

Nutritional, Antioxidant and Antibacterial Properties of *Tirmania nivea*, A Wild Edible Desert Truffle from Tunisia Arid Zone

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Abstract

Nutritional composition, antioxidant and antibacterial properties of *Tirmania nivea*, a desert truffle largely distributed in Southern Tunisia, were evaluated. Carbohydrates were the most abundant macronutrients (57.83 g/100 g DM) followed by proteins (28.81 g/100 g DM) in *T. nivea* truffle. The ash content was 5.06 g/100 g DM, and potassium, calcium, phosphorus, magnesium and iron were found to be particularly abundant. The results of ascorbic acid, total carotenoids and total anthocyanins expressed on a dry mass truffle were 10.63 mg/100 g, 1.17 mg/100 g and 29.1 mg/100 g, respectively. Organic extracts of *T. nivea* contained relatively important amounts of total phenolics and flavonoids. The methanolic extract displayed the highest DPPH• radical-scavenging activity (IC₅₀: 0.26 mg/ml) and lipid peroxidation inhibitory activity (IC₅₀: 0.51 mg/ml), and also exhibited remarkable inhibitory activity against seven species of bacteria whose minimum inhibitory concentration values ranged from 0.36 to 1.32 mg/ml.

Keywords: *Tirmania nivea*; Functional food; Nutrients; Antioxidant activity; Antibacterial activity

Introduction

Desert truffles, which are edible mycorrhizal fungi constituted a popular food in many cultures due to their medicinal and nutritional properties and they have become very attractive as a functional food [1]. Their geographical distribution was limited to arid and semi-arid lands, especially in North-Africa and Middle East. Truffles are generally known as “Kamah” in Arabic, which literally means hidden. The local population living in the arid and semi-arid areas of the Mediterranean basin used desert truffles as a meat substituent. *Tirmania nivea* is one of the highly regarded truffles due to its musky smell, delicacy and soft tissues. Ascocarps of *T. nivea* grown underground (hypogeous) and when they reached maximum size at maturity, they cracked the ground surface. Ascocarps of *T. nivea* have a roughly spherical shape and their skin was creamy white or light brown. *T. nivea* were not eaten raw. They were peeled, cut into cubes or slices, cooked and presented in many ways. Moreover, they can be ground and added to other dishes as a supplement [2,3].

Several studies on the chemical composition of desert truffles showed that they are rich in proteins, carbohydrates, dietary fiber, fatty acids and minerals as well as many beneficial phytochemicals. The protein content, which averages 20% of the dry mass in desert truffles, is more important than in most vegetables and other fungi [2,4-7]. In addition to truffles' nutritional importance and their aroma and flavor, truffles represented a vast and yet largely unexploited source of therapeutic compounds with antioxidant, anti-inflammatory, antimicrobial, immune-suppressor and anti-carcinogenic properties [8-10]. Indeed, the reported biological activities of truffles could have positive effects in the development of their added-value.

The objective of this study was to increase our knowledge about the nutritional properties of *T. nivea* truffle from Tunisia arid zone. Moreover, antimicrobial and antioxidant activities of organic and aqueous extracts were investigated.

Materials and Methods

Chemicals

DPPH• and chemical standards were purchased from Sigma Aldrich Co. (St. Louis, USA). All other chemicals and reagents used

were of analytical grade and were obtained from Merck (Darmstadt, Germany).

Truffles

Tirmania nivea fruiting bodies were collected during the months of March and April 2013 from southern area of Tunisia (Medenine, Benguerdenne: latitude 32°57'09"N, longitude 11°38'26"E, with arid climate characterized by a mean rainfall of 150 mm/year). Voucher specimens [Tn01] were deposited at the Arid Lands Institute of Medenine (Tunisia). After harvest, the fresh truffles were dried on the shade until constancy of the mass (20 days), then ground into fine powder and stored at ambient temperature in a dry place and in the dark until use.

Physicochemical and mineral composition

The samples were analyzed for moisture, proteins, fat, carbohydrates and ash using the AOAC procedures [11]. Different mineral constituents (potassium calcium, magnesium, iron, sodium, manganese and copper) were analyzed separately using an atomic absorption spectrophotometer (Hitachi Z6100, Tokyo, Japan). The total phosphorus content was determined using a molybdenum-blue colorimetric method [5].

Chemical analysis and antioxidant activities

The dried truffle powder (25 g) was soxhlet-extracted successively using three solvents of increasing polarity as follows: petroleum ether, followed by chloroform and methanol during 6 h for each solvent. The

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volume of each solvent used was 300 ml, which was then evaporated using a rotary evaporator and the residual solvents were removed by flushing with nitrogen. Finally, the obtained extracts were kept in the dark at +4°C until further analysis. For water extraction, the fungi powder (50 g) was macerated during 24 h in 200 ml distilled water, with continuous stirring at room temperature. Then, the macerate was filtered through Whatman No.1 filter paper. The same procedure was repeated twice with the obtained residue, and then the total filtrate (macerate) was lyophilized. Moreover, a hot water extract was prepared by mixing 50 g of powdered truffles with 200 ml of distilled water at 50°C. The mixture was stirred for 3 h and then, the extract was filtered and lyophilized. After that, total phenolics, flavonoids and tannins were measured in *T. nivea* extracts as previously described [12,13]. Total phenolics content was expressed as mg gallic acid equivalent (GAE)/g extract. Flavonoids and tannins contents were expressed as mg catechin equivalent (CE)/g extract. The dried truffles of *T. nivea* were also subjected for ascorbic acid, total carotenoids and total anthocyanins contents, which were estimated as previously described [2,14,15]. The DPPH• radical-scavenging and the β-carotene/linoleic acid assays of *T. nivea* extracts were measured as previously described [16,17]. Results of DPPH• radical-scavenging and β-carotene/linoleic acid bleaching assays are presented by IC₅₀ values (mg/ml), and defined as the extract concentration needed to scavenge 50% of DPPH• and to obtain 50% inhibition of β-carotene peroxidation, respectively. All tests were carried out in triplicate and the results were averaged.

Antimicrobial activity

Antimicrobial activities of *T. nivea* extracts were tested against seven strains of bacteria: three Gram-negative (*Salmonella typhimurium* (NRRLB4420), *Escherichia coli* (ATCC19115) and *Pseudomonas aeruginosa* (ATCC27853)) and four Gram-positive (*Enterococcus faecalis* (ATCC29212), *Staphylococcus aureus* (ATCC25923), *Staphylococcus epidermis* (CIP106510) and *Bacillus subtilis* (ATCC168)). Microorganisms were obtained from the culture collection of the Arid Lands Institute of Medenine (Tunisia). Minimum inhibitory concentration (MIC) values, which represent the lowest extracts concentration that preventing visible growth of microorganisms, were determined as previously described [17]. All tests were performed in Mueller-Hinton broth (MHB) medium supplemented with 5% dimethylsulfoxide (DMSO). Bacterial strains were cultured overnight in MHB at 37°C. Tubes of MHB containing various extracts were inoculated with 10 μl bacterial inoculums adjusted to 10⁶ colony forming units (cfu)/ml of bacteria cells. Then, they were incubated under shaking conditions (120 rpm) for 24 h at 37°C. Control tubes without tested samples were assayed simultaneously. All tests were carried out in triplicate and the results were averaged.

Statistical analysis

SPSS (version 12.1, SPSS, Chicago, IL, USA) was used for statistical analysis. Data are expressed as means ± SD. To assess the variation of the variables among samples, a one-way Analysis Of Variance (ANOVA) was performed. Statistical significance between means was determined using Duncan's multiple range tests and set at *p*<0.05.

Results and Discussion

Nutritional quality

The results of the nutrient composition expressed on a dry mass basis were presented in Table 1. The average moisture content of the fresh truffle was 77.63 g/100 g, which was in close agreement with those reported for fresh *T. nivea* from various Middle Eastern origins [2,3].

Carbohydrates were the most abundant macronutrients (57.83 g/100 g) in *T. nivea* truffle as was found previously for other desert truffles, which contained approximately 60% of carbohydrates [18]. Table 1 shows that *T. nivea* truffle contained a relatively high fat content (6.78 g/100 g) as compared to those reported for other desert truffles whose fat contents ranged from 2.81 to 7.42% [1]. Obtained results also demonstrated that protein content of *T. nivea* truffle (28.81 g/100 g) was comparable to the value reported for *Terfezia boudieri* truffle from arid region of Tunisia (26.12 g/100 g) [7]. Besides, it was reported that essential amino acids are present in appreciable amounts in desert truffles [3]. These findings qualify these truffles as a rich source of protein and justify the practice of the local population of using them as a meat substitute. The ash content in *T. nivea* truffle was 5.06 g/100 g, which was close to those mentioned for other desert truffles such as *Terfezia clavaryi* and *Tirmania pinoyi* [19]. Concentrations of different minerals were presented in Table 2. Potassium, calcium, magnesium, phosphorus and iron were found to be particularly abundant in *T. nivea* truffle as was previously reported for European truffles [1]. Thus, the intake of *T. nivea* could be expected to contribute a large proportion of the essential mineral requirement in the body. Among their ecological roles, desert truffles are well known to help their associated plants in mineral acquisition by taking up many important minerals.

Antioxidant potential of *T. nivea* truffle

Vegetables and fruits are rich sources of antioxidants, such as vitamin A, vitamin C, vitamin E, carotenoids, anthocyanins, flavonoids and polyphenolic compounds, which prevent free radical damage. In fact, it is considered that consumption of foods rich in antioxidants, in the context of a balanced diet, is associated with the prevention of many degenerative diseases [20]. Therefore, the chemical constituents contributing towards antioxidant activities in *T. nivea* were investigated. The results of ascorbic acid, total carotenoids and total anthocyanins expressed on a dry mass basis were presented in Table 1. Obtained results showed that ascorbic acid content (10.63 mg/100 g), total carotenoids content (1.17 mg/100 g) and total anthocyanins content (29.1 mg/100 g) in *T. nivea* truffle were higher than the values reported for different desert truffles [2]. Previous works showed that phenolics were the major antioxidant compounds found in the mushroom extracts, as compared to ascorbic acid and β-carotene

Moisture ^a	77.63 ± 0.10
Fat ^b	6.78 ± 1.50
Proteins ^b	28.81 ± 0.63
Carbohydrates ^b	57.83 ± 0.51
Ash ^b	5.06 ± 0.78
Ascorbic acid ^c	10.63 ± 0.28
Total carotenoids ^c	1.17 ± 1.53
Total anthocyanins ^c	29.1 ± 0.14

^a(g/100 g fresh mass); ^b(g/100 g dry mass); ^c(mg/100 g dry mass)

Table 1: Macronutrients, ascorbic acid, total carotenoids and total anthocyanins of *T. nivea* (n=3).

K	1263.12 ± 1.56
Ca	427.51 ± 2.21
Mg	366.12 ± 0.27
Fe	217.05 ± 1.21
Na	33.01 ± 0.10
P	287.06 ± 0.17
Mn	3.20 ± 0.63
Cu	0.77 ± 1.33

Table 2: Mineral concentrations (mg/100 g dry mass) in *T. nivea* (n=3).

[21,22]. To better study the antioxidant properties of *T. nivea* truffle, the dried mushroom was extracted using three solvents of increasing polarity (petroleum ether, chloroform and methanol). Besides aqueous extracts at ambient temperature (macerate) and at 50°C (hot water extract) were prepared. Then, total phenolics, flavonoids and tannins contents were measured in the truffle extracts (Table 3). The yield of extractable compounds relative to the mass of dried fungi material ranged from 0.54 g/100 g (macerate extract) to 26 g/100 g (methanolic extract) (Table 3). Methanolic extract has the highest total phenolics (211.22 mg GAE/g extract) and flavonoids (74.52 mg CE/g extract) contents. Whereas, the highest content of tannins was recorded in the chloroformic (23.18 mg CE/g extract) extract (Table 3). Values of phenolic compounds in *T. nivea* truffle were within the range of values previously reported in some wild mushrooms [1], but much higher than the value (13.19 mg GAE/g extract) reported for the methanolic extract of *T. pinoyi* [23]. Interestingly, the total phenolic content of *T. nivea* (1.39 g GAE/100 g FM) was very high as compared to other phenolic-rich foods such as cherries (44.3-87.9 mg GAE/100 g FM), strawberries (59.8-93.7 mg GAE/100 g FM) or onions (142-428 mg GAE/100 g FM) [24-26]. The high values of phenolic compounds in desert truffles could be explained by their natural habitat characterized by many harsh environmental conditions. In deserts, for example, contrasting conditions may occur on the same day, i.e., very cold nights and very hot days. Therefore, the ability of certain plants or wild mushrooms to withstand stressful conditions is probably due to their ability to neutralize the reactive oxygen species by increasing the level of antioxidants, especially phenolic compounds [3].

T. nivea extracts were subjected to DPPH• radical-scavenging and lipid peroxidation inhibitory activities (Table 3). Organic extracts were able to effectively reduce the stable free radical DPPH• (IC₅₀: 0.26-0.31 mg/ml) and to inhibit the linoleic acid oxidation (IC₅₀: 0.41-0.86 mg/ml), as compared to aqueous extracts. The methanolic extract containing the highest amounts of total phenolics and flavonoids showed the highest antioxidant potential as compared to other extract. *T. nivea* presented more interesting antioxidant potential than *T. pinoyi* whose methanolic extract showed moderate DPPH• radical-scavenging

scavenging activity (IC₅₀: 6.41 mg/ml) and lipid peroxidation inhibition (IC₅₀: 28.38 mg/ml) [23].

Antimicrobial activity of *T. nivea* truffle

The antimicrobial activity of *T. nivea* truffle extracts against seven species of bacteria was assessed by evaluating the determination of minimum inhibitory concentration (MIC) values (mg of extract/ml of medium). As can be seen in Table 4, truffle extracts showed varying degrees of antibacterial activity against all tested strains. Chloroformic and methanolic extracts showed the interesting antimicrobial activities (MIC: 0.25-2.1 mg/ml) as compared to aqueous and petroleum ether extracts. Table 4 shows that methanolic extract seems to be the most effective on the most tested strains. Hussain and Al-Ruqaie [19] reported that methanolic extract of *Tirmania* truffles has antimicrobial activity against a wide range of both gram positive and gram negative bacteria. Furthermore, it was reported that the methanolic extract of *Tirmania* truffles has been considered to provide the higher antimicrobial inhibitory activity as compared to water and ethyl acetate extracts [9,10,19]. Thus, desert truffle extracts might be used to control the microbiological quality of processed foods, since several questions about the safety of chemical additives used for food preservation were raised. In fact, Stojković et al. [23] reported that methanolic extract of *Tirmania pinoyi* truffle successfully inhibited the growth of *Staphylococcus aureus* in chicken soup, kept at room temperature and in a refrigerator, in a dose dependent manner.

Conclusion

The present paper is a contribution to the studies on the nutraceutical potential of *T. nivea*, a wild edible desert truffle from Tunisia arid zone. More importantly, *T. nivea* truffle seems to be a good source of several important nutrients and phytochemicals, such as phenolics, minerals and proteins. *T. nivea* could provide a healthy meat alternative that satisfies the nutritional requirements, especially for non-meat eaters. Furthermore, this truffle could be considered as antioxidant-rich food, that are currently in demand for their beneficial effects on the general state of health and/or to reduce the risk of some oxidative stress related

	Petroleum ether	Chloroform	Methanol	Macerate	Hot water extract
Yield (g/100 g dry mass)	0.57 ± 0.02 ^c	3.78 ± 0.20 ^b	26.0 ± 0.15 ^a	0.54 ± 0.02 ^c	0.73 ± 0.03 ^c
Total phenolics ^A	169.22 ± 0.21 ^b	132.53 ± 0.10 ^c	211.22 ± 0.18 ^a	23.31 ± 1.27 ^d	27.73 ± 1.23 ^d
Flavonoids ^B	43.81 ± 0.25 ^{bc}	61.86 ± 0.16 ^b	74.52 ± 0.12 ^a	22.17 ± 0.32 ^d	23.97 ± 1.22 ^d
Tannins ^B	19.21 ± 0.23 ^b	23.18 ± 0.12 ^a	19.78 ± 1.16 ^b	8.13 ± 0.23 ^c	8.23 ± 0.41 ^c
DPPH• radical-scavenging assay (IC ₅₀ , mg/ml)	0.31 ± 0.32 ^a	0.29 ± 0.03 ^a	0.26 ± 0.07 ^a	1.36 ± 0.21 ^b	1.59 ± 0.62 ^{bc}
β-carotene/linoleic acid assay (IC ₅₀ , mg/ml)	0.86 ± 0.011 ^b	0.61 ± 0.01 ^b	0.41 ± 0.02 ^a	3.10 ± 0.01 ^c	3.0 ± 0.09 ^c

^{a,b,c,d}Values with same superscript letters in the same row are non-significant at $p < 0.05$; ^Amg gallic acid equivalents (GAE)/g extract; ^Bmg catechin equivalents (CE)/g extract.

Table 3: Extraction yields, total phenolics, flavonoids, tannins and antioxidant activity assays of *T. nivea* extracts (n=3).

Tested microorganisms	Petroleum ether	Chloroform	Methanol	Macerate	Hot water extract
Gram-positive					
<i>E. faecalis</i>	2.25	1.80	0.71	5	5
<i>S. aureus</i>	5.03	2.0	0.43	6	6
<i>S. epidermis</i>	2.10	1.25	0.98	8	8
<i>B. subtilis</i>	2.80	0.25	0.36	8	8
Gram-negative					
<i>S. typhimurium</i>	4.01	2.0	1.32	8	8
<i>E. coli</i>	3.20	2.10	0.61	6	6
<i>P. aeruginosa</i>	3	1.60	1.16	6	6

Table 4: Antibacterial activity (minimum inhibitory concentration, mg/ml) of *T. nivea* extracts (n=3).

diseases. In perspective, it is important to identify phenolic compounds in *T. nivea* by Liquid Chromatography-High Resolution Electrospray Ionization Mass Spectrometry technique. Moreover, studies on *T. nivea* truffle domestication and cultivation were important in order to increase its production as natural functional food. In addition, studies on truffle incorporation into the formulation of conventional foods could be considered as an interesting approach that may significantly improve their antioxidant capacity and therefore they could become a "preventive model" for disease prevention.

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