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Investigation of Unsaturated Fatty Acids and Antioxidant Activity of Desert Truffles *Picoa lefebvrei* from Iran

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Abstract: Fatty acids, especially the unsaturated fatty acids (USFAs), are important as nutritional substances and metabolites in living organisms. Many kinds of fatty acids play an important role in the regulation of a variety of physiological and biological functions. Chemical characteristics, fatty acid composition and antioxidant activity of *Picoa lefebvrei* truffle oil were evaluated in this study. The hexane extract of the truffle *Picoa lefebvrei*, which was collected from northwestern Iran, was obtained by extraction in a Soxhlet apparatus. The fatty acids were converted to methyl esters and determined by GC and GC/MS systems. The main components were 9,12-octadecadienoic acid (ω-6) (41.03 %), 9-octadecenoic acid (ω-9) (34.35%) and hexadecanoic acid (palmitic acid) (10.82%). The hexane extract from the *Picoa lefebvrei* truffle detected as an important source of unsaturated fatty acids (75.38%). The antioxidant activity of the extract was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The results indicate the methanolic extract of this desert truffle possess considerable antioxidant activity.

Keywords: *Picoa lefebvrei*, Fatty acid, ω-6, DPPH.

Introduction

Desert truffles are a complex family of mycorrhizal hypogeous fungi, mainly containing species of the genera *Balsamia*, *Picoa*, *Terfezia*, *Tirmania* and *Tuber*, whose geographical distribution is limited to arid and semi-arid areas such as Iran, Tunisia, Egypt, Italy, France, and so on [1]. Desert truffles in Iran are locally known as "Donbalan" and usually appear after the rainy season in the months of early March to late April. 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 carbohydrate [2].

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, antiinflammatory, antimicrobial, anti-carcinogenic, immune-suppressor and antimutagenic properties [3,4]. 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,

Carbohydrates, DNA and proteins, resulting in cell death and tissue damage [5]. 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

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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 contributes to the protection against cancer, cardiovascular disease, diabetes, and other aging-related diseases by reducing oxidative stress [6]. The purpose of the present study was to determine the chemical composition of fatty acids and antioxidant activities of the hypogeous fungi in Iran.

Results and Discussion

Chemical composition of P. lefebvrei truffle:

Composition of the fatty acids of *P. lefebvrei* truffle was investigated using GC and GC/MS techniques. Percentages and retention time of the identified fatty acids are listed in (Table **1**) in the order of their elution on the HP-5MS column. The chromatographic analysis resulted in the identification of four fatty acids, accounting for 90.01% of the total fat content. Interestingly, the *P. lefebvrei* truffle was characterized by its higher content of the essential linoleic acid (ω-6) (41.03%). Furthermore, oleic acid (ω-9) and palmitic acid represent 34.45% and 10.82%, respectively (Table **1**). The unsaturated fatty acid contents (75.38 %) were higher than saturated ones (14.63 %). The high percentage of unsaturated fatty acids are largely attributed to ω- 6 and ω- 9 was the most predominant fatty acids. The ratio of total unsaturated fatty acid (UFA): SFA (saturated fatty acid) was 5.2 (Table **1**). Unsaturated fatty acid has also been reported to be as effective as Poly Unsaturated Fatty Acids (PUFAs) in the reduction of low density lipoprotein cholesterol in humans [7].

Table 1. Fatty acid composition of *P.lefebvrei* truffle

No.[a]	Compounds	Concentration (%)[a]	Rt[b]
1	Hexadecanoic acid, methyl ester (C16:0)	10.82	30.704
2	9,12-Octadecadienoic acid, methyl ester (C18:2 ω-6)	**41.03**	33.885
3	9-Octadecenoic acid, methyl ester (C18:1 ω-9)	**34.35**	33.998
4	Octadecanoic acid, methyl ester (C18:0)	3.81	34.467

[a] The numbering refers to the elution order of compounds from a HP-5MS column and the percentages were obtained by FID peak-area normalization.

[b] Rt: Retention time

Antioxidant properties of P. lefebvrei truffle

Fruits and vegetables are rich sources of antioxidants, such as vitamin C, vitamin A, 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 is beneficial in preventing cardiovascular diseases, especially atherosclerosis [8]. Therefore, chemical constituents contributing toward antioxidant activities were investigated. 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 *P. lefebvrei* truffle was subjected to DPPH radical-scavenging activity, presented by IC_{50} values (Table **2**). The methanolic extract containing the highest amounts of phenolics, showed the highest DPPH radical-scavenging activity (Table **2**). These results were in agreement with the fact that free radical-scavenging activity is greatly influenced by the phenolic compounds of the sample [9]. DPPH assay results showed good correlations with the total phenolic contents of the truffles, measured by the Folin-Ciocalteau assay: (r^2= 0.891, p< 0.0001).

Experimental

Chemicals and reagents

Trolox (water soluble equivalent of vitamin E) and Quercetin were obtained from Acros Organics. Acetic acid glacial, dimethyl sulphoxide, Folin-Ciocalteau reagent, hexane, methanol, sodium acetate and sodium carbonate were purchased from Merck. Gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrochloric acid 32% were obtained from Sigma–Aldrich.

Table 2. Total phenolic contact and radical scavenging capacity of methanolic extract of *P. lefebvrei*

Samples	DPPH Radical-scavenging Assay IC_{50} (mg/ml)	Total phenolic content (mg GAE/g Extract)
Methanolic extract	2.7 ± 0.19	159.67 ±0.2
Quercetin	0.72 ± 0.47	-

Values represent the mean of three experiments ± SD. Quercetin was tested as a reference compound in the DPPH assay.

Truffles:

Truffles of *P. lefebvrei* were collected during the months of March and April 2015 from the northern section of the Zanjan province, Iran. Voucher specimens of the sample have been deposited in the herbarium.

Lipid extraction:

The truffle lipids were extracted using n-hexane as previously described [10]. After lipids extraction, methyl esters of the fatty acids were prepared as follows. A sample containing 50 mg lipids was dissolved in 500µl n-hexane. Then, 200µl of potassium hydroxide 2M in methanol was added and the solution was mixed for 5 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.

Fatty acid analyses:

GC analysis was performed on a Shimadzu 15A gas Chromatograph equipped with a split/splitless injector (250°C) and a flame ionization detector (250°C). N_2 was used as carrier gas (1 ml/min) and the capillary column used was DB-5 (50 m × 0.2 mm, film thickness 0.32 µm). The column temperature was kept at 60 °C for 3 min and then heated up to 220°C with a rate of 5 °C/min and kept constant at 220°C for 5 min. The relative percentages of the characterized components are given in Table **1**. GC-MS analysis was performed using a Hewlett Packard 5973 with an HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm). The column temperature was kept at 60°C for 3 min and programmed to 220°C at a rate of 5°C/min and kept constant at 220°C for 5 min. The flow rate of helium as carrier gas was 1 ml/min. MS were taken at 70 eV. The fatty acids were identified by comparing their retention times and mass peaks with those of standard methyl ester mixtures and by the NIST-Wiley library data search. Relative percentage amounts were calculated from the peak area using a Shimadzu C-R4A chromatopac without the use of correction factors.

Preparation of P. lefebvrei extracts:

The dried truffle powder (20 g) was macerated during 48 h in 200 ml methanol /water (90/10), with continuous stirring at room temperature. The extract was filtered and then concentrated in a rotary evaporator in less than 10 min. Powders were weighed to calculate the yield, and kept on 20°C until used. Shortly before each experiment, the powder was dissolved in methanol at the desired concentration and tested for antioxidant activity and total phenolic content.

Assessment of total phenolic content:

Total phenol content in sample extracts was determined by the Folin–Ciocalteau calorimetric method [11]. A sample of stock solution (1.0 mg/ml) was diluted in methanol to final concentrations of 1000, 800, 60, 400, and 200 µg/ml. A 0.1 ml aliquot of the sample was pipetted into a test tube containing 0.9 ml of methanol, then 0.05 ml Folin-Ciocalteu's reagent was added, and the flask thoroughly shaken. After 3 min, 0.5ml of 5% Na_2CO_3 solution was added and the mixture was allowed to stand for 2 h with intermittent shaking. Then, 2.5 ml of methanol was added and the mixture was left to stand in the dark for 1 h. The absorbance measurements were recorded at 765 nm. The same procedure was repeated for the standard Gallic acid solutions and the concentration of total phenolic compounds in the extracts was expressed as mg of Gallic acid equivalent per g of sample. Tests were carried out in triplicate and the Gallic acid equivalent value was reported as mean ± SD of triplicate.

DPPH radical scavenging assay:

Radical scavenging activity of *P. lefebvrei* extracts against the stable free radical DPPH was measured as described previously [12]. Briefly, 0.1mM solution of DPPH was prepared in methanol and 4 ml of this solution was added to 1mL of sample solution in DMSO at different concentration 1.25, 2.5, 5μM. Thirty min later, the absorbance was measured at 517 nm. The Lowered absorbance of the reaction mixture indicated higher free radical scavenging activity and was calculated as per the following equation:

$\%I = [(A_{DPPH} – A_P) /A_{DPPH}] \times 100$

Where A_{DPPH} and A_P were the absorbance of the DPPH solutions containing methanol and sample extract, respectively. The dose-response curve was plotted by using the software Sigma Plot for Windows version 8.0 and IC_{50} values for extract was calculated. Quercetin was tested at the final concentration of 10 μM and used as the reference compound.

Conclusions

The results obtained from the present study indicated that the oil of *P. lefebvrei* truffle contained a high source of poly-unsaturated fatty acids (PUFAs). These results also showed that the methanol extract of this sample contained significant antioxidant activities. 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. The identification, characterization and purification of their bioactive compounds were critical because these compounds could be used as potential therapeutic agents.

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