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Original Article

Chemical Composition and Nutritional Value of Edible Moroccan Truffles

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ABSTRACT

Background and objectives: Truffles have a unique nutritional profile and are known to play an important role in terrestrial ecosystems. Mediterranean countries, including Morocco, are the location of an abundant harvest of truffles. This study investigated the chemical composition and the nutritional value of edible Moroccan truffles. **Materials and methods:** We studied the nutritional profile, and we performed a phytochemical screening of Tirmania pinoyi, Terfezia claveryi, Terfezia oligosperma, Terfezia rosea, and Terfezia arenaria collected from different areas from Morocco. **Results and conclusions:** The results showed that the truffles that we analyzed are a good source of carbohydrates, lipids and dietary fibers. The phytochemical screening that we performed afterwards revealed that these Moroccan truffles contain numerous secondary metabolites such as saponins, alkaloids, sterols, coumarins, and tannins. These results demonstrate the nutritional value of these truffles that can support a healthy and well-balanced diet. The secondary metabolites that we detected in our samples can make an important pharmacological contribution that could be the subject of future investigations for therapeutic purposes.
Keywords: Truffles, Fungi, Phytochemical screening, Nutritional value, Morocco.

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INTRODUCTION

Truffles are produced by many genera of fungi belonging to the Ascomycetes class [1]. They represent the hypogeous ascocarps of these fungi [2].

There are hundreds of different kinds of truffles, and many species of them are edible [3]. Truffles are one of the oldest forms of food used by Arabs [4]. Yet, beyond their status as a luxurious culinary element, truffles harbor a much deeper significance, encompassing both nutritional and therapeutic value that often remains elusive. They have a unique nutritional profile and are known to play an important role in terrestrial ecosystems [5]. They are healthy foods that are low in calories and fat and rich in fiber, proteins, vitamins, and minerals [6,7]. They are well known for their nutritional importance, especially when compared with meat and fish and are very appreciated in several Middle-Eastern, North-African, and Mediterranean countries where they have a prominent place in the local diet and traditional medicine of native people and Bedouins [8,9].

The nutritional value of truffles, whether black or white, is nothing short of astonishing. Despite their low-calorie and low-fat nature, they are abundant sources of essential vitamins such as vitamin C, potassium, and magnesium. These nutrients play pivotal roles in maintaining optimal health, bolstering the immune system, regulating blood pressure, and fostering cardiovascular well-being [9].

Mediterranean countries, including those of North Africa, are the location of an abundant harvest of edible truffles known by several different names: "*Terfass*," "*Torfa's*", "*Desert truffles*", and "*Truffles*". Moroccan truffles, with their distinct character and profound connection to the land, offer far more than mere gustatory delight. Indeed, beneath their unassuming appearance lies a wealth of nutritional and therapeutic benefits that warrant a thorough exploration. Moreover, the versatility of Moroccan truffles extends beyond their culinary applications. These remarkable fungi can also be harnessed and commercialized as dietary supplements, amplifying their potential impact on human health. As the global interest in natural and holistic remedies grows, the utilization of truffles as dietary supplements offers a promising avenue for delivering their therapeutic benefits to a wider audience [10].

Three truffle areas are identified in Morocco: the "A" zone corresponding to the forest of the Maamora Rabat, the "B" zone corresponding to highlands of eastern Morocco, and the "C" zone located in the east of the city of Safi (plain of Abda) [9]. Truffles are represented in Morocco by several species belonging to the genera *Tuber*, *Terfezia*, *Tirmania*, *Picoa* and *Delastria* and constitute an important food for the local population [11,13].

However, it is within their intricate composition and therapeutic properties that the true allure of Moroccan truffles resides. For centuries, these enigmatic fungi have been integral to traditional medicine due to their documented anti-inflammatory, antioxidant, and even aphrodisiac attributes. The bioactive compounds inherent in truffles have been subject to scientific inquiry for their potential in combatting inflammation, mitigating oxidative stress, and enhancing cognitive function [12].

The present work aimed to perform a comparative analysis of the nutritional properties of popular Moroccan truffles. We have also performed a phytochemical screening of these different species in order have an idea of their main constituents and, thus, better understand their pharmacological

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properties. We carried out this research on the following species: *Tirmania pinoyi, Terfezia claveryi, Terfezia oligosperma, Terfezia rosea,* and *Terfezia arenaria.*

MATERIAL AND METHODS

Samples collection and preparation

Samples were collected from different regions of Morocco in March 2024. *Terfezia oligosperma, Terfezia rosea,* and *Terfezia arenaria* were harvested in the forest of Maamora near the city of Rabat, while the collection of *Terfezia claveryi* and *Tirmania pinoyi* took place in the eastern region of Morocco. The collected samples were transported to the laboratory of analytical chemistry of the faculty of medicine and pharmacy (Mohammed V University of Rabat), and thoroughly cleaned, peeled, sliced, weighed, and carefully dried (at 45°C) for one night. Then, the samples were micronized to a fine powder and stored at ambient temperature in a dry and dark place until analysis. All analyses were performed in triplicate.

Water content

The water content was determined by three grams of each fresh sample [3]. The samples of each species were weighed, dried at 105°C for 3 hours, and then reweighed to calculate the water content.

Humidity content

The humidity content was determined on three grams of each dried powder sample by oven-drying at 40°C. The weighing was carried out every 30 minutes until the samples reached a constant mass. The samples were allowed to cool at room temperature in a desiccator before being reweighed. The humidity content was then calculated [14].

Total Ash

The determination of the total ash was performed as follows: One gram of each sample was placed in a tared crucible, weighed accurately, and slowly carbonized using a muffle-oven at 550°C until the sample turned into white ash and reached a constant weight. The ash was weighed, and the percentage of the total ash was calculated [15]

Lipid content

The lipid content of each sample was determined using Soxhlet extraction method. The solvent used was petroleum ether. Three grams of dried samples were weighed and secured in a Soxhlet extraction thimble. The extraction lasted 3 hours. At the end of the extraction, the solvent evaporated using the rotavapor. The flask was cooled and reweighed, then the percentage of crude lipid was calculated [16].

Total carbohydrates

The anthrone colorimetric method was used for the determination of total carbohydrates [17]. When dissolved in concentrated sulfuric acid, anthrone reacts with carbohydrate solutions to yield a green blue color. After colling, the absorbance of this colored solution is then measured in a spectrophotometer at 620 nm [18].

Dietary fiber

Pectin was extracted from ten grams of dry powder of each species sample according to method of Lefsih [19].

Cellulose was determined by treating three grams of dry sample of each species with an alkali solution (NaOH 4%). The mixture was heated at a reflux temperature for 2 hours. Then, the solid was filtered and washed using distilled water. This treatment was performed thrice. An acid hydrolysis treatment was conducted on the fibers after alkali treatment [20].

Phytochemical screening

The phytochemical screening showed the presence of different classes of bioactive secondary metabolites [21].

Phenolic Derivatives

One gram of powder of each species was weighed, to which 2 ml of distilled water and 6 ml of acetone were added. The preparation was placed in a water bath at 60°C for 5 minutes. A few drops of $FeCl_3$ were added to the filtered extract. The appearance of green or blue indicates the presence of the phenolic derivatives [22].

Flavonoids

The presence of flavonoids was tested in powdered of each species extract: One gram of powder was added to 10 ml of methanol using the cyanidin reaction [21]. The appearance of an orange color indicates the presence of flavones.

Tannins

Approximately, one gram of powder was homogenized into 10 ml of methanol and then filtered. A few drops of ferric chloride (1%) were added. Blue-black or brownish-green color indicated the presences of catechetical or gallic tannins, respectively [23]. Positive results of the presence of catechetical tannins were confirmed by obtaining a red coloration after addition of hydrochloric acid to the extract and heating at 100°C during five minutes (Bate- Smith reaction).

Anthracenics

The presence of anthracenics was tested by Borntrager reaction [24]. One gram of powder of each species was treated with a hot solution of NaOH 5% followed by acidification with acetic acid and dissolution in toluene. When a clear phase is separated, it is afterwards treated with ammonia solution.

Alkaloids

The characterization of alkaloids was based on their capacity to combine heavy metals or iodine (Dragendoff's reagent, Mayer's reagent and Iodoplatinate test) [25]. The Samples (approximately three grams of dried powder) were dissolved in 30 mL of 2% sulfuric acid, then, a few drops of Dragendorff reagent were added to the filtrate. The appearance of a colored precipitate indicates a positive reaction [26].

Quinones

One gram of dry powder of each sample was placed in a tube with petroleum ether. After agitation and a rest of 24 hours, extracts are filtered and concentrated in a rotavapor. The presence of quinones is confirmed by the addition of a few drops of NaOH (1/10), which results in the aqueous phase turning yellow, red or purple [23].

Saponins

The foam index was used to screen the presence of saponins [27]. Two grams of dry powder of each species were used to prepare a decoction by boiling for 30 min. with 100 ml of distilled water. Then, the resulting solution was divided into 10 tubes from 1ml to 10 ml of decoction. The content of each tube was adjusted to 10 mL with distilled water. Each tube was shaken vigorously in a horizontal position for 15 seconds. After 15 min vertical position, persistent foam measurement was obtained. The foam index was calculated by the following formula:

I = Foam level (cm) in the 9^{th} tube x 10/0,09

The presence of saponins was confirmed by an index exceeding 100 [25].

Coumarins

The coumarins are detected using the following test: One gram of the dry powder of each sample was weighed into a test tube, covered with filter paper impregnated with dilute NaOH. The tube was then placed in a boiling water bath for a few minutes. The filter paper is then examined under UV light at 365 nm. Yellow fluorescence indicates the presence of coumarins [28].

Sterols

Sterols were identified by Liebermann-Buchard reaction [23]. Two grams of each sample were dissolved in 1 ml of hot acetic anhydride. Then, 0.5 ml of concentrated sulfuric acid was added. The appearance, at the interphase, of a purple ring, turning blue then green, indicates a positive reaction [29].

RESULTS

Truffles have a high nutritional value through their carbohydrates, lipids and minerals composition. Our results show that carbohydrates were the most abundant macronutrients. The results are summarized in Table 1.

The dietary fiber composition of the different studied truffles is detailed in Table 2. The pectin content is six times larger than the cellulose. "*Tirmania pinoyi*" is the species that contains the highest content of dietary fiber.

Phytochemical screening of the truffle species powders is summarized in Table 3.

DISCUSSION

Compared to the other species studied, *Terfezia rosea* has the highest water content. Generally, the water content of the fresh samples of the studied truffles was high. This result is in line with those obtained by other authors [3, 13, 30]. The high-water content promotes bacterial growth, which accelerates deterioration. This involves taking the necessary measures for the conservation of truffles. Moisture content is almost the same for the five studied species. These low values content means that these truffles have a low power of incorporation of water.

The total ash of the studied sample is consistent with the result other authors [13, 31, 32]. In our study, the ash content does not exceed 5.5% for all the studied species. Total ash contains "physiological ash", which comes from the truffle tissue itself, and "non-physiological ash", which often comes from environmental contamination such as sand and soil [13]. This value is an excellent indicator of the nutritional quality and the purity of truffles [15].

Lipid levels obtained for the five studied species ranged from 3.5% to 5.15%. Our results are similar to the findings reported by El Houssaine et al. [33] but are slightly higher than the lipid content of *Terfezia claveryi* described by Bokhary et al. [34].

The carbohydrate content obtained by Anthrone method ranged from 67.1% to 81.88% on dry weight basis. The highest value was observed with *Terfezia arenaria*, while the lowest value was observed with *Terfezia rosea.* The results for the different species studied are consistent with the results obtained by other authors [13,31]. Carbohydrates constitute more than half of truffle dry matter. This group comprises various compounds: sugars (monosaccharides, their derivatives, and oligosaccharides) and both reserve and construction polysaccharides (glycans) [35,36].

Table 2 shows the dietary fibers content which is represented by pectin and cellulose. The pectin content is much higher than the cellulose content. The highest value of dietary fibers is observed in *Tirmania pinoyi* species. This result is consistent with the pectin content obtained in the study of Bouatia et al. [13]. The other results are in line with those obtained by other authors [30]. Dietary fibers content has been shown to impact all the aspects of gut physiology and to be essential for a healthy diet. Initial observations linking dietary fiber to health praised the benefits of consumption of fiber-rich foods rather than dietary supplementation with fibers [37]. They are effective antioxidants because of their scavenging properties, chelators of metal ions, and

Table 1. Nutrient content (in g/100g of DM or FM) of the studied species (mean ± SD).

Nutriments	*Tirmania pinoyi*	*Terfezia claveryi*	*Terfezia oligosperma*	*Terfezia rosea*	*Terfezia arenaria*
Water content (FM)	72.90 ± 1.84	62.90 ± 0.40	77.55 ± 2.05	84.07 ± 1.81	78.55 ± 1.18
Humidity content (DM)	1.56 ± 0.44	1.28 ± 0.01	0.66 ± 0.25	1.33 ± 0.10	1.40 ± 0.03
Total ash (DM)	5.24 ± 0.06	4.59 ± 0.36	3.95 ± 0.10	4.29 ± 0.14	5.01 ± 0.24
Lipid content (DM)	4.87 ± 0.36	3.42 ± 0.49	3.84 ± 0.70	4.54 ± 0.09	5.15 ± 1.74
Total carbohydrates (DM)	68.78± 0.46	75.33± 0.01	73.7± 0.15	67.1± 0.02	81.88 ± 0.67

Table 2. Dietary Fiber content (in g/100g of DM) of the studied species (mean ± SD).

Fibers	*Tirmania pinoyi*	*Terfezia claveryi*	*Terfezia oligosperma*	*Terfezia rosea*	*Terfezia arenaria*
Pectin	8.66 ± 0.49	6.67 ± 0.40	6.01 ± 0.21	4.66 ± 1.01	7.33 ± 0.65
Cellulose	0.88 ± 0.01	0.89 ± 0.38	1.04 ± 0.04	0.88 ± 0.15	0.87 ± 0.67

Table 3. Phytochemical screening of powdered studied truffles.

Chemical constituents	*Tirmania pinoyi*	*Terfezia claveryi*	*Terfezia oligosperma*	*Terfezia rosea*	*Terfezia arenaria*
Phenolic derivatives	+++	++	++	+++	++
Flavonoids	+++	++	++	++	++
Tannins	+++	++	++	-	++
Anthracenics	+	-	-	-	-
Alkaloids	+++	++	-	++	+++
Quinones	+++	++	++	+	++
Saponins	+++	+++	+++	+++	+++
Coumarins	+++	+	+++	+	+++
Sterols	+	+	-	+	+

Precipitate or coloration: +++ Appreciable amount; ++: moderate amount; +: trace amount; -: complete absence.

may protect tissues against free oxygen radicals and lipid peroxidation [38]. Pectin is a soluble, non-cellulosic fiber polysaccharide composed mainly of galacturonic acid. The gel forming effects of pectin is known to increase the binding of bile acids, thus, reducing their toxicity to intestinal mucosa in pathologic state[39]. Pectin was reported to exert a variety of effects on the gastrointestinal tract such as: maintaining the morphology and structure of the intestinal villi, increasing lipase activity delaying gastric emptying time, and increasing intestinal transit time [40].

The phytochemical study of the most popular Moroccan truffles is reported in table 3. The results obtained show that all the species studied contain phenolic derivatives. *Tirmania pinoyi* and *Terfezia rosea* are particularly rich in phenolic derivatives. Polyphenols are synthesized by plants as secondary metabolites and are usually synthesized as defense mechanisms against stressors such as pathogens [41]. They were thoroughly investigated recently since they were found to have beneficial properties such as being strong antioxidant [22]. Numerous studies have demonstrated the importance of naturally occurring dietary polyphenols in promoting cardiovascular health and emphasized the significant role these compounds play in limiting the effects of cellular aging [41].

For humans, several health beneficial properties of dietary flavonoids are recognized for their antioxidant and anti-proliferative effects which may protect the body from various diseases, such as cancers, cardiovascular disease, and inflammation [2]. Flavonoids with antioxidant properties could act on the blood vessels to prevent atherosclerosis, which is one of the main complications in diabetes [42]. In our study, all studied species were shown to contain flavonoids, especially *Tirmania pinoyi.*

Studies of the flavonoid composition of truffles are very rare. Only a few works on Tunisian species have been published [43].

In our study, chemical tests carried out revealed the presence of coumarins. "*Terfezia claveryi*" and "*Terfezia rosea*" are the species in which the quantity of coumarins detected was the lowest. The presence of coumarins may explain the antifungal effect of truffles [27,43].

The tannins tests on our samples were also positive. The presence of tannins, endowed with tissue renewing properties, was shown to contribute in healing wounds caused by diabetes [42].

The anthracenic derivatives are found in plants, either in free form or in the combined form of anthracenic Heterosides. Their distribution is very limited as they are found only in some families of angiosperms such as Liliaceae and Fabaceae. They have laxative properties.

In our study, the tests for anthracenic derivatives were negative, except for *Tirmania pinoyi* for which the test was slightly positive. This result is in line with that obtained in the study of Hamza et al. [21].

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. They often have strong pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Typical examples are cocaine, caffeine, nicotine, morphine, and quinine [43]. Except for *Terfezia oligosperma*, all our samples showed positive reaction to alkaloids. This result is consistent with the fact that most medicinal plants are shown to contain alkaloids during phytochemical screenings [4].

Quinones are a class of natural and synthetic compounds that have several beneficial effects. They exert an antioxidant activity which improves general health conditions. They constitute, as vitamins, a class of molecules preventing and treating several illnesses such as osteoporosis and cardiovascular diseases [45]. All the species that we studied gave a positive reaction to the quinones test. In another study, *Terfezia boudieri* was also shown to contain quinones [21].

Saponins are very abundant in all the species that we studied. The biological activity of saponins is attributed to their amphipathic properties. As might be expected from their chemical diversity, saponins have antimicrobial and/or anti-herbivore activity and so may play a role in plant defense [46]. Saponins also possess a range of important pharmaceutical properties (anti-inflammatory, antifungal, antibacterial, anti-parasitic, anti-cancer, and antiviral activities) [47]. Our results for saponins are in accordance with another research on the same subject [48].

Sterols regulate the fluidity of membranes and probably play a role in the adaptation of membranes to temperature. In addition, sterols participate in the control of membrane-associated metabolic processes, which involves the action of a few specific sterols. They also act as substrates for a wide variety of secondary metabolites such as the glycoalkaloids, and saponins [49]. The sterols present in truffle tubers, even though their concentration is low as shown by the results of our study, possess an interesting profile [16].

Tannins are one of the most important families of secondary metabolites. They are defined as phenolic compounds of high molecular weight, found in leaves, barks, and wood in the form of insoluble or soluble tannin-protein complexes [50]. Indeed, tannins have beneficial effects on protein metabolism in ruminants, decreasing rumen degradation of dietary protein and increasing absorption of amino acids in the small intestine [50]. The antimicrobial property of tannins has been well documented. The anticancer activity of tannins may be linked to their antioxidant property by protecting cellular components from oxidative damage, including lipid peroxidation [51]. Our work shows that the studied species contain tannins in their chemical composition, except for *Terfezia rosea*. This result is consistent with that found by Boufeldja et al. [52].

CONCLUSION

Moroccan truffles that we analyzed in this study were found to be a good source of carbohydrates, lipids and dietary fibers. These results make them excellent food that can support a healthy and well-balanced diet. To achieve a more comprehensive understanding of the nutritional and compositional aspects of Moroccan truffles, further research is imperative. Analytical and spectral studies offer promising avenues for deeper exploration, allowing us to unravel the intricate tapestry of bioactive compounds and essential nutrients that these truffles encompass. Such endeavors are pivotal in bridging the gap between traditional wisdom and modern scientific inquiry. The use of these truffles for flavoring and preserving foods will be of interest for further study. The phytochemical screening revealed that numerous compounds are present as saponins, alkaloids, sterols, coumarins, and tannins. These results demonstrate the nutritional value of this type of truffles as well as their pharmacological contribution, which could be the subject of many future studies for therapeutic purposes.

As we navigate the uncharted terrain of these Moroccan truffles, our current study lays the foundation for subsequent investigations, fostering a holistic comprehension of their multifaceted contributions to culinary excellence and human well-being. Embracing this call for continued research, we look forward to a future enriched by deeper insights, where the true essence of these truffles is fully illuminated, guiding us towards a more profound appreciation of their cultural heritage and inherent benefits.

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