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ORIGINAL ARTICLE

Nutraceutical potential, antioxidant and antibacterial activities of *Terfezia boudieri* Chatin, a wild edible desert truffle from Tunisia arid zone

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Abstract Nutritional composition, antioxidant and antibacterial properties of *Terfezia boudieri* Chatin, a desert truffle largely distributed in Southern Tunisia were evaluated. Carbohydrates were the most abundant macronutrients (62.03 g/100 g) followed by proteins (26.12 g/100 g) in *T. boudieri* truffle (dry mass). The ash content was 4.49 g/100 g dry mass and potassium, phosphorus, iron, and calcium were found to be particularly abundant in *T. boudieri*. The fat content of the truffle was 8 g/100 g dry mass, characterized by its higher content of the essential linoleic acid (*n*-6) (54.18%). Eight volatile compounds were identified by GC and GC/MS and they were dominated by fatty acids. The results of ascorbic acid, total carotenoids and total anthocyanins expressed on a truffle dry mass were 12.20 mg/100 g, 1.43 mg/100 g and 35.40 mg/100 g, respectively. The methanolic extract, containing the highest amounts of phenolics and flavonoids, displayed the highest DPPH radical-scavenging activity (IC_{50} = 0.20 mg/ml) and the lipid peroxidation inhibitory activity (IC_{50} = 0.36 mg/ml). Furthermore, the truffle extracts were tested for their antibacterial activity against seven species of bacteria. The methanolic extract also exhibited remarkable inhibitory activity on the tested strains, which minimum inhibitory concentration values ranged from 0.25 to 1.3 mg/ml.

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1. Introduction

Mushrooms were a popular food in many cultures and they have become very attractive as a functional food (Wang and Marcone, 2011). Truffles represented a complex family of hypogeous fungi, mainly containing species of the genera

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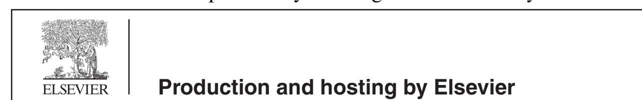

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Picoa, *Tirmania*, *Tuber* and *Terfezia*. Their geographical distribution was limited to arid and semi-arid lands, mostly in countries around the Mediterranean basin. In addition, some truffle species have been found in South Africa, in North America, in Japan, and in China (Trappe and Sundberg, 1977). Among the ascomycete truffles, *Tuber* and *Terfezia* were known as excellent edible fungi with a considerable economic importance (Morte et al., 2006). The popularity of truffles was due to their flavor, delicious taste, and potential health benefits. Wild edible fungi were also considered as valuable food in their own right due to their nutritional value. In fact, truffles were a rich source of protein, amino acids, fatty acids, minerals and carbohydrates (Bokhary and Parvez, 1993).

In addition to truffles' nutritional importance and their aroma and flavor, truffles represented a vast and yet largely unexploited source of therapeutic compounds with anti-inflammatory, antioxidant, antimicrobial, immune-suppressor, anti-mutagenic and anti-carcinogenic properties (Murcia et al., 2002; Janakat et al. 2004, 2005). In fact, the reported biological activities of truffles have drawn scientific attention as they were believed to have positive effects in the development of value-added truffles or truffle-related products.

Endogenous metabolic processes in the human body might produce highly reactive free radicals, especially reactive oxygen species (ROS) capable of oxidizing biomolecules, including lipids, DNA, carbohydrates and proteins, resulting in cell death and tissue damage (Dubost et al., 2007). On a cellular basis, damage provoked by these free radical formations was usually protected by oxidative enzymes as well as compounds such as ascorbic acid, tocopherols and phenolics. When the mechanism of antioxidant system becomes unbalanced by factors such as deterioration of physiological functions, this leads to diseases and accelerated aging. For that reason, the regular intake of natural antioxidants contribute to the protection against cancer, cardiovascular disease, diabetes, and other aging-related diseases by reducing oxidative stress (Kris-Etherton et al. 2002).

Terfezia boudieri Chatin, a North African species, grows in the South East of Tunisia and constitutes an important food for the local population. In the present work, we aimed to increase our knowledge about the nutritional properties (protein content, lipid and mineral composition) of *T. boudieri* truffle from Tunisia arid zone. Analysis of volatile compounds by gas chromatography (GC) and GC/mass spectrometry (MS) techniques was carried out. Moreover, antimicrobial and antioxidant activities were also investigated for aqueous and organic extracts of *T. boudieri*.

2. Methods

2.1. Chemicals and spectrophotometric measurements

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 purchased from Carlo Erba Reagenti SpA (Starda Rivoltana, Italy), Merck (Darmstadt, Germany) and Kemika (Zagreb, Croatia). Spectrophotometric measurements were performed by UV–VIS spectrophotometer (UV mini-1240) model Shimadzu.

2.2. Truffles

Truffles of *T. boudieri* were collected during the months of March and April 2009 from Southern area of Tunisia (Medenine, Benguerdenne: latitude 32°57′09″N, longitude 11°38′26″ E, with an arid climate characterized by a mean rainfall of 150 mm/year). After harvest, the fresh truffles were dried in the shadow, until constancy of the mass (20 days). Then, they were ground into fine powder and stored at ambient temperature in a dry place and in the dark until use.

2.3. Volatile compounds' extraction

The truffle dry matter was submitted to hydrodistillation for 4 h using a Clevenger-type apparatus and the collector solvent used was *n*-hexane (2 ml). After evaporation of the solvent under nitrogen flow, the volatile compounds were stored in sealed vials protected from the light at −20 °C until analysis by GC and GC/MS.

2.4. Lipids' extraction

The truffle lipids were extracted using chloroform/methanol as previously described by Zouari et al. (2010). After lipids' extraction, methyl esters of the fatty acids were prepared as follows. A sample containing 50 mg lipids was dissolved in 500 μl *n*-hexane. Then, 200 μl of potassium hydroxide 2 M in methanol was added and the solution was mixed for 2 min in a vortex mixer. After phase separation, the upper layer of *n*-hexane containing the fatty acid methyl esters was analyzed by GC and GC/MS.

2.5. Volatiles' and fatty acids' analyses

2.5.1. Gas chromatography

The volatiles and fatty acids were analyzed using a Hewlett–Packard 5890 series II gas chromatograph equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d.; film thickness 0.25 μm; Hewlett–Packard, Palo Alto, CA, USA) and connected to a flame ionization detector (FID). The column temperature was programed at 50 °C for 1 min, then 7 °C/min to 250 °C, and then left at 250 °C for 5 min. The injection port temperature was 240 °C and that of the detector 250 °C (split ratio: 1/60). The carrier gas was helium (99.995% purity) with a flow rate of 1.2 ml/min, and percentages of the constituents were calculated by electronic integration FID peak areas, without the use of response factor correction. Retention indices (RI) were calculated for separate compounds relative to C_7–C_{25} *n*-alkane mixture (Aldrich Library of Chemical Standards, Saint–Louis, Missouri, USA).

2.5.2. Gas chromatography/mass spectrometry

The volatiles or fatty acids were also analyzed by GC/MS, using a Hewlett–Packard 5890 series II gas chromatograph. The fused HP-5MS capillary column (the same as that used in the GC analysis) was coupled to a HP 5972A masse-selective detector (Hewlett–Packard). The oven temperature was programed at 50 °C for 1 min, then 7 °C/min to 250 °C, and

then left at 250 °C for 5 min. The injection port temperature was 250 °C and that of the detector was 280 °C (split ratio: 1/100). The carrier gas was helium (99.995% purity) with a flow rate of 1.2 ml/min, and the analyzed sample volume was 2 μl. The mass spectrometer conditions were as follows: ionization voltage 70 eV; ion source temperature, 150 °C; electron ionization mass spectra were acquired over the mass range of 50–550 *m*/*z*.

2.5.3. Volatiles' and fatty acids' identification

The volatiles and fatty acids were identified by comparing the mass spectral data with spectra available from the Wiley 275 mass spectral data with spectral libraries (software, D.30.00, Palo Alto, California, USA). Further identification confirmations were made referring to retention indice (RI) data generated from a series of known standards of n-alkane mixture (C_7–C_{25}) and to those previously reported in the literature (Adams, 2001).

2.6. Physicochemical composition and mineral concentration

Dry matter was determined by oven-drying at 105 °C to constant mass (AOAC, 1997). Crude proteins were analyzed according to the Kjeldhal method. A factor of 6.25 was used for conversion from total nitrogen into crude protein (AOAC, 1997). The fat content was determined by Soxhlet extraction with hexane for 8 h at the boiling point of the solvent. The ash content was determined by combustion of the samples at 550 °C for 8 h. Then, different mineral constituents such as (potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), sodium (Na), manganese (Mn) and copper (Cu)) were analyzed separately using an atomic absorption spectrophotometer (Hitachi Z6100, Tokyo, Japan). Total phosphorus (P) content was determined using a molybdenum-blue colorimetric method (Murcia et al., 2003). The carbohydrate content was estimated by difference of mean values:

$$\text{Carbohydrate} = [\text{total solids} - (\text{protein} + \text{lipids} + \text{minerals})].$$

2.7. Preparation of *T. boudieri* extracts

The dried truffle powder (25 g) was soxhlet-extracted successively using solvents of increasing polarity as follows: petroleum ether, followed by dichloromethane, chloroform, ethyl acetate and finally with methanol during 6 h for each solvent. The volume of each solvent used is 300 ml which was then evaporated under vacuum and the residual solvent was 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. After that, 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.

2.8. Chemical analysis

The aqueous and the organic fractions of the truffles were selected for preliminary chemical analysis to explore the major classes of phytoconstituents. Phenolic content of *T. boudieri* extracts was determined by the Folin–Ciocalteu method (Dewanto et al. 2002). Gallic acid monohydrate was used as standard for the calibration curve. Phenolic content was expressed as mg gallic acid equivalent (GAE)/g extract. Flavonoid content of the samples was determined as previously described (Dewanto et al., 2002) and catechin was used as standard. The results were expressed as mg catechin equivalent (CE)/g extract. Tannin content in different extracts was also measured using the modified vanillin assay described by Sun et al. (1998). Tannin content was expressed as mg catechin equivalent (CE)/g extract. The dried truffles of *T. boudieri* were also subjected for carotenoid and anthocyanin contents which were estimated as previously described by Lichtenthaler and Wellburn (1983) and by Rodriguez-Saona and Wrolstad (2001), respectively. Furthermore, ascorbic acid content was determined according to Al-Laith (2010). All tests were carried out in triplicate and the results were averaged.

2.9. DPPH radical-scavenging and antioxidant activities

The DPPH radical-scavenging activity and the antioxidant activity using β-carotene/linoleic acid assay of *T. boudieri* extracts were measured as previously described (Yildirim et al., 2001; Koleva et al., 2002; Zouari et al., 2011). The DPPH radical-scavenging activity and the antioxidant activity of *T. boudieri* extracts were presented by IC_{50} values, defined as the concentration of the extract needed to provide 50% inhibition. Therefore, IC_{50} values were calculated from the graph plotting DPPH radical-scavenging activity or antioxidant activity percentages against extract concentration. Lower IC_{50} values reflected better scavenging or antioxidant activities.

2.10. Antimicrobial activity

2.10.1. Microbial strains

Antimicrobial activities of *T. boudieri* 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), *Staphlyococcus aureus* (ATCC25923), *Staphlyococcus epidermis* (CIP106510) and *Bacillus subtilis* (ATCC168)). Microorganisms were obtained from the culture collection of the (Arid Lands Institute of Medenine, Tunisia).

2.10.2. Determination of the minimum inhibitory concentration (MIC)

MIC values, which represent the lowest extract concentrations that prevent visible growth of microorganisms, were determined as described previously (Zouari et al. 2011). 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 10^6 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 samples were realized simultaneously. All tests were carried out in duplicate and the results were averaged.

2.11. Statistical analysis

Excel (Microsoft Corporation, USA) and SPSS (version 12.1, SPSS, Chicago, IL, USA) were 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 $p < 0.05$.

3. Results and discussion

*3.1. Physicochemical composition of **T. boudieri** truffle*

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 78.23 g/100 g which in close agreement with those reported for fresh desert truffles (*Tirmania nivea*) from various Middle Eastern origins (Al-Laith, 2010). However, moisture content as high as 93% was reported for some American edible mushrooms (Dubost et al. 2007). Carbohydrates were the most abundant macronutrients (62.03 g/100 g) in *T. boudieri* truffle as was found previously for other desert truffles which contain approximately 60% carbohydrates (Kagan-Zur and Roth-Bejerano, 2008). The protein content of *T. boudieri* truffle (26.12 g/100 g) was found to be higher than the value reported for *Terfezia claveryi* ascocarps (16 g/100 g) (Bokhary and Parvez, 1993). The ash content in *T. boudieri* truffle was 4.49 g/100 g which is close to those (from 4.9 to 5.9%) previously reported for different desert truffles such as *T. claveryi*, *T. nivea*, and *Tirmania pinoyi* (Hussain and Al-Ruqaie, 1999). Table 2 presents the concentrations of different minerals (K, Ca, Mg, Fe, Na, P, Mn and Cu) in *T. boudieri* truffle. Potassium, phosphorus, iron, and calcium were found to be particularly abundant in *T. boudieri* truffle as was previously reported for European truffles (Wang and Marcone, 2011). The intake of *T. boudieri* could be expected to contribute a large proportion of the essential mineral requirement in the body. Table 1 shows that *T. boudieri* truffle contains a high fat content (8 g/100 g) as compared to those (from 2.81% to 7.42%) reported for different desert truffles (Wang and Marcone, 2011). Composition of the fatty acids of *T. boudieri* truffle was investigated using both GC and GC/MS techniques. Percentages and retention indices of the identified fatty acids are listed in Table 3 in the order of their elution on the HP-5MS column. The global chromatographic analysis resulted in the identification of four fatty acids, accounting for 99.9% of the total fat content. Interestingly, the *T. boudieri* truffle was characterized by its higher content of the essential linoleic acid (n-6) (54.10%). Furthermore, oleic acid (n-9) and palmitic acid represent 22% and 20.40%, respectively (Table 3).

Table 1 Macronutrients, ascorbic acid, cartenoids and anthocyanins of *T. boudieri* truffle ($n = 3$).

Moisture[a]	78.23 ± 0.60
Fat[b]	8.01 ± 1.30
Proteins[b]	26.12 ± 0.67
Carbohydrates[b]	62.03 ± 0.58
Ash[b]	4.49 ± 0.78
Ascorbic acid[c]	12.20 ± 0.46
Carotenoids[c]	1.43 ± 1.03
Anthocyanins[c]	35.40 ± 0.18

[a] (g/100 g Fresh weight).
[b] (g/100 g Dry weight).
[c] (mg/100 g Dry weight).

Table 2 Mineral concentrations (mg/100 g dry weight) in *T. boudieri* truffle ($n = 3$).

K	1512.60 ± 1.41
Ca	474.28 ± 3.18
Mg	126.19 ± 1.33
Fe	170.29 ± 2.06
Na	26.89 ± 0.78
P	322.70 ± 0.64
Mn	3.01 ± 0.44
Cu	0.92 ± 0.15

3.2. Volatile compounds of T. boudieri truffle

In addition to their nutritional value, truffles have gained popularity as they have a unique aroma profile which was highly desirable in various food. The volatile compounds of *T. boudieri* truffle were extracted by hydrodistillation and analyzed by GC and GC/MS techniques. The identified compounds, their percentages and their experimental retention indices are listed in Table 4. Eight compounds were identified, accounting for 98.29% of the total volatiles. The volatile profile identified in the *T. boudieri* truffle seems to be original since, volatiles were dominated by fatty acids. In fact, palmitic acid (65.21%) and linoleic acid (15.7%) were the major compounds. Aldehydes such as hexanal (1.18%) and phenols such as thymol (2.65%) were found to be minor compounds. More than 200 volatile compounds have been reported from various truffle species. However, the volatile compounds identified so far, by GC/MS analysis, are hydrocarbons, alcohols, aldehydes, esters, ketones, benzene derivatives and sulfur compounds (Culleré et al. 2010). Bellesia et al. (1998) found that the major contributors to the aroma profile of *Tuber melanosporum* from central Italy were four volatile compounds including two aldehydes (2- and 3-methylbutanal) and two alcohols (2- and 3-methylbutanol). Moreover, Bellesia et al. (2001) found that octen-3-ol (mushroom alcohol) was the main volatile product of the *Tuber borchii* truffle.

3.3. Antioxidant properties of T. boudieri truffle

3.3.1. Phenolic, flavonoid and tannin contents

Vegetables and fruits are rich sources of antioxidants, such as vitamin A, vitamin C, vitamin E, carotenoids, anthocyanins, flavonoids and phenolic compounds, which prevent free radical damage, reducing the risk of chronic diseases. Thus, the consumption of dietary antioxidants from these sources

Table 3 Fatty acid composition of *T. boudieri* truffle (n = 3).

No.[a]	Compounds	Concentration (%)[a]	RI[b]
1	Hexadecanoic acid, methyl ester (C16:0)	20.40	1926
2	9,12-Octadecadienoic acid, methyl ester (C18:2 *n*-6)	54.10	2094
3	9-Octadecenoic acid, methyl ester (C18:1 *n*-9)	22.0	2099
4	Octadecanoic acid, methyl ester (C18:0)	3.40	2125

[a] The numbering refers to elution order of compounds from a HP-5MS column and their percentages were obtained by FID peak-area normalization. Fatty acids were methylated before analysis by GC and GC/MS.
[b] RI, retention indices calculated against C_7–C_{25} *n*-alkanes mixture on the HP-5MS column. Results are mean values of duplicate injection of their samples. Standard deviations from means did not exceed 1% of absolute values.

Table 4 Mean percentage of *T. boudieri* truffle volatile compounds.

No.[a]	Compounds	Concentration (%)[a]	RI[b]
1	Hexanal	1.18	799
2	Naphthalene	0.34	1186
3	Thymol	2.65	1307
4	Tetradecanoic acid (C14:0)	1.60	1762
5	Hexadecanoic acid (C16:0)	65.21	1964
6	9,12-Octadecadienoic acid (C18:2 *n*-6)	15.70	2141
7	Ethyl linoleate	2.13	2152
8	Octadecanoic acid (C18:0)	9.48	2163
	Total	98.29	

[a] The numbering refers to elution order of compounds from a HP-5MS column and their percentages were obtained by FID peak-area normalization.
[b] RI, retention indices calculated against C_7–C_{25} *n*-alkanes mixture on the HP-5MS column. Results are mean values of duplicate injection of their samples. Standard deviations from means did not exceed 1% of absolute values.

is beneficial in preventing cardiovascular diseases, especially atherosclerosis (Hu, 2000). Therefore, chemical constituents contributing toward antioxidant activities were investigated. The results of ascorbic acid, carotenoids and anthocyanins expressed on a dry mass basis were presented in Table 1. Ascorbic acid content (12.2 mg/100 g dry weight) was higher than those (from 1.56 to 9.6 mg/100 g) reported for different desert truffles (Sawaya et al. 1985; Al-Laith, 2010). Our results showed that carotenoid content (1.43 mg/100 g) in *T. boudieri* truffle, was also higher than the average value (0.68 ± 0.24 mg/100 g) reported for different desert truffles (Al-Laith, 2010). Furthermore, anthocyanin content (35.4 mg/100 g) in *T. boudieri* truffle was higher than those (from 4.5 to 23.6 mg/100 g) reported for other desert truffles species (Al-Laith, 2010).

Previous works showed that phenolic compounds were the major antioxidant components found in the mushroom extracts, as compared to ascorbic acid and β-carotene (Mau et al. 2004 ; Barros et al. 2007). To better study the antioxidant properties of *T. boudieri* truffle, the dried mushroom was extracted using various solvents of increasing polarity. After that, contents of phenolics, flavonoids and tannins were measured in the truffle extracts (Table 5). The yield of extractable compounds relative to the mass of dried fungi material ranged from 0.6 g/100 g (petroleum ether extract) to 21 g/100 g (methanol extract) (Table 5). Phenolic and flavonoid contents in the truffle extracts varied from 31.21 to 159.67 mg GAE/g and from 25.46 to 96.18 mg CE/g, respectively. Methanol extract has the highest phenolic and flavonoid contents. The highest content of tannins was recorded in the ethyl acetate (18.79 mg CE/g) and in the chloroform (17.81 mg CE/g) extracts (Table 5). The contents of phenolic compounds measured in *T. boudieri* truffle were found within the range of those previously reported for wild mushrooms (Wang and Marcone, 2011). The highest contents of phenolic compounds in the *T. boudieri* truffle extracts could be the cause of their important antioxidant activity.

3.3.2. DPPH radical-scavenging activity

Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. The free radical DPPH possesses a characteristic absorption at 517 nm (purple in color), which decreases significantly on exposure to radical-scavengers (by providing hydrogen atoms or by electron donation). Extracts of *T. boudieri* truffle were subjected to DPPH radical-scavenging activity, presented by IC_{50} values (Table 5). These extracts were able to effectively reduce the stable free radical DPPH with IC_{50} values ranging from 0.2 to 1.8 mg/ml, whereas the IC_{50} value of the synthetic antioxidant butylhydroxyanisol was 11 μg/ml. The methanolic extract containing the highest amounts of phenolics and flavonoids, showed the highest DPPH radical-scavenging activity (Table 5). These results were in agreement with the fact that free radical-scavenging activity is greatly influenced by the phenolic compounds of the sample (Cheung et al. 2003).

3.3.3. β-Carotene bleaching by linoleic acid assay

The potential of *T. boudieri* extracts to inhibit lipid peroxydation was evaluated using the β-carotene/linoleic acid bleaching test, which measures the extracts' capacity for inhibiting the conjugated diene hydroperoxide formation upon linoleic acid

Table 5 Extraction yields, phenolics, flavonoids, tannins and antioxidant activity assays (IC_{50} values) of *T. boudieri* truffle ($n = 3$).

Extracts	Yield (g/100 g dry weight)	DPPH radical-scavenging assay (mg/ml)	β-carotene/linoleic acid assay (mg/ml)	Phenolics (mg GAE/g extract)	Flavonoids (mg CE/g extract)	Tannins (mg CE/ g extract)
Petroleum ether	0.60^{e}	0.30 ± 0.24^{a}	0.73 ± 0.01^{b}	137.22 ± 0.21^{b}	46.18 ± 0.21^{bc}	12.51 ± 0.13^{c}
Dichloromethane	3.26^{ab}	0.60 ± 0.21^{c}	1.20 ± 0.01^{c}	108.35 ± 0.36^{c}	79.82 ± 0.16^{b}	11.76 ± 0.41^{c}
Chloroform	4.60^{b}	0.30 ± 0.12^{a}	0.68 ± 0.01^{b}	105.96 ± 0.12^{c}	70.26 ± 0.26^{b}	17.81 ± 0.32^{a}
Ethyl acetate	1.20^{c}	0.40 ± 0.90^{b}	0.78 ± 0.01^{b}	108.35 ± 0.26^{c}	95.12 ± 0.43^{a}	18.79 ± 0.28^{a}
Methanol	21.0^{a}	0.20 ± 0.02^{a}	0.36 ± 0.02^{a}	159.67 ± 0.08^{a}	96.18 ± 0.52^{a}	11.01 ± 0.46^{c}
Macerate	0.76^{e}	1.20 ± 0.19^{d}	2.70 ± 0.01^{d}	31.21 ± 1.02^{d}	25.46 ± 0.82^{d}	7.75 ± 0.73^{d}
Hot water extract	0.82^{e}	1.80 ± 0.07^{cd}	3.10 ± 0.02^{e}	31.86 ± 0.73^{d}	28.83 ± 1.02^{d}	7.09 ± 0.81^{d}

Table 6 Antibacterial activity (minimum inhibitory concentration, mg/ml) of *T. boudieri* truffle extracts.

Tested microorganisms	Petroleum ether	Dichloromethane	Chloroform	Ethyl acetate	Methanol	Macerate	Hot water extract
Gram-positive							
E. faecalis	2	1.2	1.2	2.5	0.625	6	6
S. aureus	5	2	2	5	0.516	8	8
S. epidermis	2	1.25	1.25	2	1.25	8	8
B. subtilis	3	0.25	0.25	3	0.25	8	8
Gram-negative							
S. typhimurium	3	2	2	3	1.2	8	8
E. coli	2.5	2	2	2.5	0.625	6	6
P. aeruginosa	2	1.3	1.3	2	1.3	5	5

oxidation. Results presented in Table 5 shows that *T. boudieri* extracts were efficient to inhibit the linoleic acid oxidation with IC_{50} values ranging from 0.36 to 3.1 mg/ml. Although the chemical butylhydroxyanisol presented the highest antioxidant activity (IC_{50} value = 8.2 μg/ml), natural compounds were of growing interest as compared with synthetic ones. As was found for the DPPH radical scavenging activity, the methanolic extract showed the highest antioxidant activity (Table 5). In previous studies, the antioxidant activities of methanolic extract of several commercial and medicinal mushrooms have been reported (Mau et al. 2004). These studies also claimed that the methanolic extracts of mushroom species presented the highest antioxidant activity on the lipid peroxidation.

3.4. Antimicrobial activity of T. boudieri truffle

The antimicrobial activity of *T. boudieri* 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 6, truffle extracts showed varying degrees of antibacterial activity against all tested strains. The MIC values of microbial strains were in the range of 0.5–8 mg/ml depending on the nature of the extract and the bacterial strain. Table 6 shows that methanolic extract seems to be the most effective on the tested strains. Furthermore, *B. subtilis*, *S. aureus*, *E. faecalis*, and *E. coli* were the most susceptible bacteria for this truffle extract. Hussain and Al-Ruqaie (1999) reported that methanolic extract of *Terfezia* truffles had antimicrobial activity against a wide range of both Gram-positive and Gram-negative bacteria. Furthermore, some researchers have also investigated the impact of extract solvents on the antimicrobial activity of truffles (Hussain and Al-Ruqaie, 1999; Janakat et al. 2004, 2005). The methanol extract of *Tirmania* truffles has been considered to provide the most antimicrobial inhibitory activity as compared with water and ethyl acetate extracts of *Tirmania* truffles.

4. Conclusion

The present paper is a contribution to the studies of the nutraceutical potential, lipid chemical profile, minerals and volatile compounds of *T. boudieri*, a wild edible desert truffle from Tunisia arid zone. Interestingly, *T. boudieri* truffle seems to be a good source of several important nutrients such as essential minerals and fatty acids. Furthermore, this truffle could be considered as antioxidant-rich food which is currently valued by consumers for their positive impact on the detrimental effects of ROS-induced oxidative stress responsible for many human degenerative diseases. Besides, *T. boudieri* truffle possesses an important antibacterial activity which may add value to truffle-related food. The identification, characterization and purification of their bioactive compounds were critical because these compounds could be used as potential therapeutic agents. Moreover, studies on *T. boudieri* truffle cultivation were important in order to increase its production.

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