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Nutritional and antioxidant profile of red truffles (*Terfezia claveryi*) and white truffle (*Tirmania nivea*) from southwestern of Algeria

Boufeldja Wahiba[1,3], Tehami Wafaà[1], Kherraf Asmaà[1], Abbouni Bouziane[2] and Benali Mohammed[1]

[1]Biotoxicology Laboratory, Department of Biology, Faculty of Natural Sciences and Life, University of Djilali Liabes, Sidi Bel Abbes 22000, Algeria
[2]Molecular Microbiology and Proteomic Laboratory, Department of Biology, Faculty of Natural Sciences and Life, University of Djilali Liabes, Sidi Bel Abbes 22000, Algeria
[3]University Center of Naâma, 45000 – Naâma, Algeria

ABSTRACT

The aim of this study was to determine content of primary and secondary metabolites and evaluates the antioxidant activity of methanol extracts of dried ascocarps from two species of truffles, Terfezia claveryi and Tirmania nivea which were harvested in Chellala-El Bayadh- Algerian desert. Quantitative determination of sugars, proteins and lipids shows that T. claveryi was very rich in these compounds with levels of 06.04±0.46; 12.87±0.09; 03.95 ± 0.543 g/100 g DM respectively. However, T. nivea present lower rates for the same respective compounds of 5.01±0.09; 11.97±0.03 and 2.15±0.093 g/100 g DM, respectively. The extraction of total polyphenols was made by steeping in methanol. The results showed the richness of both species in polyphenols ranging between 15.4±0.11 mg EAG/g for T. claveryi and 19.74±0.69 mg EAG/g for T. nivea. The contents of flavonoids and tannins were respectively 12.03±0.27 to 12.07±0.79 mg EC/g and 2.3±0.27 to 8. 28±0.02 mg EQ/g. T. claveryi and T. nivea extracts exhibit a comparable inhibitory concentrations of DPPH radical (IC50), respectively 1.02±0.002 and 1.01±0.002 mg /ml but remained significantly below (P <0.05) that of ascorbic acid (0.08±0.002 mg/ml). As well our truffles were rich in Vit.C and carotenoids. These show that the area factor affects the biochemical composition of truffles, even if they belong to different species. They keep biochemical and antioxidant characteristics nearly identical.

Keywords: *Terfezia claveryi*, *Tirmania nivea*, nutritional profile, secondary metabolites, antioxidant activity.

INTRODUCTION

Desert truffles locally called «Al-Terfess or Al-Kamaà» were the fruiting of some hypogeous fungi, edible for thousands of years and belonging to the class of *Ascomycota* [1]. They live in mycorrhizal symbiotic association with herbaceous or shrubby plants of the family Cistaceae especially *Helianthemum* and *Cistus genera*. These are seasonal wild mushrooms, of exceptional weather conditions, nutritious and great socio-economic importance. They are widely encountered around the Mediterranean (Turkey, Italy and Spain), North Africa (Algeria, Tunisia, Morocco and Libya) but also in the Middle East (Saudi Arabia, Kuwait, Iraq, Iran, Lebanon, Syria, and Jordan). The people of these latter regions are the largest consumers of truffles this because of their medicinal and organoleptic properties as well as their protein content (20-27%), fiber (7-13%), fat (3-7.5%), ascorbic acid (2-5%), and minerals [2,3]. According to Shavit desert truffle contains no toxic compound [4]. Besides their nutritional importance, their aroma and their unique flavor, truffles also have various biological activities, antiviral, antimicrobial, hepatoprotective, anti-mutagenic, anti-inflammatory and antioxidant [5]. On the other hand, the chemical composition and biological activities of Algerian truffles are not studied in deepened manner. Three species exist in this country; *Terfezia*, *Tirmania* and *Picoa* which are distributed in the semi-arid, steppe and Saharan zones [6]

The main objectives of the studies intended to identify and promote truffles harvested in the Algerian southwestern area on the one hand by determining the composition of primary and secondary metabolites, on the other the antioxidant activity of methanolic extracts by scavenging free radical 2,2-diphenyl 1-picrylhydrazyl (DPPH) and reduction of iron (FRAP).

MATERIALS AND METHODS

Sampling of truffles

Several outputs were made towards to Chelala El-Bayadh station (33.0331 Latitude, Longitude 33 0.0556183 1 '59 "North, 0 3' 20" East) from February to April. We have, for this purpose, followed the directions and advice of the nomads of the region in addition to observations of soil surfaces that were often swollen and cracked. Also the strong dominance of the genus *Helianthemum* locally called *Regig* was a good indicator of the presence of these fungi. The identification of species was made by macroscopic and microscopic observations of ascocarps and asci and with comparison descriptions and classification of Trappe and Astier [7, 8, 9] .Fresh harvested truffles was cleaned, dried for 20 days then ground into fine powder and stored at room temperature in dry place away from light until use [10]

Products and chemical reagents

The gallic acid, quercetin, catechin, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), the Folin-Ciocalteu reagent, bovine serum albumin, 2,6-dichlorophenol-indophenol (DCPIP), were from Sigma-Aldrich (Germany). All other chemicals and solvents were of analytical grade.

Determination of primary metabolites

The moisture content was determined at 105 °C for 3 h according to AOAC [11]. The ash or mineral content was determined using the method described by Audigie and Dupont [12]. The total sugars were assayed in 1 g of sample dispersed in 10ml of dimethyl sulfoxide solution (DMSO 25%). The mixture was incubated in a boiling water bath for 15 min and then 0.1 ml is diluted in 9.9 ml of distilled water. 0.5 ml of this mixture was homogenized with 0.5 ml of 5% phenol. And we added 2 ml of H_2SO_4 (75%) and we incubated in a boiling water bath in the dark for 15 min. the optical densities is at 492 nm against a standard range of Glucose Proteins were determined using the method of Lowry et al [13]. Bovine serum albumin standard solution was used. The proteins extract was obtained by adding 0.226 g of ammonium sulfate to 1mL of aqueous extract of the simple. After incubation at room temperature for 12 h, the solution was centrifuged at 13 400 g at 4°C for 20 min. The resulting supernatant was discarded and the pellet was taken up in saline phosphate buffer (pH 7.4), and then stored at -20°C until assay [14].The lipid content was determined using the method of Femenia et al using hexane and heating to reflux in a Soxhlet extractor [15]. According to Stojković et al energy was calculated as follows: Energy (kj) = 17 × (g proteins + g carbohydrates) + 37 × (g fat) [16].

Determination of secondary metabolites

Preparation of methanol extracts

10 g of sample were mixed with 100 ml of 80% methanol. The mixture was left stirring macerate at room temperature for 48 hours. The contents were filtered and then dried using a rotary evaporator (Heidolph Germany).

Evaluation of total polyphenol

The method adopted for the determination of total polyphenols was described by Waterhouse **[17]**, using Folin-Ciocalteu reagent produces a blue color, whose maximum absorption at 760 nm was proportional to the amount of polyphenols present in previews. The results were expressed in milligrams equivalent gallic acid per gram dry weight of truffle residue (EAG mg / g).

Total flavonoids

The flavonoid content of methanolic crude extracts was determined by spectrophotometry according to the method described by Kim et al [18]. A quantity of 490µl of methanol is mixed with 10µl of each extract then concentrated with 0.3 ml of a solution of nitrite sodium $NaNO_2$ 5%. And then 0.2 ml of $AlCl_3$ 10% were added. After 5 minutes 1 ml of NaOH (1M) was added to the mixture. Absorbance was measured at 510 nm. Flavonoid content was estimated from a calibration curve obtained under the same conditions with catechin at different concentrations (0.01 to 1mg / ml).

Tannin

50 µl of extract were added to 1.5 ml of the vanillin/methanol solution (4%) and then vortexed . 750µl hydrochloric acid were then added and the mixture was allowed to react at room temperature for 20 min. Reading was done by spectrophotometry at 550nm [19] (SpectroScan 40)

Carotenoids

Different methanolic extracts at appropriate concentrations (10 g/l) were analyzed by spectrophotometry UV/VIS (SpectroScan 40) at 470, 653 and 666 nm. The concentrations of carotenoids and chlorophylls a (C_a) and b (C_b) were determined according to the equations reported by Lichtenthaler and Wellburn [20] as follows:

Total carotenoids (mg/ml) = $1000 \times Abs_{470}$-2.86 C_a- $(129.2 \times C_b)/245$)
Chlorophyll a (mg/ml) = $15.65 \times Abs_{666}$-7.34 Abs_{653}
Chlorophyll b (mg/ml) = $27.05 \times Abs_{653}$-11.21 Abs_{666}
The total carotenoids are expressed in mg/ml of extract.

Ascorbic acid (vitamin C)

The dosage of ascorbic acid was carried out according to the method described by Klein and Perry [21] and modified by Yen et al [22]. A sample of extract was prepared from 0.5 g of substrate with 10mL oxalic acid 1% then centrifuged for 15 min at 3000g. Then 5 ml of supernatant were mixed with 9 ml of 2,6-dichlorophenol-indophenol (DCPIP) at 0.2 mM. This reaction medium was mixed for 15 seconds and the absorbance was made at 515nm.

Antioxidant Activity

DPPH radical scavenging activity

The anti-radical activity of truffles extracts was performed by the test of reducing the 1, 1-diphenyl-2 picrylhydrazyl (DPPH) (Sigma-Aldrich, Germany) according to the protocol of Blois [23]. This reduction in capacity was determined by a decrease in absorbance induced anti-radical substances [24]. Briefly, 0.1 ml of extract truffles was added to 2.9 mL of methanol solution containing DPPH radical. The mixture was shaken and incubated at room temperature in the dark for 30 minutes. Radical reduction which changes from purple ($DPPH^{\bullet}$) to yellow (DPPH-H) was measured at 517 nm. Methanol was used as a reference solution and the vitamin C as a standard. Percent inhibition was calculated using the following formula:

$$IP\% = [(At_0 - AT_{30}) / At_0 \times 100)]$$

At_0: absorbance of control (containing no antioxidant) 30 minutes
AT_{30}: Absorbance of the extracts measured after 30 minutes.
For each extract was determined the IC50, concentration truffles extracts capable of inhibiting 50% of DPPH.

Ferric-reducing antioxidant power analysis

The reducing power of truffle extracts was determined by the method of Oyaizu [25] .50µl of extract of different concentrations were added to 1 ml of potassium ferricyanide ($K_3Fe(CN_6)$) at 1% and 1 ml of a 1M phosphate buffer (pH=6). After incubation at 50°C for 20 min, 1 ml of trichloroacetic acid 10% was added to stop the reaction. 1 ml of this solution was added to 1 ml of distilled water and 0.2 ml of an iron chloride solution ($FeCl_3$) at 0.1%. The absorbance of the various samples was read using a spectrophotometer (SpectroScan 40) at 700 nm against a blank. Ascorbic acid was used as a standard in the same conditions at different concentrations (0.5 to 10 mg/ml).

Statistical analysis

The results were expressed as mean followed by the standard error. The ANOVA test was used for comparison of results and the probability of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Macroscopic and microscopic identification of truffles, based on morphological characteristics of ascocarps and asci, reveals two species; *Terfezia claveryi* locally called red truffle and *Tirmania nivea* called white truffle or Benhoureche (**Figure 1**).

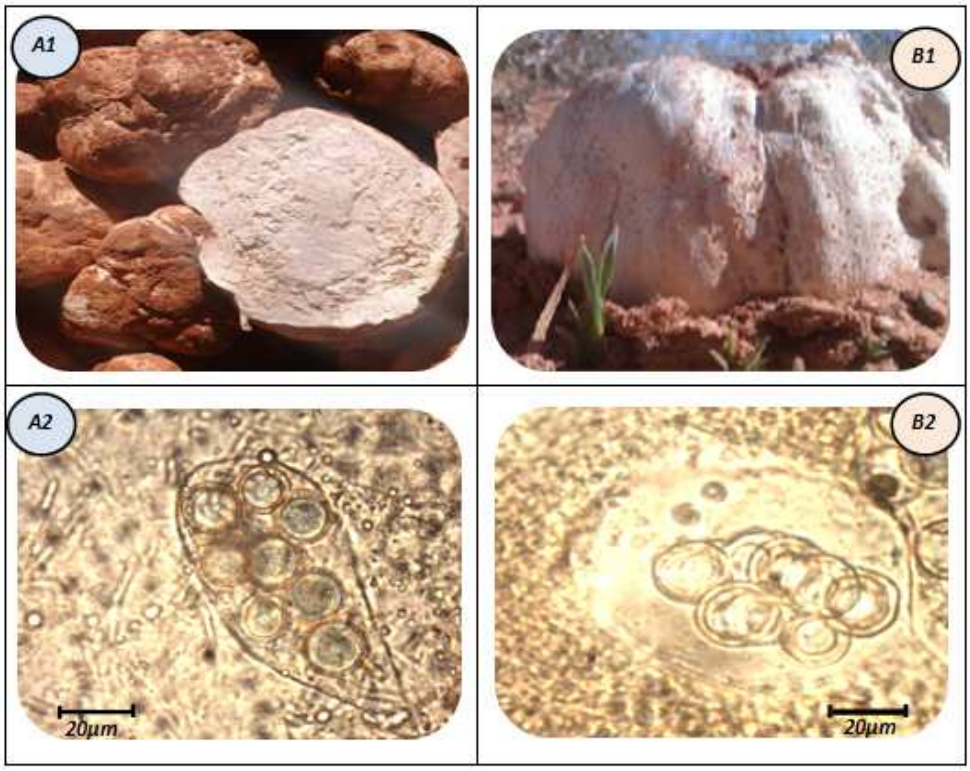

Figure 1: Macroscopic and microscopic observation of ascocarps and asci (x100) of *Terfezia claveryi* (A1, A2) and *Tirmania nivea* (B1, B2)

According to Ibn Tatto [26], *T. nivea* was endemic species of North Africa. Also, *T. claveryi* was a fungus that was found in abundance in Algeria [27], for both species harvested from the same area, the ascocarp was hypogeous with a lobed shape, a diameter of 4 to 10cm and a relatively smooth surface. Compared to the works of Janex-Favre et al, Josserand and Khabar [28, 29, 30], our species *T. claveryi* and *T. nivea* present different characteristics. *T claveryi* has a brownish-yellow peridium and a gleba with spongy appearance and yellow-pinkish color. Asci were globular and finely reticulated containing 7-8 globular spores. As for *T. nivea*, the peridium was milky white color, also a gleba with spongy appearance but white fleshy color. Asci of *T. nivea* were pear-shaped having 5 to 8 spores somewhat ovoid.

Nutritional value of truffles

The results of the nutrient composition are listed in **Table 01**. The humidity is important for Tc which represents 73.5±02.5%. This rate supports those reported by the work of Hussein and Al-Ruqaie [31], concerning the chemical composition of Fresh truffles from Saudi Arabia., *Tirmania nivea*, from the Middle East were higher in moisture compared to our varieties [32]. Our truffles also have a significant ash content of 5.5% almost similar to that reported by Hussein et al and Sawaya et al [31,33] ,who reported amounts ranging from 4.64 to 6.39% ash at the unpeeled truffle of Saudi Arabia. These results were consistent with the ecological role of truffles that were known to provide their symbiotic plants (Helianthemums) mineral elements such as P, N, Zn, K, Cu, Sr, and S [34,35] . *T. claveryi* was richer in proteins, sugars and lipids than *T. nivea*. However, it remains low in protein compared to the same species of Saudi Arabia, which contains 24.96% [31] and low in sugars compared to *T.claveryi* of Iran (28%) [36], our *T.claveryi* was richer in fat (3.95%) than that of Saudi Arabia (2%) [37] 0The nutritional value of *T.nivea* was small compared to the nutritional profile of the same species described by Al-Laith [32]. These results show that truffles have little fat but high level in proteins and mineral salts, conferring them, the qualifier of dietary food choices in human nutrition. Various studies have shown that dietary characteristics ascocarps truffles change from one species to another according to age, region, type of soil and climatic factors [38, 39, 40, 41]

Table 01: Nutritional and energetic values of *T. claveryi* and *T. nivea* ascocarps (g/100g DM)

Truffle species	humidity	ashes	Total sugar	Total protein	Total fat	Energy (kJ)
T. nivea	68 ±02	05,5 ±0,65	05,01 ±0,09	11,97 ±0,03	02,15 ±0,093	368,21 ±0,071
T. claveryi	73,5 ±02,5	05,5 ±0,20	06,04 ±0,46	12,87 ±0,09	03,9 ±0,543	467,62 ±0,364

Values are expressed as mean ± standard deviation. The comparisons were made using ANOVA test with P <0.05

Determination of secondary metabolic compounds

The methanol extracts of two species of truffles were different colors but with a common smell weakly spicy. Their respective yields relatively close, were of the order of 25±0.02 and 22±0.21% for *T. claveryi* and *T. nivea*. These high yields can be explained by their richness in phenolic compounds [41]

The evaluation of total polyphenols revealed significant amounts in *T. claveryi*, (15.4±0.11 mg GAE/g) and in *T. nivea* (19.74±0.69 mg GAE/g).

For flavonoids, the contents were similar for both species and were respectively 12.03 ± 0.27 mg CE/g and 12.07 ± 0.79 mg CE/g for *T. claveryi* and *T. nivea*.

The tannins showed, respectively, for both species, quantities of 8.28±0.02 mg QE/g and 2.3±0.27 mg QE/g **(Figure 2).**

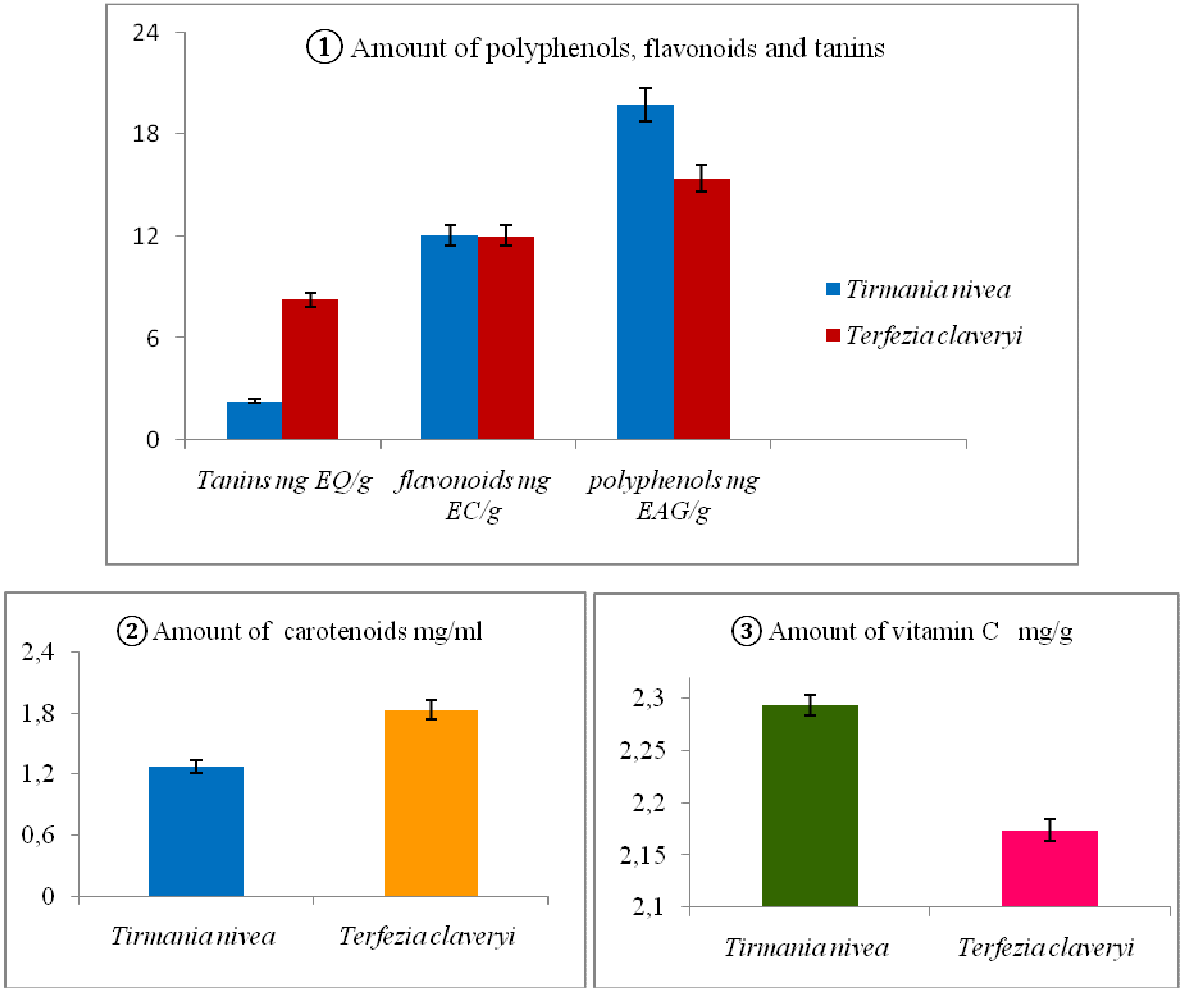

Figure 2: Contents of secondary metabolites in *T. claveryi* and *T. nivea*. ① Polyphenols, flavonoids and tannins, ② carotenoids, ③ vitamin C (n=3, ANOVA test, P<0,05)

Vitamin C content in *T. claveryi* (2,174±13 mg/g) is lower than that observed in *T. nivea* (2,294±15 mg/g) (P <0.05), However, we noted an increase regarding the amount of carotenoids in *T. claveryi* (1.83±0.04 mg/mL) compared with *T. nivea* (1.27±0.04 mg/mL) **(Figure 2)**. According to Al-laith [32] , the average contents of total polyphenols, flavonoids and vitamin C in the species *T. nivea* from different regions are, respectively, 1328±167, 293±32 and 9.6±0.15 mg/100 g of dry weight. Regarding carotenoids, the average concentration was 681±245 g/100 g dry weight. This explains the richness of *T. nivea* of Algeria in secondary metabolites. In a study of Sawaya et al[33], vitamin C content was observed with different percentages, respectively 1.8% and 5.1% for *T. claveryi* and *T. nivea*. Recent research of Beara et al [42] showed that the composition of secondary metabolites of two species of Serbian truffle *Tuber aestivum* and *Tuber magnatum* was influenced by the type of solvent and the type of extraction. Their

average levels of polyphenols and flavonoids were respectively 15.2 mg GAE/g DM, and 0.32 mg QE/g DM. Our species of desert truffles appear richer in these compounds that these species.

Evaluation of antioxidant activity of the extracts of truffles

DPPH radical scavenging method

Carotenoids are powerful antioxidants that protect cells against free radical attack [43] Similarly for vitamin C in addition to its stimulating effect on the immune system, it has an important antioxidant effect [44]. The increase in the antiradical activity against the DPPH depends significantly on the concentrations, generally we observed that the DPPH radical-scavenging effect increased as the concentration of the extract increased [45] induced by anti-radical substances, such as polyphenols, flavonoids and tannins that reduce fading and DPPH due to their ability to give hydrogen [24]. The methanol extracts of our species have trapped the free radical DPPH in dose-dependent manner. In addition, they showed lower activity than that of the ascorbic acid used as a reference antioxidant. These extracts showed trapping powers DPPH radical with almost similar rates of 42.06% and 43.78% respectively for *T. claveryi* and *T. nivea* at a concentration of 1 mg/ml **(Figure 3)** ($P<0.05$). According to Mau et al [46] reduction potentials of the methanol extracts of *Coriolus versicolor* fungi, *Ganoderma lucidum* and *Ganoderma tsugae*, were respectively 24.6%, 67.6% and 73.5% at the concentration of 0.64 mg/ml. As shown in **Figure 4** the methanol extracts of our truffles were endowed with antioxidant power moderate and comparable since their IC50 was relatively low compared to that of ascorbic acid.

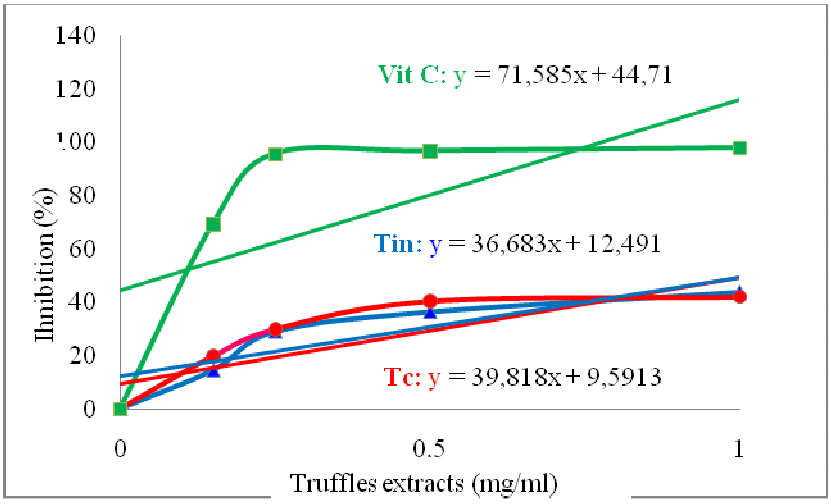

Figure 3: Inhibition Concentration of 50% DPPH by vitamin C (Vit C) and the methanolic extracts of truffles *T. claveryi* (Tc) and *T. nivea* (Tin)

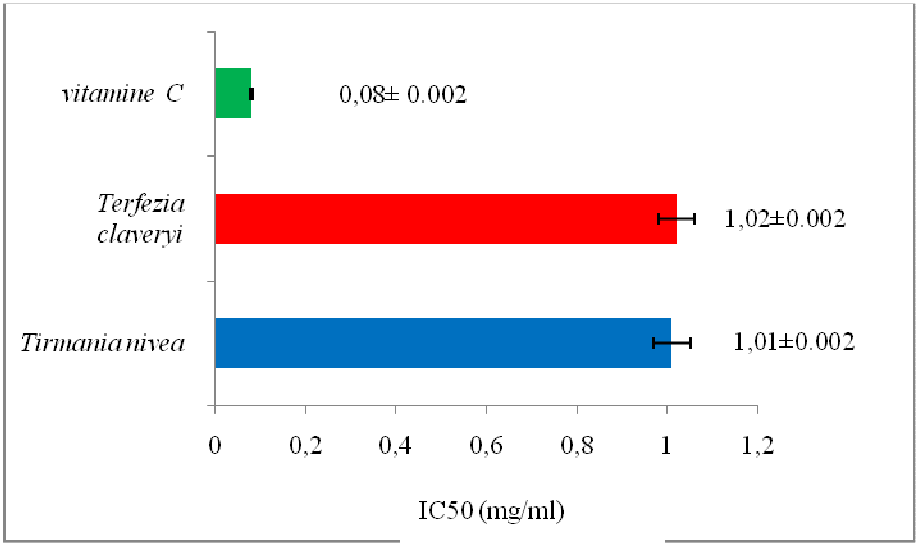

Figure 4: IC50 of methanol extracts of truffles and vitamin C (mg/ml)

Iron reduction test (FRAP)

The antioxidant activity of methanol extracts of truffles was evaluated using the method of FRAP. The latter was a simple test, rapid and reproducible. In this test, the yellow color of this solution changes to green or blue depending on the power reduction of each extract. The presence of the reducing in extracts causes reduction of Fe^{3+} ferricyanide complex to form Fe^{2+} ferrous. Therefore, Fe^{2+} can be assessed by measuring the increase in the density of the blue color in the reaction medium at 700 nm. The largest absorbance indicates the largest reduction of power [47] According to our results the increase in the reduction of iron is proportional to the concentrations used in our extracts 0.05, 0.075, 0.15, 0.25, 0.5 and 1 mg / ml **(Figure 5).**According to Yen and Duh, 1993, the power of reduction is associated with the antioxidant activity. Although the activities of our truffles are less important than that of the positive control (P<0,05) ,they remain considerable and confirm the results obtained by other researchers who show that the reducing power of extracts méthanoliques of *T.claveryi* and *T.nivea* is less important than that of the other species of mushrooms [46,48]

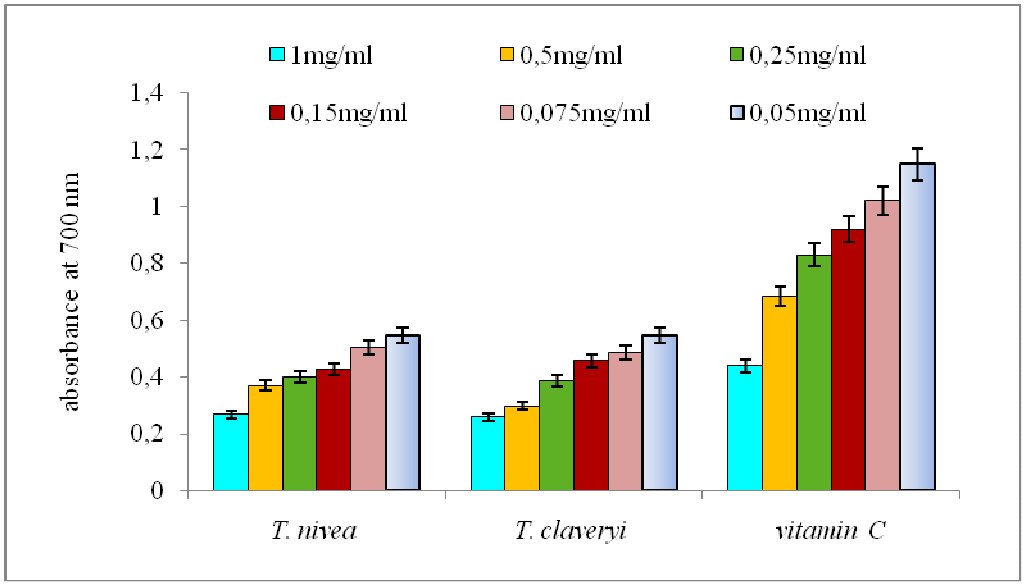

Figure 5: Antioxidant Activities of methanolic extracts of *T. claveryi*, *T. nivea* and vitamin C by the reduction of iron
Values are means followed by the standard deviation with P<0.05 indicates a significant difference

CONCLUSION

Truffles or Terfess was a luxury food and the exorbitant price explains their scarcity. They have a sensory quality appreciated in Algeria like other countries where they grow. In terms of basic nutritional principles, the truffle was an excellent source of protein, fat and minerals. The species *T. claveryi* from Chellala region was rich in primary metabolites with almost similar proportions for secondary metabolites and the antioxidant activity in comparison with *T. nivea*. These chemical differences were probably due to the species difference *T.claveryi* and *T.nivea* which under the soil and climate specific effect maintain symbiotic relationships with herbaceous plants in the region that may affect them differently. We used in these preliminary works crude methanol extracts for determination of secondary metabolites. The detailed characterization of these fractions can also open a way for the possible identification of compounds giving these truffles therapeutic benefits in addition to nutritional ones.

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