and the sample heated to 120˚C for 20 minutes then cooled to room temperature. After cooling, the volume was completed to 250 ml with deionized water and filtered. The element contents were determined using flame photometer (Na, K and Ca content) and atomic absorption spectrometer (Fe, Zn, Cu, Cd, Ni, Cr and Pb).

2.2.2.3. Isolation of essential oil:
The essential oils of a 300g sample of each species were recovered by hydrodistillation for 4 hours in a Clevenger type device. The collector solvent utilized was n-hexane (2.5 ml). After evaporation, the oils were dried over anhydrous sodium sulfate and kept in the refrigerator in sealed vials shielded from light until GC and GC/MS analysis.

2.2.2.4. Gas chromatography/mass spectrometry
Agilent technologies 6890N gas chromatography system interfaced to a 5975B mass spectrometer was used for analysis of the samples. The separation was performed on a 30cm x 0.25 i.d. narrow bore silica capillary column coated with 0.25 µm film HP-5MS. The injector and the detector temperatures were set at 250 and 280°C, respectively. The oven temperature was held at 30 °C for 1 min, and programmed from 30 to 120°C at 4°C min^{-1} then to 240°C at 6°C min^{-1} then to 270 °C at 6°C min^{-1} and finally maintained at 280°C for 2 min. Split injection was conducted with a split ratio of 2:1. Helium was used as carrier gas, and flow-rate was 14.3ml.min^{-1}. The mass spectra were recorded over a range of 50-550 amu at 0.345 $scan^{-1}$. The inlet and ionization source temperature were 270°C. The relative percentage amounts of separated compounds were calculated from total ion chromatogram by a computerized integrator.

2.2.3. Statistical Analysis
All values were presented as means ± S.E.M. Statistical analysis was performed using the "SSPS" statistic computer software. A difference in the mean values of $p<0.05$ or less was regarded to be statistically significant.

3. Results and Discussion
In this work, the antioxidant and metal levels were determined in solvent extracts from *T. claveryi* and *T. boudieri*. Also the content of essential oils of both species was analyzed. In addition, GC-MS analysis was utilized to establish the extracts primary chemical composition.

3.1 Antioxidant Activity
The DPPH free radical trapping technique was used in our work to demonstrate the in vitro antioxidant activity of the phenolic components in *Terfezia claveryi* and *Terfezia boudieri*.

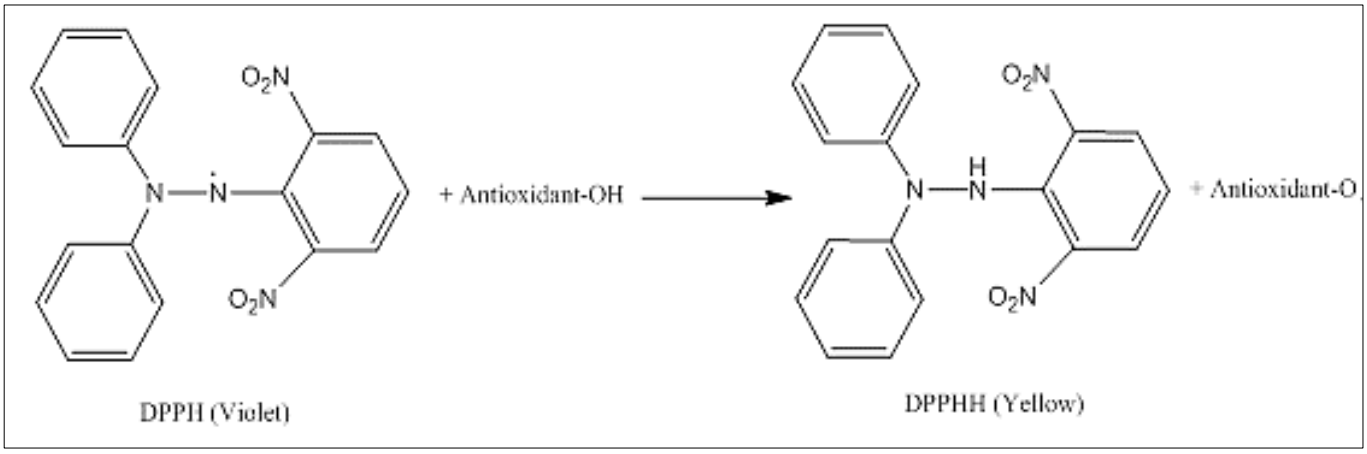


Fig 1: Reaction mechanism of an antioxidant with DPPH

The antioxidant capacity of the aqueous extracts was measured using the IC50, which reflects the concentration of extracts that neutralizes 50% of the DPPH radicals. The IC50 was computed using the equations of the linear regression between percentage change curve inhibition (%I) and extract concentrations. The lower the IC50 value, the stronger the antioxidant activity of a substance. The findings are an average of three experiments (Table 1).

Table 1: The antioxidant capacity of Quercetin reference and aqueous Terfezia extracts expressed as IC50 standard deviation (in mg/mL).

IC50 mean±SD (mg/mL)	
Aqueous Extract *T. claveryi*	0.69±0.002
Aqueous Extract *T. Boudieri*	0.68±0.001
Quercetin	0.012±0.001

Extracts of *T. claveryi* and *T. boudieri* have been shown to exhibit significant levels of radical scavenging activity. In this investigation, both species aqueous extracts demonstrated almost equal DPPH radical scavenging activity, with IC50 values of 0.69 mg/mL and 0.68 mg/mL, respectively, whereas the positive control quercetin had an IC50 value of 0.012 mg/mL. Aqueous extracts of *Terfezia claveryi* from Iraq, on the other hand, had a significantly lower IC50 of 0.058 mg/mL [34], whereas *T. boudieri* truffle from Tunisia had a higher IC50 for the macerate of 1.20 mg/mL [35], and *Terfezia claveryi Chatin* ascocarps macerate from Algeria [36] had a much higher IC50 of 22.16 mg/mL.

Endogenous machinery in cells neutralizes Reactive Oxygen species (ROS) and prevents their buildup. At larger quantities, however, ROS disrupts several life processes by affecting cellular macromolecules. The accumulation of intracellular ROS is thought to be one of the fundamental drivers of oncogenic transformation and cancer growth, and it also plays a role in aging-related cardiovascular and neurological illnesses, as well as many metabolic diseases [37]. Antioxidants are divided into two categories: natural and synthetic. Natural antioxidants have sparked renewed interest in the development of novel ways to safeguard cellular processes and minimize excessive oxidative stress in recent years. Epidemiological studies have shown that consuming more natural antioxidants (vitamins, carotenoids, and phenolics) in one's daily diet helps protect against cardiovascular disease, cataract, cancer, and aging-related illnesses [38]. Because a variety of plants are high in antioxidants and micronutrients, dietary antioxidant supplementation is believed to protect against the oxidative stress that leads to disease development. However, the most often employed synthetic antioxidants to preserve food are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butyl hydroquinone (TBHQ). These synthetic antioxidants, which are commonly used in the food business, are inexpensive, efficient, pure, readily accessible, and safe if used at the legal quantities. A few studies, on the other hand, have discovered that the chemical compounds utilized as synthetic antioxidants may be contributors to various health issues including urticaria, eczema, angioedema, and increased levels of cholesterol in blood at high levels of ingestion [39]. As a result of these concerns, various researches have been conducted to establish an alternative industry strategy that might replace synthetic antioxidants or, at the very least, reduce their use as food additives. One of the solutions is to include natural antioxidant sources into food items. This has led to a rise in market demand for natural antioxidants found in plants [40, 41]. The truffle antioxidant research will provide new information on the potential health benefits of a truffle-rich diet. Furthermore, antioxidant components can be created in fruit bodies, mycelium, and culture, isolated, and included into a meal regimen to protect our bodies from ROS agents.

3.2. Mineral Content

Iron concentrations were identified at significant levels among the micro-elements of the two truffles when compared to other trace elements examined. *Terfezia claveryi* and *Terfezia boudieri* had iron content of 125.88 and 150.63 mg/100 g dry weight, respectively (Table 2). In both species, the additional elements were zinc and copper in decreasing order. *Terfezia boudieri* exhibited greater iron levels, although *Terfezia claveryi* had higher zinc and copper levels. Our findings are more or less consistent with the ranges reported by previous researchers [42, 43]. Toxic elements including lead, chromium, nickel, and cadmium were also tested, but quantities were below the detection limit of the method employed (Table 2).

Table 2. Mean metal content of *T.claveryi* and *T. boudieri* (mg/100g dry weight ± standard deviation)

Metal	*T. claveryi*	*T. boudieri*
Na	645.34±10.19	600.57±8.45
K	3135.12±22.04	2705.73±36.51
Ca	50.42±1.04	250.14±3.99
Fe	125.88±3.39	150.63±3.84
Zn	13.65±0.27	11.51±0.18
Cu	11.52±0.19	5.75±0.11
Cd	0	0
Ni	0	0
Cr	0	0
Pb	0	0

Cd, Ni, Cr, and Pb were all analysed but not detected (the concentrations were under the detection limit of the method used).

Minerals are necessary for the normal functioning of living organisms. They are structural components of numerous proteins and enzymes, constituents of various bioactive compounds, nerve transmission regulators, and can assist in muscle contraction, osmotic pressure, and water-salt balance in the human body [44]. Mineral deficiency is a widespread issue in both poor and developed countries. Micronutrient deficiencies impact over two billion people worldwide, resulting in poor health, a high death and morbidity rate, and a high incidence of morbidity. Over 60% of the world's population is iron deficient, 30% is zinc deficient, and 15% is selenium poor; magnesium, calcium, and copper deficiencies are also frequent in certain developing nations [45, 46]. Minerals are categorized as macro-elements, which require more than 100 mg/day, and micro-elements, which require less than 100 mg/day. The macro-elements include potassium, sodium, magnesium, calcium, nitrogen, and phosphorus, while the micro-elements are manganese, copper, iron, zinc, cobalt, and selenium, among others. The macro-elements are structural and functional components of cellular and basal metabolism, as well as in the maintenance of acid-base balance in organisms. Trace or micro-element elements help important biological reactions by acting as cofactors for numerous enzymes and as centres for enzyme and protein structure stabilization [47]. However, due to rapid industrialization, many truffles become contaminated with heavy metals such as cadmium, chromium, and lead, which can have profoundly negative effects on human health including kidney problems, neurobehavioral and developmental disorders, high blood pressure, and even lung cancer [48]. Excess micronutrient consumption is also hazardous; for example, excessive zinc consumption might reduce copper and iron absorption. Excess copper also causes toxicity in humans because it produces reactive oxygen species such as superoxide, hydrogen peroxide, and the hydroxyl radical that might damage proteins, lipids, and DNA [49]. As a result, it is critical to investigate the presence of such minerals in each dietary item.

Desert truffles nutritional value varies by country, hence the nutritional value of *T. claveryi* from Iraq differs greatly from that of *T. claveryi* from Saudi Arabia [50]. The mineral element levels of the truffle are proportional to the quantity of soil elements present. Normally, the interaction between the mycorrhizal plant and the soil determines how the element levels of mushrooms fluctuate. While mushroom mycelium gives the plant with required minerals, it also obtains the item that the plant need. Growth conditions, variety, genetic variables, harvesting time, soil qualities, regional variances,

and testing processes may all have a role in cultivar mineral differences [51].

Mean concentrations of macro- (sodium, potassium, and calcium) and micro-elements (iron, zinc, and copper), as well as heavy metals (Cd, Ni, Cr, and Pb) were measured in the Terfezia species under investigation (Table 2). Truffles consumption might be expected to supply a significant amount of the body's necessary mineral requirements. The potassium concentration of the samples was higher than the amounts of other minerals, which was consistent with prior investigations [35, 52]. For the examined species, the decreasing order of macro-element concentration was K > Na > Ca. The amount of "heavy metals" needed by living organisms varies. Humans require iron, cobalt, copper, manganese, molybdenum, and zinc, however too much of these elements can harm the body. Other heavy metals, such as mercury and lead, are poisonous elements that may cause major ailments in animals if they accumulate in their systems over time. Copper (Cu) is an important metal that is necessary for hemoglobin production and metabolic growth catalysis. It is also a part of several metalloenzymes. Iron (Fe) is a biochemically important metal. It is well known that a sufficient iron content in the diet is critical for lowering the occurrence of anemia. Iron is a component of hemoglobin, the oxygen-carrying pigment found in erythrocytes. Zinc (Zn) is an essential metal that is found in a wide range of enzymes, where it plays catalytic, structural, and regulatory functions [53].

3.3. Essential Oil Content

Essential oils are concentrated sources of phenolics, polyphenolics, monoterpenes, diterpenes, sesquiterpenes, flavonoids, esters, and a variety of other phytochemicals. The chemistry of the essential oil is defined by these ingredients, which are divided into two groups: hydrocarbons and oxygenated molecules. A comparison screening was carried out in this section of the study based on the prior analytical results as well as the previous literature survey for both species. Percentage differences were recorded for the key chemical ingredients as well as chemical classes.

The essential oil produced was pale yellow in color and rich in secondary metabolites. Figures 2 and 3, show the chromatograms of essential oil components extracted from both species.

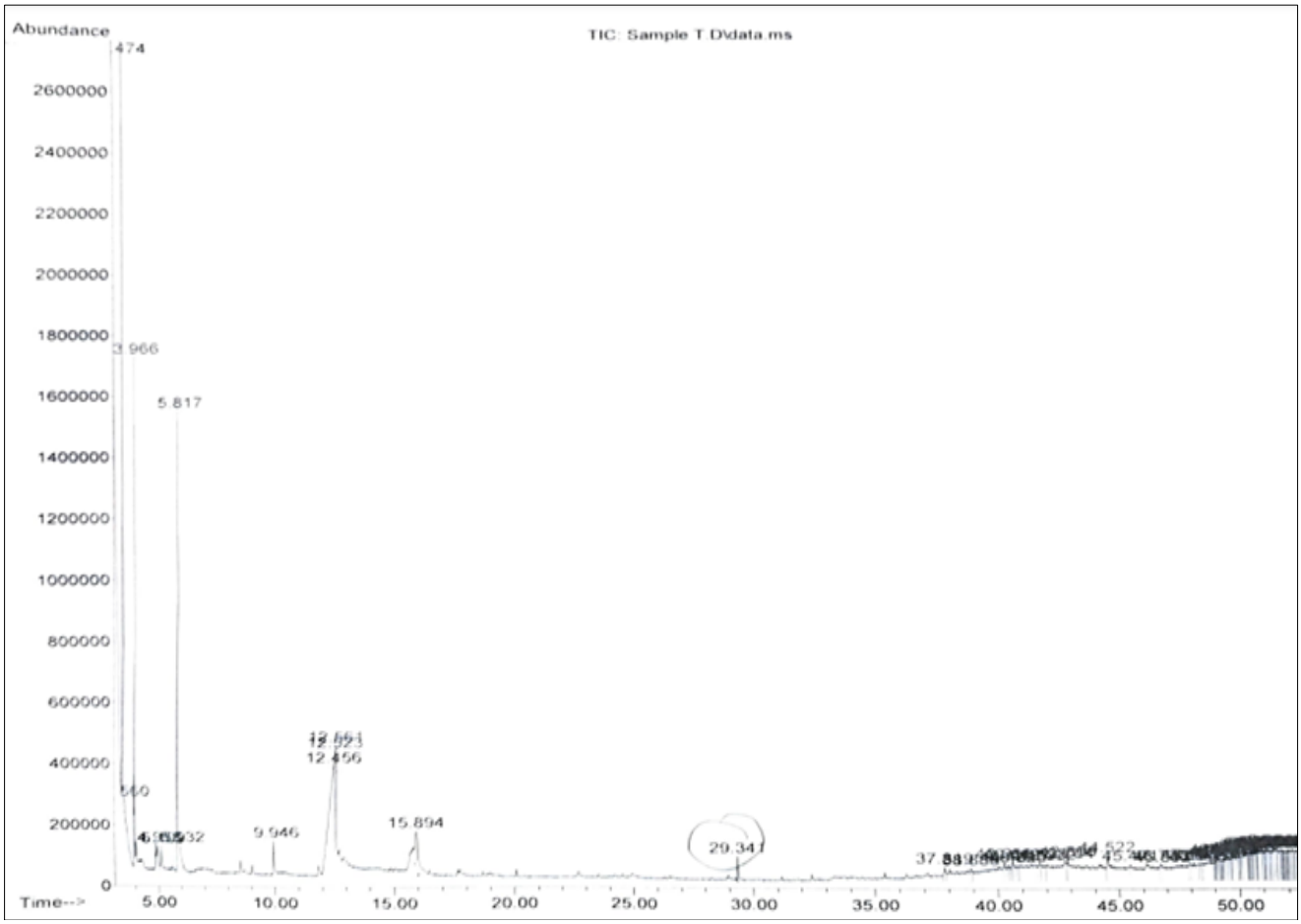


Fig 2: Total Ion Chromatogram of essential oil components in *Terfezia claveryi* species by GC/MS analysis

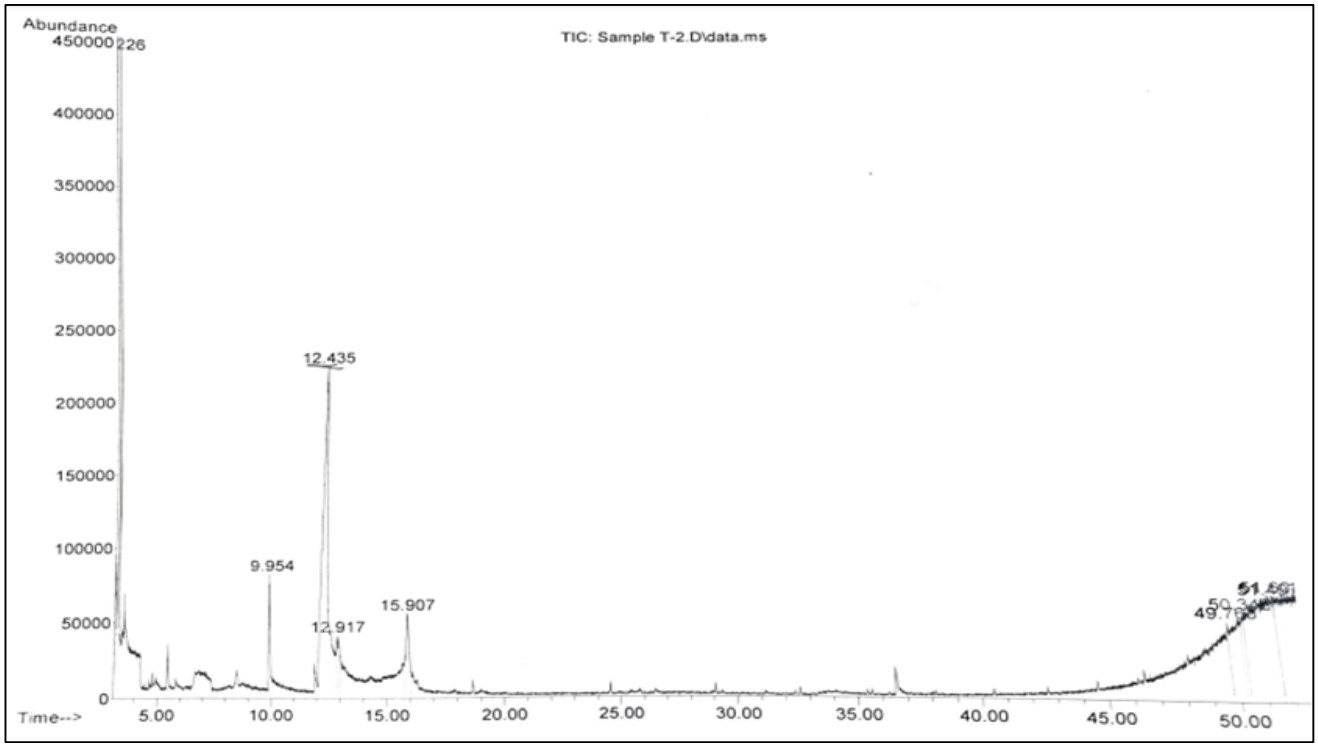
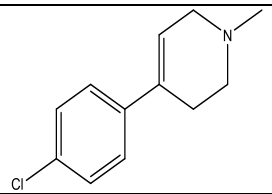
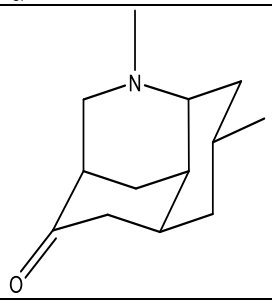
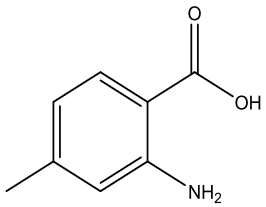
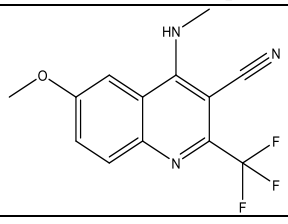


Fig 3: Total Ion Chromatogram of essential oil components in *Terfezia boudieri* species by GC/MS analysis

Table 3: Major compounds detected in the essential oil of *T. claveryi* listed in the order of elution

No.	RT (min)	% of total amount	Component	Chemical Structure	Chemical Class
1.	5.839	3.98	octane		Hydrocarbon
2.	6.674	1.29	1-methyl-4,4-chlorophenyl- 1,2,3,6-tetrahydropyridine		Nitrogenous aromatic compounds
3.	7.247	1.24	Luciduline 3,5-ethanoquinolin-10-one-1,7-dimethyl-decahydro- [3R-(3.α., 4a.beta., 5α., 7 beta, 8a .beta.)]		Tricyclic sesquiterpenes
4.	9.954		2-amino-4-methyl benzoic acid		Aromatic compounds
5.	12.917	1.22	6-methoxy-4-methylamino-2-trifluoromethylquinoline-3-carbonitrile		Aromatic compounds

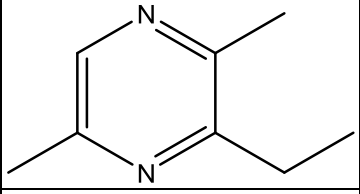
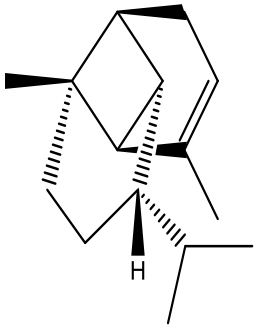
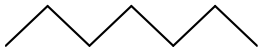
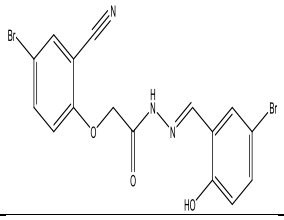
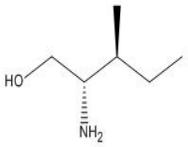
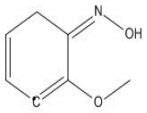
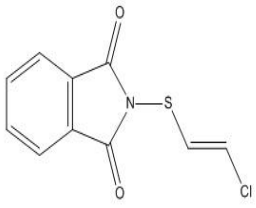
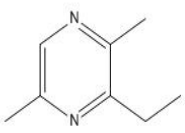
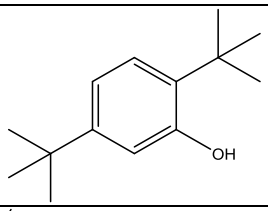
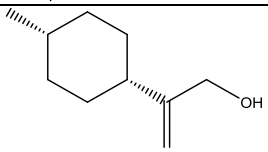
6.	15.907	3.98	3-ethyl-2,5-dimethyl- pyrazine		Nitrogenous aromatic compounds
7.	31.217	0.86	Copaene		Tricyclic sesquiterpenes
8.	35.811	1.15	6,10,14-trimethyl-2-pentadecanone		Hydrocarbon

Table 4: Major Compounds detected in the essential oil of *T. boudieri* listed in the order of elution

No.	RT (min)	% of total amount	Component	Chemical Structure	Chemical Class
1.	3.476	10.58	heptane		Hydrocarbon
2.	3.968	8.968	2-(4-Bromo-2-cyanophenoxy)-N`-(5-bromosalicylidene) acethydrazide		Nitrogenous phenolic compound
3.	4.884	0.74	isoleucinol		Monoterpene alcohol
4.	9.948	1.60	Methoxy phenyl oxime		Imines
5.	12.563	8.34	2-(2-chlorovinylsulfanyl) isoindole		Nitrogenous aromatic Compounds
6.	15.893	2.02	3-ethyl-2,5-dimethyl pyrazine		Aromatic Amines

7.	29.339	0.93	2,5-bis(1,1-dimethyl ethyl)phenol		Aromatic compounds
8.	40.045	1.16	Cis-p-menth-8-(10)-en-9-ol		Monoterpene alcohols

In this study, the components were identified using GC/MS by comparing fragmentation patterns and retention indices to those of authentic samples, and they are reported in the order of elution from a SE-30 fused-silica capillary column. More than fifty components were discovered in *Terfezia claveryi* oils, seven of which were major compounds that made up a significant portion of the oil, as well as a large number of smaller components. While in *Terfezia boudieri*, more than eighty components were discovered in the oils, eight of which were significant compounds. There was a lot of variation in the class of the individual separated components, their amounts, and their designated activities. Tables 3 and 4 demonstrate the qualitative and quantitative findings of determining the volatile oil content of the two species. As indicated in Tables 3&4, GC-MS for volatile analysis found peaks including acids, alcohols, ketones, lactones, ethers, hydrocarbons, aromatics, monoterpenes, sesqui-terpenes, nitrogen, and sulfur, with alcohols and ketones being the most abundant classes in *T. boudieri* and *T. claveryi*, respectively. *T. boudieri* has a high concentration of alcohols, with one example being cis-p-menth-8-(10)-en-9-ol. *T. claveryi*, on the other hand, contained much less alcohol. Ketones were the most common volatile class in *T. claveryi*, with 6,10,14-trimethyl-2-pentadecanone as an example. Sesqui-terpene hydrocarbons were found in higher concentrations in *T. claveryi* than in *T. boudieri*, contributing to the distinct aroma. The contents of the two species may explain the great antioxidant effect of both. Phenolics, terpenes and sesquiterpenes are essential secondary metabolites in plants with antioxidant activity that terminate free radicals by different mechanisms.

Desert truffles (*Tirmania nivea*, *Terfezia boudieri*, *Terfezia claveryi*, and *Picoa lefebvrei*) are related to the highly valued European truffles, but their fragrance and tastes are less powerful [54]. The presence of sulfur compounds, such as dimethyl disulphide and dimethyl sulphide, which impart sulfurous characteristics, is thought to be responsible for the European Tuber truffles distinct scent qualities. They are also linked to the existence of 1-octen-3-ol, a fatty acid derivative that provides an earthy-mushroom fragrance [55, 24] discovered 36 volatiles in six separate white and black truffle species, including aldehydes, terpenes, esters, alkanes, alcohols, and ketones, with regional origin variation. Few studies have shown the chemical composition of volatile organic compounds (VOC) in desert truffle species as *Terfezia claveryi*, *Terfezia boudieri*, and *Tirmania nivea* [55, 56].

4. Conclusion

One of the most important aspects of global research is the quest for more potent natural supplements and antioxidant agents. According to the findings, Terfezia species, owing to their high phenolic and mineral content profile, may be considered a source of phytochemical substances. The discovery, characterization, and separation of Terfezia bioactive components is crucial. The findings suggested that *T.claveryi* and *T.boudieri* could be used as functional foods, and they also pave the way for a more detailed characterization of the main bioactive compounds in extracts that are responsible for their potential medical activity, allowing these truffles to provide therapeutic as well as nutritional benefits. Natural antioxidants are important in the defense against oxidative stress because they are antimutagenic, anticarcinogenic, antiinflammatory, and neuroprotective. Terfezia extracts antioxidant activity appears to be depending on extract concentration and the solvent used to prepare them.

Ethical considerations

Ethical clearance of this study was obtained from the LIU SOP research committee: 2022RC-005-LIUSOP.

Conflicts of Interest

The authors would like to certify that there are no known conflicts of interest related with this publication.

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