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

Amina Hamza1, Hamida Jdir2 and Nacim Zouari3*

1Range Ecology Laboratory, Arid Lands Institute of Medenine, Medenine, Tunisia
2Laboratory of Enzyme Engineering of Lipases and Biochemistry, Engineering National School of Sfax (ENIS), University of Sfax, Sfax, Tunisia
3High Institute of Applied Biology of Medenine, University of Gabes, Medenine, Tunisia

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/g/100, 1.17 mg/g/100 g and 29.1 mg/g/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, Benguerdene: latitude 32°57'09'', longitude 11°38'26'', 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

---

*Corresponding author: Zouari N, High Institute of Applied Biology of Medenine, Medenine, University of Gabes, Medenine, Tunisia. Tel: +21624862279; E-mail: znacim2002@yahoo.fr

Received June 22, 2016; Accepted July 01, 2016; Published July 04, 2016

Citation: Hamza A, Jdir H, Zouari N (2016) Nutritional, Antioxidant and Antibacterial Properties of *Tirmania nivea*, A Wild Edible Desert Truffle from Tunisia Arid Zone. Med Aromat Plants 5: 258. doi: 10.4172/2167-0412.1000258

Copyright: © 2016 Hamza A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
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. 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 claveryi 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.

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.

| Moisturea | 77.63 ± 0.10 |
| Fatb | 6.78 ± 1.50 |
| Proteinsb | 28.81 ± 0.63 |
| Carbohydratesb | 57.83 ± 0.51 |
| Asha | 5.06 ± 0.78 |
| Ascorbic acidc | 10.63 ± 0.28 |
| Total carotenoidsd | 1.17 ± 1.53 |
| Total anthocyaninsd | 29.1 ± 0.14 |

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).
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• (IC$_{50}$: 0.26-0.31 mg/ml) and to inhibit the linoleic acid oxidation (IC$_{50}$: 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 activity (IC$_{50}$: 6.41 mg/ml) and lipid peroxidation inhibition (IC$_{50}$: 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-Ruqie [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, Stojkovic 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 diseases.
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.

**Acknowledgements**

Special thanks go to Miss Amina Gammoudi (ISBAM) for her kind help with English.

**References**

1. Wang S, Marcone MF (2011) The biochemistry and biological properties of the world’s most expensive underground edible mushroom: Truffles. Food Res Int 44: 2567-2581.
2. Al-Laith AAA (2010) Antioxidant components and antioxidant/antiradical activities of desert truffle (*Tirmania nivea*) from various Middle Eastern origins. J Food Compos Anal 23: 15-22.
3. Al-Laith AAA (2014) Nutritional and antioxidant properties of the white desert truffle *Tirmania nivea* (Zubaidi). In: Kagan-Zur V (eds.). Desert Truffles, Soil Biology, Springer-Verlag, Berlin Heidelberg 38: 275-297.
4. Dabbour IR, Takruri HR (2002) Protein quality of four types of edible mushrooms found in Jordan. Plant Foods Hum Nutr 57: 1-11.
5. Murcia MA, Martinez-Tome M, Vera AM, Morte A, Gutiérrez A, et al. (2003) Effect of industrial processing on desert truffles *Terfezia claveryi* and *Picoa juniperi* Vitt.: proximate composition and fatty acids. J Sci Food Agric 83: 535-541.
6. Yildiz A, Yesil OF, Yavuz O, Karakaplan M (2005) Organic elements and protein in some macrofungi of south east Anatolia in Turkey. Food Chem 89: 605-609.
7. Hamza A, Zouari N, Zouari S, Jdir H, Zaidi S, et al. (2016) Nutraceutical potential, antioxidant and antibacterial activities of *Terfezia boudieri* Chatin, a wild edible desert truffle from Tunisia and zone. Arab J Chem 9: 383-389.
8. Murcia MA, Martinez-Tomé M, Jiménez AM, Vera AM, Honrubia M, et al. (2002) Antioxidant activity of edible fungi (truffles and mushrooms): losses during industrial processing. J Food Prot 65: 1614-1622.
9. Janakat S, Al-Fakhiri S, Salil AK (2004) A promising peptide antibiotic from *Terfezia claveryi* aqueous extract against *Staphylococcus aureus* in vitro. Phytother Res 18: 810-813.
10. Janakat SM, Al-Fakhiri SM, Salil AK (2005) Evaluation of antibacterial activity of aqueous and methanolic extracts of the truffle *Terfezia claveryi* against *Pseudomonas aeruginosa*. Saudi Med J 26: 952-955.
11. AOAC (1997) Official Methods of Analysis. 16th edn. Washington, DC: Association of Official Analytical Chemists.
12. Sun B, Richardo-da-Silvia JM, Spranger I (1998) Critical factors of vanillin assay for catechins and proanthocyanidins. J Agric Food Chem 46: 4267-4274.
13. Dewanto V, Wu X, Adom KK, Lu RH (2003) Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J Agric Food Chem 50: 3010-3014.
14. Lichtenthaler HK, Wellburn AR (1983) Determination of total carotenoids and chlorophylls a and b of leaf in different solvents. Biochem Soc Trans 11: 591-592.
15. Rodriguez-Saona LE, Wrolstad RE (2001) Unit F1.1: anthocyanins. Extraction, isolation, and purification of anthocyanins. In: Wrolstad RE (ed.), Current Protocols in Food Analytical Chemistry, John Wiley and Sons, New York, USA, pp. 1-11.
16. Yildirim A, Mavi A, Kara AA (2001) Determination of antioxidant and antimicrobial activities of Rumex crispus L. extracts. J Agric Food Chem 49: 4083-4089.
17. Zouari N, Fakhfakh N, Zouari S, Bougatæf A, Neffati M, et al. (2011) Chemical composition, angiotensin I-converting enzyme inhibitory, antioxidant and antimicrobial activities of essential oil of Tunisian Thymus algeriensis Boiss. et Reut. (Lamiaceae). Food Bioprod Process 89: 257-265.
18. Kagan-Zur V, Roth-Bejerano N (2008) Desert truffles. Truffles 1: 32-37.
19. Hussain G, Al-Ruqaij IM (1999) Occurrence, chemical composition, and nutritional value of truffle: An overview. Pakistan J Biol Sci 2: 510-514.
20. Hu FB (2002) Dietary pattern analysis: a new direction in nutritional epidemiology. Curr Opin Lipidol 13: 3-9.
21. Mau JL, Huang PN, Huang SJ, Chen CC (2004) Antioxidant properties of methanolic extracts from two kinds of Antrocll amphantora mycelia. Food Chem 86: 25-31.
22. Barros L, Ferreira MJ, Queiros B, Ferreira ICFR, Baptista P (2007) Total phenols, ascorbic acid, 6-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chem 103: 413-419.
23. Stojkovic D, Reis FS, Ferreira ICFR, Barros L, Gamočičja J, et al. (2013) *Tirmania pinioyi*: Chemical composition, in vitro antioxidant and antibacterial activities and in situ control of *Staphylococcus aureus* in chicken soup. Food Res Int 53: 56-62.
24. Usenik V, Fabick J, Štampar F (2008) Sugars, organic acids, phenolic composition and antioxidant activity of sweet cherry (*Prunus avium* L.). Food Chem 107: 185-192.
25. Reikda K, Kahanzadeh S, Deschenes M, Levassuer A, Charlie MT, et al. (2005) Antioxidant capacity and phenolic content of selected strawberry genotypes. HortScience 40: 1777-1781.
26. Lu, Wang J, Al-Qadiri HM, Ross CF, Powers JR, et al. (2011) Determination of total phenolic content and antioxidant capacity of onion (*Allium cepa*) and shallot (*Allium oschaninii*) using infrared spectroscopy. Food Chem 129: 637-644.