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Comparative Chemical Composition, Phytochemical Content, Mineral Profile, and Antimicrobial Activity of Two Libyan Desert Truffles and Oyster Mushroom

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Abstract

Desert truffles are seasonal edible fungi valued in North Africa and the Middle East, but comparative data for Libyan material remain limited. This study compared white desert truffle (Tirmania nivea), red desert truffle (Terfezia claveryi), and commercial oyster mushroom (Pleurotus sp.) with respect to extraction yield, phytochemical composition, proximate composition, mineral content, and antimicrobial activity. Dried samples were macerated in water, ethanol, or chloroform (10 g/100 mL, 24 h). Moisture, ash, protein, and fat were determined by gravimetric, Kjeldahl, and Soxhlet procedures. Total phenolics and flavonoids were measured colorimetrically, minerals were quantified after wet digestion, and methanolic extracts were screened by agar-well diffusion against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans. Ethanol produced the highest yield from T. claveryi (19.2%), while chloroform produced the lowest yield from T. nivea (6.4%). Oyster mushroom had the highest protein content (28.07%), whereas the two truffles contained more total phenolics (96.2-98.9 mg gallic acid equivalents/g) and flavonoids (100.1-148.6 mg quercetin equivalents/g) than oyster mushroom (53.0 and 20.4 mg/g, respectively). Oyster mushroom also showed the highest reported concentrations of Ca, Mg, K, and Fe. All methanolic extracts inhibited the test organisms; the largest zone was observed for T. claveryi against E. coli (25 mm). Nevertheless, omnibus ANOVA did not show significant differences among extracts for any tested microorganism ($p=0.079-0.310$). The findings identify complementary nutritional and phytochemical advantages among the three edible fungi.

Keywords: Tirmania nivea; Terfezia claveryi; oyster mushroom; phenolic compounds; flavonoids; mineral composition; antimicrobial activity

1. Introduction

Desert truffles are hypogeous ascomycetes adapted to arid and semi-arid environments. Species of Tirmania and Terfezia form mycorrhizal associations, commonly with Helianthemum species, and are harvested after seasonal rainfall across North Africa and the Middle East. In addition to their culinary value, these fungi contribute to local food traditions, rural livelihoods, and the ecological functioning of drylands [1-3].

The fruiting bodies of desert truffles contain protein, carbohydrates, dietary fibre, minerals, unsaturated fatty acids, phenolic compounds, and other secondary metabolites. Their composition varies with species, maturity, soil, host plant, climate, harvest season, storage, and analytical basis [2,4-8]. Tirmania nivea is generally described as a white desert truffle, whereas Terfezia claveryi develops a darker reddish-brown fruiting body. Both species have been investigated for antioxidant and antimicrobial properties, but published values show substantial geographical and methodological variability [4,7-12].

Oyster mushrooms (Pleurotus spp.) provide a useful cultivated comparator because they are widely available, protein-rich, and recognized as sources of minerals and bioactive metabolites [13-15]. Direct comparison with desert truffles can therefore clarify whether the nutritional and phytochemical advantages of these foods are complementary rather than interchangeable.

Extraction solvent is an important determinant of measured chemical and biological activity. Water and aqueous alcohols preferentially recover polar metabolites, whereas less-polar solvents recover different fractions. Consequently, extraction yield alone does not represent biological potency, and antimicrobial screening should be interpreted together with extract concentration, vehicle controls, positive controls, and minimum inhibitory concentration measurements [10,16-18].

Despite the culinary importance of desert truffles in Libya, integrated comparative data on their chemical composition and antimicrobial effects are scarce. The present study therefore compared T. nivea, T. claveryi, and oyster mushroom by determining solvent extraction yield, qualitative phytochemicals, proximate composition, total phenolics, total flavonoids, selected minerals, and in vitro antimicrobial activity.

2. Materials and Methods

2.1 Study materials and sample collection

White desert truffle (Tirmania nivea) and red desert truffle (Terfezia claveryi) fruiting bodies were collected from the Al-Dafniya area of Misurata and the Al-Hamada area of Zintan, Libya, during February-March sampling periods reported for 2020, 2021, and 2023. Commercial oyster mushroom was purchased from local markets. Samples were transported to the laboratories of the Faculty of Science, Misurata University. Species assignments followed the source study; voucher specimen numbers and molecular authentication were not reported.

Visible soil was removed, samples were washed with distilled water, sliced with a stainless-steel knife, dried at room temperature, milled, and stored in tightly closed, light-protected containers at 4 °C until analysis.

2.2 Solvent extraction and extraction yield

For each fungal material, 10 g of dried powder was separately macerated in 100 mL of distilled water, ethanol, or chloroform. Suspensions were continuously stirred with a magnetic stirrer for 24 h, filtered, and concentrated under reduced pressure using a rotary evaporator. Dried extracts were stored at 4 °C in the dark. Extraction yield was calculated as 100 × (mass of dried extract/mass of dried starting material). Methanolic extracts were prepared for antimicrobial testing and redissolved in DMSO to the working concentration described in the source protocol.

2.3 Qualitative phytochemical screening

Water, ethanol, and chloroform extracts were screened using standard colour or precipitation reactions for carbohydrates, alkaloids, phenols, flavonoids, glycosides, anthocyanins, tannins, terpenes, anthraquinones, steroids, proteins, and amino acids. Responses were recorded semiquantitatively as absent (-), weak (+), moderate (++), or strong (+++).

2.4 Proximate composition

Moisture was determined from 3 g portions dried at 105 °C to constant mass. Ash was measured after incineration of 3 g portions at 550 °C. Crude protein was determined by the Kjeldahl method using a nitrogen-to-protein conversion factor of 6.25. Crude fat was measured by Soxhlet extraction of 2 g samples with petroleum ether for 16 h. Procedures were based on AOAC methods [19].

2.5 Total phenolic and flavonoid contents

Total phenolic content (TPC) was determined using the Folin-Ciocalteu assay. One millilitre of methanolic extract was mixed with 1 mL of 10% Folin-Ciocalteu reagent; after 3 min, 2 mL of 20% sodium carbonate was added and the volume was adjusted to 10 mL. After 30 min in the dark at room temperature, absorbance was read at 765 nm. Gallic acid (10-240 mg/L) was used for calibration, and results were expressed as mg gallic acid equivalents per g (mg GAE/g) [20].

Total flavonoid content (TFC) was measured by an aluminium chloride colorimetric assay. One millilitre of extract was mixed with 0.5 mL of aluminium chloride solution and diluted to 10 mL with 70% methanol.

After 40 min, absorbance was measured at 433 nm. Quercetin (10-240 mg/L) served as the calibration standard, and results were expressed as mg quercetin equivalents per g (mg QE/g) [21].

2.6 Mineral analysis

One gram of dry material was wet-digested with 5 mL concentrated nitric acid at 120 °C for 3 h. After cooling, 1.5 mL hydrogen peroxide was added, followed by a further 30 min of heating; the digestion cycle was repeated, and the final digest was diluted to 25 mL with distilled water. Calcium, magnesium, potassium, phosphorus, iron, and manganese were quantified using atomic absorption and flame-photometric procedures as specified in the source study. Results were reported in ppm.

2.7 Antimicrobial assay

Antimicrobial activity was evaluated by agar-well diffusion against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans. Mueller-Hinton agar was used for bacteria and blood agar for C. albicans. After inoculation, wells were cut with a sterile cork borer and filled with 100 µL of each methanolic extract. Plates were incubated at 37 °C for 24 h for bacteria and 72 h for C. albicans. Inhibition-zone diameters were measured in millimetres.

2.8 Statistical analysis

Data were analysed in SPSS version 24. Results were treated as three replicates per fungal material. One-way analysis of variance (ANOVA) was followed by the least significant difference (LSD) procedure for pairwise comparisons. Statistical significance was defined as $p<0.05$. Because replicate-level raw data were not available in the thesis document, the present article reports the tabulated summary values and the archived ANOVA outputs.

3. Results

3.1 Extraction yield

Extraction yield depended on both fungal material and solvent (Table 1). The highest yield was obtained from red truffle with ethanol (19.2%), followed by oyster mushroom with water (18.0%). The lowest yield was obtained from white truffle with chloroform (6.4%). Across the three materials, the effect of sample type was significant within water [$F(2,6)=48.064$, $p<0.001$], ethanol [$F(2,6)=393.301$, $p<0.001$], and chloroform [$F(2,6)=216.367$, $p<0.001$] extractions.

Table 1. Solvent extraction yields of the studied fungi.

Solvent	White truffle (%)	Red truffle (%)	Oyster mushroom (%)
Water	15.4	16.0	18.0
Ethanol	9.0	19.2	16.8
Chloroform	6.4	12.4	14.0

3.2 Phytochemical screening and proximate composition

Qualitative screening showed a broad distribution of carbohydrates, alkaloids, phenols, flavonoids, glycosides, terpenes, anthraquinones, steroids, proteins, and amino acids. Anthocyanins were not detected in red truffle, and tannins were not detected in oyster mushroom. Signal intensity varied by solvent, with water and ethanol generally producing stronger reactions for polar constituents.

Oyster mushroom contained the most protein (28.07%), while white and red truffles contained 17.90% and 14.89%, respectively (Table 2). Fat was not detected in red truffle and remained low in oyster mushroom. Red truffle had the highest moisture and ash values. ANOVA indicated significant differences for moisture

[F(2,6)=12.505, p=0.007], ash [F(2,6)=5.945, p=0.038], protein [F(2,6)=92.135, p<0.001], and fat [F(2,6)=24.348, p=0.001].

Table 2. Proximate composition and colorimetric phytochemical contents.

Parameter	White truffle	Red truffle	Oyster mushroom
Moisture (%)	8.00	10.30	7.76
Ash (%)	1.88	3.60	1.30
Crude protein (%)	17.90	14.89	28.07
Crude fat (%)	5.36	ND	2.04
Total phenolics (mg GAE/g)	96.2	98.9	53.0
Total flavonoids (mg QE/g)	148.6	100.1	20.4

ND, below the detection limit. TFC mapping follows the English abstract and archived SPSS outputs; the pre-submission note documents a conflicting label in the thesis results chapter.

The two desert truffles had similar reported TPC values (96.2-98.9 mg GAE/g), both markedly higher than oyster mushroom (53.0 mg GAE/g). TFC was highest in white truffle (148.6 mg QE/g), followed by red truffle (100.1 mg QE/g) and oyster mushroom (20.4 mg QE/g). Between-material effects were significant for both TPC [F(2,6)=311.559, p<0.001] and TFC [F(2,6)=919.849, p<0.001].

Figure 1. Total phenolic and total flavonoid contents of the studied fungi.

3.3 Mineral profile

Oyster mushroom showed the highest reported concentrations of calcium, magnesium, potassium, and iron (Table 3). White truffle had the highest phosphorus concentration, although the differences in phosphorus were small. Manganese was below the detection limit in both truffles and was detected at 0.017 ppm in oyster mushroom. Significant between-material differences were found for K [F(2,6)=1032.186, p<0.001], Mg [F(2,6)=5059.831, p<0.001], and Ca [F(2,6)=12839.596, p<0.001], but not for P (p=0.347), Fe (p=0.468), or Mn (p=0.180).

Table 3. Selected mineral concentrations in the studied fungi (ppm).

Mineral	White truffle	Red truffle	Oyster mushroom
Calcium (Ca)	41.700	58.110	91.520
Magnesium (Mg)	45.650	56.890	81.890
Potassium (K)	19.800	15.300	45.600
Phosphorus (P)	2.950	2.160	2.890
Iron (Fe)	2.187	5.990	7.684

Mineral	White truffle	Red truffle	Oyster mushroom
Manganese (Mn)	ND	ND	0.017

ND, below the detection limit of the analytical instrument.

3.4 Antimicrobial activity

All three methanolic extracts produced measurable inhibition zones against the four test microorganisms (Table 4; Figure 2). Red truffle showed the largest zone against E. coli (25 mm) and C. albicans (18.5 mm). Oyster mushroom produced the largest zone against S. aureus (20 mm) and P. aeruginosa (17 mm). White truffle showed zones of 13-20 mm.

Despite these descriptive differences, omnibus ANOVA did not detect significant between-extract effects for E. coli [$F(2,6)=2.616$, $p=0.152$], S. aureus [$F(2,6)=1.500$, $p=0.296$], P. aeruginosa [$F(2,6)=4.000$, $p=0.079$], or C. albicans [$F(2,6)=1.431$, $p=0.310$]. These results support broad screening activity but not a statistically established rank order of antimicrobial potency.

Table 4. Inhibition-zone diameters produced by methanolic extracts (mm).

Fungal material	E. coli	S. aureus	P. aeruginosa	C. albicans
White truffle	20	16	15	13
Red truffle	25	19	15	18.5
Oyster mushroom	15	20	17	15

Figure 2. Antimicrobial inhibition-zone diameters of the methanolic extracts.

4. Discussion

The extraction data demonstrate a strong interaction between fungal material and solvent. Ethanol maximized recovery from red truffle, while water produced the highest yield from oyster mushroom and chloroform consistently produced lower yields. This pattern is consistent with the abundance of polar carbohydrates, phenolics, peptides, and other hydrophilic constituents in edible fungi. However, extraction yield cannot be equated with bioactive-compound concentration because non-active macromolecules may contribute substantially to dried extract mass. Contemporary truffle studies similarly show that solvent polarity changes the chemical profile and measured antioxidant or antimicrobial response [7,10,11,16].

The proximate results indicate complementary nutritional strengths. Oyster mushroom had the highest crude protein content, whereas the truffles supplied moderate protein and relatively little fat. Protein contents previously reported for desert truffles vary widely, commonly reflecting dry- versus fresh-weight expression and differences among species and collection sites. Sawaya et al. reported protein values of approximately 20-27% for Saudi desert truffles [5], while Hamza et al. reported 28.81 g/100 g dry matter for Tunisian T. nivea [4]. The present white and red truffle values were lower, but they remain nutritionally meaningful. The oyster-mushroom protein value agrees with the general recognition of Pleurotus species as protein-rich foods [13-15].

The most distinctive truffle feature was the high reported content of colorimetrically measured phenolics and flavonoids. TPC was comparable between T. nivea and T. claveryi, and both exceeded the oyster-mushroom value. Studies of T. nivea, T. boudieri, and T. claveryi likewise identify phenolic and flavonoid fractions as major contributors to antioxidant capacity [7-12,22]. Nevertheless, Folin-Ciocalteu values are operational indices rather than compound-specific measurements because reducing substances other than phenolics can react with the reagent [20]. The aluminium chloride assay is also sensitive to flavonoid

structure and calibration standard [21]. HPLC-DAD or LC-MS profiling should therefore be used to confirm the specific metabolites underlying the reported TPC and TFC values.

Oyster mushroom contained the highest reported Ca, Mg, K, and Fe concentrations. These findings are directionally compatible with the capacity of mushrooms to accumulate minerals from their substrate [13-15].

All extracts produced zones of inhibition, with the largest numerical response observed for T. claveryi against E. coli. Previous studies have described antimicrobial activity in methanolic or other organic extracts of Tirmania and Terfezia species [4,10,11,16-18,23]. Phenolics, peptides, terpenoids, sterols, and other constituents may contribute through multiple mechanisms.

5. Conclusion

The three edible fungi displayed complementary profiles. Libyan T. nivea and T. claveryi were notable for high reported total phenolic and flavonoid contents, whereas oyster mushroom showed the highest protein and several mineral concentrations. Methanolic extracts from all materials inhibited E. coli, S. aureus, P. aeruginosa, and C. albicans in agar-well screening,

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التركيب الكيميائي المقارن، والمحتوى الكيميائي النباتي، والتركيب المعدني، والنشاط المضاد للميكروبات لنوعين من الكمأة الصحراوية الليبية وفطر المحار

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الملخص

تُعدّ الكمأة الصحراوية من الفطريات الموسمية الصالحة للأكل وذات القيمة الغذائية في شمال أفريقيا والشرق الأوسط، إلا أن البيانات المقارنة المتعلقة بالأنواع الليبية منها لا تزال محدودة. الهدف من هذه الدراسة مقارنة الكمأة الصحراوية البيضاء (Tirmania nivea)، والكمأة الصحراوية الحمراء(Terfezia claveryi) ، وفطر المحار التجاري (Pleurotus sp.) من حيث مردود الاستخلاص، والتركيب الكيميائي النباتي، والتركيب التقريبي، والمحتوى المعدني، والنشاط المضاد للميكروبات.

خضعت العينات المجففة لعملية النقع باستخدام الماء أو الإيثانول أو الكلوروفورم بنسبة 10 غ/100 مل لمدة 24 ساعة. كما جرى تقدير محتويات الرطوبة والرماد والبروتين والدهون باستخدام الطرق الوزنية، وطريقة كيلدال، وطريقة سوكسليت. وقُدّرت المركبات الفينولية الكلية والفلافونويدات باستخدام الطرق اللونية، بينما قُدّرت العناصر المعدنية بعد إجراء الهضم الرطب. كذلك جرى تقييم النشاط المضاد للميكروبات للمستخلصات الميثانولية باستخدام طريقة الانتشار بالحفر في الآغار ضد كل من Escherichia coliو Staphylococcus aureusو Pseudomonas aeruginosaو Candida albicans.

حقق الإيثانول أعلى مردود استخلاص من T. claveryi بلغ 19.2%، في حين أعطى الكلوروفورم أقل مردود استخلاص من T. niveaبلغ 6.4%. وسجّل فطر المحار أعلى محتوى من البروتين بنسبة 28.07%، بينما احتوى نوعا الكمأة الصحراوية على كميات أكبر من المركبات الفينولية الكلية، تراوحت بين 96.2 و98.9 ملغ مكافئ حمض الغاليك/غ، ومن الفلافونويدات، تراوحت بين 100.1 و148.6 ملغ مكافئ الكيرسيتين/غ، مقارنة بفطر المحار الذي سجل 53.0 و20.4 ملغ/غ، على التوالي.

كما أظهر فطر المحار أعلى التركيزات المسجلة من الكالسيوم والمغنيسيوم والبوتاسيوم والحديد. وأظهرت جميع المستخلصات الميثانولية قدرة تثبيطية ضد الكائنات الدقيقة المختبرة، وسُجّلت أكبر منطقة تثبيط لمستخلص T. claveryi ضد بكتيريا E. coli، إذ بلغت 25 ملم. ومع ذلك، لم يُظهر تحليل التباين الشامل وجود فروق ذات دلالة إحصائية بين المستخلصات تجاه أي من الكائنات الدقيقة المختبرة، حيث تراوحت قيم p بين 0.079 و0.310 تُبرز هذه النتائج المزايا الغذائية والكيميائية النباتية المتكاملة للأنواع الثلاثة من الفطريات الصالحة للأكل.