Nutritional and Chemical Compositions of The Desert Truffle (Terfezia Claveryi) in Samawa City of Iraq

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Abstract

The present study was conducted to investigate the nutritional and phytochemical composition (amino acids, phenolic compounds) of Terfezia claveryi collected from Samawa city. The macro-Kjeldahl technique was used to determine the crude protein content (N6.25) of the samples. A Soxhlet device was used to determine the crude fat content. The identification of free amino acids and individual phenolic compounds were performed by an amino acid analyzer and High-Performance Liquid chromatography (HPLC). Terfezia claveryi rich in carbohydrates, proteins and low in fat, protein percentage was 17.64%. Terfezia claveryi contain twelve amino acids, nine phenolic compounds, Rutin, Gallic acid, Sinapic acids and Chlorogenic acid were 6479.035, 3737.48, 1263.303, 1151.521 µg/gm identified as the major phenolic compound respectively. The protein content is significantly higher than most vegetables, which can use as a well-balanced diet. Owing to rich amino acids and phenolic profile Terfezia claveryi can be considered as a source of therapeutic agents.

Keywords: Desert Truffle, Chemical composition, Phenolics, Amino acids.

1. Introduction

Mushrooms are commonly taken in daily diets due to their highly delectable flavor and odor, as well as their long-established nutritional and therapeutic benefits [1]. In addition, among all edible fungi, truffles are one among the most ancient delicacies are well-known for their nutritional value, particularly when compared to meat and fish, this is a healthy alternative [2]. Terfezia species, as well as other fungus such as Tirmania species, are known as desert truffles or in Iraq its call kamaa, kima or chima, depending on local dialects and can be found in various Mediterranean nations. Terfezia claveryi is a common hypogynous ascomycete found in a semi-arid region that forms a mycorrhizal association with many annual and perennial Helianthemum species [3].

Protein (amino acids), minerals, fatty acids, and carbs are abundant. Because of the nutritional and biological importance of truffle species, research on them has risen [4,5]. Protein deficiency affects a considerable portion of the population in poor countries. As a result, research efforts are focused on identifying and evaluating underutilized for these countries as a source of alternative protein crops [6]. There are 20 amino acids in all in live cells used for protein synthesis under the guidance of genes are unique in that they are essential to all living things as peptide and protein building blocks [7]. Truffles’ protein level, it accounts for 20% of the dry weight, is much more than that of the majority of vegetables, as well as various fungus, and so truffle eating is recommended [5].

Truffles represented a massive and mostly untapped supply of phytochemicals, in addition to their nutritional value and odour and flavour. Phytochemicals are non-nutrient bioactive plant components with health benefits that can be found in grains, fruits, vegetables and other plant foods [8]. Phenols are a diverse group of phytochemicals produced by plants’ secondary metabolism [9]. Certain mushroom species harbour rich phytochemicals such as phenolic compounds, shown to possess several medical effects such as antioxidant, antimicrobial, antitumor, anti-inflammatory, DNA protective action and immunosuppressive agents, so Many chronic diseases are prevented by these chemicals [10]. This study aims to determine the morphological features, chemical compositions and phytochemical compounds of the Terfezia claveryi from Al-Muthanna Governorate in Iraq.
2. Materials and Methods

2.1 Chemical materials

Sulphuric acid, Perchloric acid, sodium hydroxide, boric acid, hydrochloric acid (HCL 6 M), sodium citrate; OPA (ortho phethalene aldehyde); ethanol, water, (methanol, formic acid), petroleum ether, nitrogen gas, acetonitrile.

2.2 Methods

2.2.1 Collect sample

Truffles (T. claveryi) were purchased from the local market of Samawa city of Iraq, it was then dried, milled into a fine powder and stored at 4°C in a dry, dark location until needed.

2.2.2 Morphological study

The physical traits of the fruiting bodies and the colour of the glebe were used to identify the Terfezia species. The ascocarp, peridium, and glebe, as well as their shape, color, and size, were studied macroscopically. In addition, more details were obtained throughout the microscopic scan of ascus and ascospore. The length and diameter of the truffles were measured using a micrometer caliper [11].

2.3 Chemical compositions of Terfezia claveryi

2.3.1 Proximate composition

AOAC techniques were used to determine the chemical composition of the samples (protein, fat, carbohydrates, moisture, and ash) [12]. With some modification. The macro-Kjeldahl technique was used to determine the crude protein content (N6.25) of the samples. Using a Soxhlet device, a known weight of powdered material was extracted with petroleum ether to determine the crude fat content. Estimating moisture based on (LOD) loss on drying. The amount of ash produced was determined by burning at 400 Celsius degrees, The difference was used to calculate total carbs.

\[
\text{CHO}\% = \frac{\text{total solids} - (\text{protein }\% + \text{fat }\% + \text{ash }\%)}{100}
\]

Energy of Terfezia claveri was calculated by the following equation:

\[
\text{Energy} \ (\text{kcal}) = 4 \times (\text{g protein + g carbohydrate}) + 9 \times (\text{g lipid})
\]

2.3.2 Amino acid extraction

Around 5 mg of solid samples were weighed with 0.01 mg accuracy, and liquid samples weighing about 100 mg were weighed with 0.01 mg accuracy. then 1 ml of 6 M hydrochloric acid solution as hydrolysis agent was added, the tube was covered and placed in the aluminum thermo block at 100°C±20°C for 24 hours for hydrolysis, Using a pipette, a volume of 100 μl of hydrolyzed is introduced in a vial placed in evaporation to remove moisture with nitrogen gas; The dried amino acid residues were dissolved in a volume of 100 μl of acetonitrile; They are derivative with a volume of 100 μl of OPA( ortho phethalene aldehyde ); The sealed vial is subjected to ultrasound for 1 minute;· The vial is placed in the thermal block at 100°C+2°C for 30 min. To complete the derivatization reaction; The vial is placed in the gas chromatograph sample stand; 10 injections of 100 μl per sample are performed [14].

2.3.3 Extraction of Phenolic Compounds by Ultrasonic

The phenolic components were extracted from a homogenized fungal sample (3 g) using a 70/30 solvent mix of ethanol and water. The extraction was carried out for 1 hour at room temperature using an Ultrasonic Bath (USA). The extraction yield was determined using 5 mL of liquid extract after filtration. The solvent was evaporated under vacuum using a rotary evaporator in Slovenia, and the mass was dried at 40°C to a constant mass. To avoid oxidative damage, dry extracts were kept in glass vials at 4°C until analysis. Reversed-phase HPLC analysis was used to quantify particular phenolic components, model SYKAMN (Germany) chromatographic system equipped with a UV detector), the column separation was Chemstation, a Zorbx Eclipse Plus- C18-OSD (25cm, X 4.6mm). The column temperature was 30°C Eluent A (methanol) and eluent B (1 percent formic acid in water (v/v)) were used in a gradient elution procedure, as following: initial 0-5 min, 40 % B; 6-15 min, 50 % B; and flow-rate of 1.2 mL/min. The amount of samples that were injected 100 μL, as well as standards was 100 μL Using an autosampler, it was completed automatically. The spectra were recorded at a wavelength of 280 nm[15].
2.4 Statistical Analysis

MINITAB version 16 used to analysis the recorded data. The statistical differences between the samples and controls were assessed using the test of one-way analysis of variance (ANOVA) and Tukey's post-test analysis was performed to evaluate significant differences among the obtained bioassay data at a 95% confidence interval. The outcome of the results was presented as the standard deviation (SD) of three repeat studies.

3. Results and Discussion

3.1 Morphological characteristic

*T claveryi* has a brownish-yellow peridium and a gleba with a spongy appearance and yellow-pinkish colour. Samples were of similar irregular spherical shape and size which detected macroscopically as shown in figure (1) this agree with [16]. In addition, ascus and ascospore detected microscopically where ascus vary in the shape (spherical and elongated) and each ascus contain eight spore presented in figure (2). The average of length and weight were 3.32, 19.83 respectively as shown in table (1).

![Macroscopic observation of ascocarps of Terfezia claveryi.](image1)

| Table 1. Physicals properties of Terfezia claveryi. |
|--------------------------------------------------|
| No. | Length cm | Weight gm  |
|-----|------------|------------|
| Terfezia claveryi | 3.32 ± 0.05 | 19.83± 0.10 |

![Microscopic image of Terfezia claveryi.](image2)
3.2 Proximate composition

Proximate analysis was done to examine if there was any difference in the composition that could alter the sample’s nutritional content and biological activity. Results revealed that protein, fats, carbohydrates, moisture and ash were 17.64%, 1.02%, 79.84%, 82% and 1.5% as shown in table (2). The yield of *Terfezia claveryi* after the drying sample was 19.88 %. The average of moisture, lipids, and ash, the findings of our research are consistent with those published in prior studies Dundar et. Al [17], where ranged [80-90,0.86-1.71,1.02-1.98 % respectively]. Also Kıvrak Ibrahim [18] found that *Terfezia Claveryi* Moisture content is considerable (83.14 g/100 g FW), this very related with our results. This indicates that mushrooms are perishable due to their high moisture content, which stimulates microbial growth and enzyme activity, going to accelerate decay. Crude protein was 17.64%, this agrees with Tejedor-Calvo et. al. [19] who found that protein percentage in high levels and varied between 14.04 and 24.15 g/100 g dw disagree with [11], who found protein percentage 3.35%. percentage of carbohydrates agree with [20], high energy contribution 399 (kcal /100 g dw) this closely related with [18] where the authors found that energy 337.74 (kcal/100 g) and disagree with [17] where they found energy ranged between (34-65 kcal/100 g) due to differences in chemical and biological soil structure, rainfall rates, atmospheric factors, and air temperature where it grow. The present study showed that *Terfezia claveryi* contained a high amount of carbohydrate, crude protein and low amount of lipids and ash.

Table 2. Chemical composition of *Terfezia claveryi* (g/100 g).

| T. claveri truffle       |       |
|-------------------------|-------|
| Moisture                | 82 ± 0.92 |
| Protein                 | 17.64 ± 0.55 |
| Carbohydrates           | 79.84 ± 1.02 |
| Lipid                   | 1.02 ± 0.064 |
| Ash                     | 1.5 ± 0.03 |
| Energy kcal             | 399 ± |
| a (g/100 g Fresh weight)|       |
| b (g/100 g dry weight)  |       |

Values are expressed as mean ± standard deviation. The comparisons were made using ANOVA test with P <0.05

3.3 Amino acids content

Truffles are naturally high in nutritional value and contain necessary Amino acids (essential amino acids for building the human body) [21]. Truffles species contained varied quantities of different types of free amino acids. Evaluation of free amino acid content was done by amino acid analyzer. twelve amino acids were detected some of it is essential such as (Leu, Met, Phe, Val), 100.9, 97.2, 102.7, 153.6 and others was nonessential (Ala, Asp, Glu, Gly, His, Ser, Tyr) 160.6,190.3, 284.2, 120.7, 33.6, 124.1, 100.3 µg/gm, were gained. Glu, Asp, Ala and Val the amino acids that were found in the highest concentrations in *Terfezia claveryi* show in a table (3), (Met) sulfur-containing amino acids, which are typically the limiting amino acids in many plant-based meals present in *T. claveryi*. The total amino acid contents were 1567.7µg/gm. Glu, Asp, Ser, Gly, Ala concentration higher than the concentration of these amino acids conducted by [18].

Figure 3. Chromatographic Amino Acid Analyzer of T. Claveryi.
Table 3. Free amino acid contents (µg/gm.) of Terfezia claveryi.

| Compound name | Amount(µg/gm.) | Time (min) |
|---------------|----------------|------------|
| Aspartic acid | 190.3 ± 0.21   | 7.93       |
| Glutamic acid | 284.2 ± 1.52   | 9.17       |
| Asparagine    | 99.5 ± 0.98    | 9.92       |
| Serine        | 124.1 ± 0.79   | 10.55      |
| Histidine     | 33.6 ± 0.09    | 11.84      |
| Glycine       | 120.7 ± 1.02   | 13.35      |
| Tyrosine      | 100.3 ± 0.95   | 13.97      |
| Alanine       | 160.6 ± 0.87   | 15.14      |
| Methionine    | 97.2 ± 0.91    | 16.55      |
| Valine        | 153.6 ± 1.39   | 17.14      |
| Phenylalanine | 102.7 ± 1.72   | 19.84      |
| Leucine       | 100.9 ± 0.98   | 22.15      |
| Total         | 1567.7         | 167.55     |

3.4 Phenolic composition

Phenolic compounds are secondary metabolites capable of exhibiting antioxidant, antimicrobial, anti-inflammatory and anticancer activities [22]. There is a limited number of studies about the phenolic compound identification and quantitation of Hypogenous Ascomycota truffles and Because of this biological importance of phenolic compounds, in this study, the phenolic profiles of Terfezia claveryi were investigated in detail. Phenolic compounds (expressed as µg/gm extract), HPLC was used to identify them. The results are presented as in Table (4). Nine phenolic compounds namely, coumarin, Sinapic acids, Catechin, Quercetin, Gallic acid, Caffeic acid, Chlorogenic acid, Rutin and Vanillin were 317.558, 1263.303, 888.779, 708.564, 3737.48, 248.655, 1151.521, 6479.035 and 302.25 were obtained respectively, Rutin, Gallic acid, Sinapic acids and Chlorogenic acid were identified as the main phenolic compound in respectively. Rutin was the predominant phenolic acid with a value of 6479.035 µg/gm. The phenolic concentration of our study was higher than those investigated by [11,18]. In previous studies, by [23] Caffeic acid, Gallic acid and Quercetin were not found in the phenolic profile.

![Figure 4. Chromatography Hplc phenolic content of T. Claveryi.](image)

Table 4. Phenolic content (µg/g dry weight) of Terfezia claveryi.

| Phenolic compounds  | Concentration(µg/gm) |
|---------------------|----------------------|
| Coumarin            | 317.558 ± 1.92       |
| Sinapic acids       | 1263.303 ± 3.58      |
| Catechin            | 888.779 ± 2.01       |
| Quercetin           | 708.564 ± 1.69       |
| Gallic acid         | 3737.48 ± 2.98       |
| Caffeic acid        | 248.655 ± 0.93       |
| Chlorogenic acid    | 1151.521 ± 1.76      |
| Rutin               | 6479.035 ± 3.48      |
| Vanillin            | 302.25 ± 0.67        |
Conclusion

The search for new potent natural supplements and antioxidant agents is one of the most imperative features of world research. According to the findings of the research, the protein content in T. claveryi is higher in comparison with most vegetables, this state provided to be high-quality food that can be used to create well-balanced meals. T. claveryi is rich in essential and nonessential free amino acids, it could be employed as a food complement or in the pharmacy sector. Owing to rich phenolic profile, coumarin, Sipanic acids, Catechin, Quercetin, Gallic acid, Caffeic acid, Chlorogenic acid, Rutin and Vanillin Terfezia claveryi can be considered as a source of phytochemical compounds. The discovery, characterization, and separation of their bioactive constituents are crucial because these bioactive compounds could be employed to extend the shelf life of food. These results suggested the potential utilization of T. claveryi as functional food, also open the way the detailed characterization main bioactive compounds in T. claveryi extracts responsible for the activity is profiled that giving these truffles therapeutic benefits in addition to nutritional ones.

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