

## Comparison of Phenolic Contents and Antioxidant Activities for Black and White Desert Truffles Spread in Syria

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### Abstract

Desert truffle is considered as a type of Syrian wild fungi that spreads heavily, and it occupies important rank in folk medicine, where its aqueous extract is used for the treatment of some eye and skin illnesses, and people prefer the use of black truffle. This work interested in studying of the most available species; *Terfezia clavaryi* (black) and *Tirmania pinoyi* (white). The extracts of the two species of truffle were prepared by maceration with water, methanol, and ethanol 70%. Their total phenolic contents (TPC) and total flavonoid contents (TFC) were analyzed using Folin-ciocalteu and Aluminum chloride methods respectively, and their antioxidant activities was tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) methods, after microscopic examination and detection of phytochemical components. Then, phenolic profile of ethanolic 70% extract of black truffle *T.clavaryi* was studied by using LC-MS/MS. The values of TPC were between 25.3-43.6 mg GAE/g dry extract and TFC were between 2.5-6.8 mg QE/g dry extract. The values of DPPH (IC<sub>50</sub>) and FRAP were between 5.6-9.0 mg/ml and 90.1-153.4 μmol AAE/g dry extract respectively. There is a great similarity in content and activity of two species, also the aqueous extract is similar to other extracts in content and activity, and this means that the method of extract preparation in traditional medicine is reliable. It has been predicted about 14 phenolic compounds in the extract; as *p*-Hydroxy benzoic acid, Syringic acid and *trans*-Cinnamic acid. As a result, both truffle species are a new rich resource of antioxidant compounds which are usable in nutritional, cosmetic, and therapeutic applications.

**Keywords:** DPPH, Flavonoids, FRAP, LC-MS/MS, Polyphenols, Truffle.

### مقارنة المحتوى الفينولي والفعالية المضادة للأوكسدة للكمامة الصحراوية السوداء والبيضاء المنتشرة في سوريا

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### الخلاصة

تعد الكمامة الصحراوية أحد أنواع الفطور المنتشرة بكثرة في البادية السورية، وهي تشغل منزلة قيمة في الطب الشعبي، حيث تُستخدم خلاصتها المائية في علاج بعض الأمراض العينية والجلدية، ويفضل الناس استخدام الكمامة السوداء. اهتم البحث بدراسة النوعين الأكثر توافراً *Terfezia clavaryi* (السوداء) و *Tirmania pinoyi* (البيضاء)، إذ حُضرت خلاصات نوعي الكمامة بالتنقيع بالماء والميثانول والإيثانول 70٪، ودُرست محتواها الفينولي الكلي TPC والفلافونويدي الكلي TFC بطريقتي فولين-سيوكالتو وكلوريد الألمنيوم على الترتيب، وتم اختبار فعاليتها المضادة للأوكسدة الكابحة للجذر الحر DPPH والمرجعة للحديد FRAP، بعد الفحص المجهرى والكشف عن المجموعات الكيميائية. ثم تم دراسة التركيب الفينولي للخلاصة الإيثانولية 70٪ لنوع الكمامة السوداء *T.clavaryi* باستخدام تقنية LC-MS/MS. تراوحت قيم TPC بين 25.3-43.6 ملغ مكافئ من حمض الغاليك لكل غرام خلاصة جافة، و TFC بين 2.5-6.8 ملغ مكافئ من الكيرسيتين لكل غرام خلاصة جافة. تراوحت قيم IC<sub>50</sub> (DPPH) و FRAP بين 5.6-9.0 ملغ/مل، و 90.1-153.4 ميكرومول AAE لكل غرام خلاصة جافة على الترتيب. وُجد تشابه كبير بين النوعين من حيث المحتوى والفعالية، إضافة إلى تشابه محتوى الخلاصة المائية وفعاليتها مع بقية الخلاصات، وهذا يعني أن طريقة تحضير الخلاصة في الطب الشعبي يمكن الاعتماد عليها. تبين وجود 14 مركباً من المركبات الفينولية التي تم التحري عنها في الخلاصة، من أهمها *p*-Hydroxy benzoic acid و Syringic acid و *trans*-Cinnamic acid. تشكل الكمامة بنوعها مصدراً جديداً غنياً بالمركبات المضادة للأوكسدة والتي يمكن الاستفادة منها بالتطبيقات العلاجية والتجميلية والغذائية.

**الكلمات المفتاحية:** الكمامة، عديدات الفينول، الفلافونويدات، DPPH، FRAP، LC-MS/MS.

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## Introduction

Truffle is one of the wild edible hypogeous macrofungi, and it belongs to the phylum Ascomycota that do not have cap, gills and stalk unlike other fungi such Basidiomycetes<sup>(1)</sup>. Truffle has hundreds of species. The species that grow in arid and semi-arid areas in Mediterranean basin and middle east are called “desert truffle”, and are classified under genus of “*Tirmania*” and “*Terfezia*” that belong to Pezizaceae family<sup>(2)</sup>. Some species were identified in Syria as *Terfezia claveryi*, *Terfezia boudieri*, *Tirmania pinoyi* and *Tirmania nivea*<sup>(3)</sup>, where they grow naturally in the desert plains such as the region between Homs and Deir ezzor. The season of gathering is between March and May. The quantity of truffles is usually varied from season to season, and it is dependent on rainfall and temperature, so truffles absent some years when weather conditions are not suitable<sup>(4,5)</sup>.

Truffles have high values of proteins, minerals, unsaturated fatty acids, and antioxidant compounds<sup>(6,7)</sup>, that candidates it to be as complements and complete food. Bedouins use boiled truffle extract in treatment of trachoma and illnesses of eyes and skin, and they consume truffle as meat alternative<sup>(8)</sup>. The application of truffle extract on infected eyes was advised by Prophet Mohammad (Peace be upon him).

Species of truffle have wide biological activities such as antioxidant<sup>(9)</sup>, antimicrobial<sup>(10)</sup>, anti-inflammatory<sup>(11)</sup>, anticancer<sup>(12)</sup>, and antidepressant<sup>(13)</sup>. Types of wild and cultivated mushrooms are well known to contain various polyphenolic compounds which are considered as an excellent antioxidants<sup>(14,15)</sup>. Some articles studied the antioxidant activity and the antioxidant compounds of the two species of desert truffle (*Terfezia calveryi* and *Tirmania pinoyi*). The same species of desert truffles from different geographical regions may not exhibit the same chemical composition, because many environmental factors such as amount of rain, soil types and climatic changes have influence on the chemical profile<sup>(16)</sup>.

Polyphenols are considered as secondary metabolites, and they are wide group of compounds that differ in structure and resources. They help the organism to defend against oxidant stress and free radicals which damage the cells<sup>(17)</sup>. The use of modern technics in extraction of polyphenols increases efficacy, save time, and decrease amounts of solvents, furthermore they are convenient for thermo-sensitive compounds<sup>(18)</sup>. DPPH method is considered as one of the most common methods to determine the antioxidant activity according to hydrogen atom or single electron transfer, and it depends on a stable organic radical (2,2-diphenyl-1-picrylhydrazyl) which changes from purple to yellow color when interacts with antioxidants<sup>(19)</sup>. Ferric reducing antioxidant power (FRAP) is another method to determine the antioxidant activity

according to single electron transfer, and it measures reducing of Fe (III) to Fe (II) by antioxidants<sup>(20)</sup>.

Desert truffle is famous fungi that has crucial value in traditional medicine, and there is a lack of information about chemical components and the importance of antioxidant activity of truffles that are harvested in Syria.

This study aims to compare between the most two abundance species according to their phenolic contents and antioxidant activities.

## Materials and methods

### Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-tri-2-pyridinyl-1,3,5-triazine (TPTZ) were from (Cayman chemical, USA). Folin-ciocalteu's phenol reagent (FC) was from (Sigma-Aldrich, Switzerland). L(+)-Ascorbic acid (AA), Ferric chloride hexahydrate, Sodium acetate anhydrous, and Sodium carbonate anhydrous were from (Panreac, Spain). gallic acid (GA) was from (Titan biotech, India). Quercetin (Q) was from (Sigma-Aldrich, USA). Aluminium chloride hexahydrate was from (Riedel-de Haen, Germany). All other chemicals were of analytical grades.

### Collection and preparation of fungi

Ascocarps were collected from desert region in the east of Homs (Palmyra and Al-Sukhna) during April 2020. They were intact and showed no sign of spoilage. The identification of two species was made advising with Dr. Fawaz Al-Azmah and Dr. Hijazi Mando from faculty of agricultural engineering, Damascus university, by macroscopic and microscopic (OLYMPUS BX41, Japan) observations for fresh ascocarps and asci as described in the literature<sup>(21,22)</sup>. Ascocarps were cleaned, peeled, and sliced. The slices were dried in an oven at (40°C) and the size was reduced mechanically, then stored at room temperature in dry place away from light until use.

### Determination of chemical composition

These reactions were conducted according to standard methods<sup>(23-25)</sup>. Two species of truffle were evaluated before extraction to determine the presence of alkaloids, anthraquinones, saponins, coumarins, flavonoids, tannins, steroid and triterpenoid glycosides. The resulting solutions were observed for color change and/or precipitate formation to indicate positive results.

### Preparation of extracts

Three extracts of each type were prepared. Namely, powder of dried truffle was extracted by maceration with water, methanol and Ethanol 70% separately (10 g of dried truffle/100 ml of solvent) at room temperature, and the extracts were exposed to ultrasonication (PHYLO USH-10D, Italy) for 15 min at the beginning of extraction. After 24 h, the extracts were centrifugated and filtrated, then the solvent was evaporated by the rotary evaporator (Great Wall, Rotary evaporator R-1001-VN, China)

at (45°C). Finally, the dried extracts were stored in a refrigerator.

#### **Determination of total phenolic content (TPC)**

Total phenolic content was estimated by Folin–Ciocalteu method adapted from (Al-laith, 2010) <sup>(9)</sup> with a slight modification. Briefly, 20 µl of the extract, standard and the blank were added to 1.58 ml H<sub>2</sub>O, followed by the addition of 100 µl concentrated FC reagent. After 3 min standing at room temperature, 300 µl of sodium carbonate buffer (200 g/l) was added. The mixture was incubated at (40°C) for 30 min. The absorbance was measured at 736 nm (pg instruments UV-VIS Spectrophotometer T80+, UK). The phenolics concentration was determined by comparing with the standard calibration curve of gallic acid ( $y=0.0009x+0.00177$ ,  $R^2=0.9961$ ), and results were reported as mg of gallic acid equivalent (GAE)/ g of dry extract.

#### **Determination of total flavonoid content (TFC)**

Total flavonoid content was estimated by aluminium chloride colorimetric assay adapted from (Sembiring *et al.*, 2018) <sup>(26)</sup> with a slight modification. Briefly, 0.5 ml of extract or standard were added to 100 µl of the 10% aluminium chloride solution and followed by 1.5 ml of 96% ethanol. Finally, 100 µl of (1 M) sodium acetate was added to the mixture. Ethanol 96% was used as blank. The mixture was incubated at room temperature for 40 min protected from light. The absorbance was measured at 415 nm. Total flavonoid content was determined by comparing with the standard calibration curve of quercetin ( $y=0.0106x+0.077$ ,  $R^2=0.9986$ ), and results were reported as mg of quercetin equivalent (QE)/ g of dry extract.

#### **DPPH radical scavenging activity**

DPPH<sup>•</sup> scavenging activity of the extracts was estimated according to (Gouzi *et al.*, 2013) <sup>(27)</sup> with a slight modification. Various concentrations of extracts or positive control (ascorbic acid) (0.1 ml) were added to 2.9 ml of methanolic solution containing DPPH radical (0.1 mM). The absorbance was measured at 515 nm after the mixture was left to stand at room temperature for 30 min in a dark place. The methanol was used as a blank. DPPH free radical scavenging activity was calculated according to the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{[(\text{Abs}_0 - \text{Abs}_1)/\text{Abs}_0] \times 100}{1}$$

where Abs<sub>0</sub> was the absorbance of the negative control (methanol), and Abs<sub>1</sub> was the absorbance of the extract or the positive control.

The extract concentration that scavenges 50% of DPPH radical (IC<sub>50</sub>) was calculated from the graph that plots the percentage of radical scavenging activity against the extract concentration, Also IC<sub>50</sub> was calculated for the positive control (Ascorbic acid).

#### **Ferric Reducing Antioxidant Power (FRAP) assay**

The reducing power of extracts was estimated according to (Benzie *et al.*, 1996) <sup>(28)</sup> with a slight modification. The FRAP reagent contained 2,4,6-tripyridyl-s-triazine (TPTZ) solution (10 mM) in HCl (40 mM), ferric chloride solution (20 mM), and acetate buffer (0.3 M) at pH 3.6 in the ratio 1:1:10 respectively. 100 µl of the extract, or standard, or blank was mixed with freshly prepared FRAP reagent (3 ml). The mixture was incubated at (37°C) for 30 min. The absorbance was measured at 595 nm. The results of FRAP assay were determined by comparing with the standard calibration curve of ascorbic acid ( $y=0.002x+0.0067$ ,  $R^2=0.9991$ ), and were reported as micromoles of ascorbic acid equivalent (AAE)/ g of dry extract.

#### **LC-MS/MS analysis of selected phenolics**

Ethanol 70% extract of *T.claveryi* was selected to qualitative detection of some phenolic compounds in it. This detection was carried out using liquid chromatography-mass spectroscopy (Agilent technologies LC-MS/MS, USA) according to procedure of (Orčić *et al.*, 2014) <sup>(29)</sup> and (Kıvrak, 2015) <sup>(6)</sup>. Data were acquired in Multiple Reaction Monitoring (MRM) mode, using the optimized compound specific parameters (precursor ion, product ion, fragmentor voltage, collision voltage). Nineteen phenolic compounds which were detected are the following: (Apigenin, Catechin, Chlorogenic acid, *trans*-Cinnamic acid, *p*-Coumaric acid, 3,4-Dihydroxybenzaldehyde, Eugenol, Ferulic acid, Gallic acid, Gentisic acid, Hesperidin, Homogentisic acid, *p*-Hydroxy benzoic acid, Protocatechuic acid, Pyrocatechol, Rutin, Syringic acid, Vanillic acid, and Vanillin).

#### **Statistical analysis**

The results were expressed as a mean of three frequencies followed by standard deviation. Statistical analysis was performed using SPSS software (version 25). One-way ANOVA (Tukey's HSD) was used for comparison between means. Values were considered significantly different when P-value<0.05.

#### **Results and Discussion**

Macroscopic and microscopic identification of truffles, based on morphological characteristics of ascocarps and asci, reveals two species: *Terfezia claveryi* (black truffle) locally called "Obaidi", and *Tirmania pinoyi* (white truffle) locally called "Zubaidi" (Figures 1-4).

According to Alsheikh <sup>(21,22)</sup>, the ascocarp of two studied species have lobed shape and diameters of 4 to 10 cm. The studied species *T.claveryi* and *T.pinoyi* present different characteristics. *T.claveryi* has a brownish-yellow peridium and a gleba with fleshy appearance and yellow-pinkish color. Asci are subglobose and contain 8 globose spores ornamented with warts. *T.pinoyi* has a white peridium, and a gleba with fleshy appearance and white color. Asci are pear-shaped and contain 8 smooth globose spores which have double outer layer.



Figure 1. An ascocarp of *Terfezia claveryi* and a cross section of it.



Figure 2. An ascocarp of *Tirmania pinoyi* and a cross section of it.

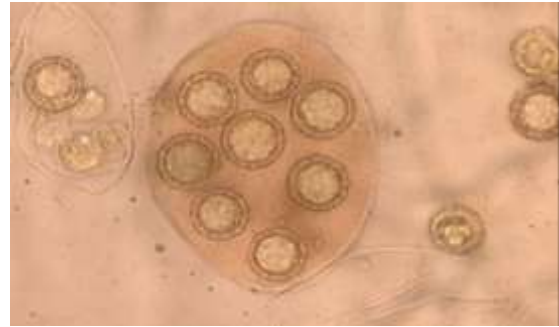


Figure 3. An ascus of *Terfezia claveryi* which contains 8 globose spores ornamented with warts (colored with alcoholic iodine, magnification  $\times 40$ ).



Figure 4. An ascus of *Tirmania pinoyi* which contains 8 smooth globose spores (colored with alcoholic iodine, magnification  $\times 40$ ).

The results of detection about phytochemical components for dried *T.claveryi* and *T.pinoyi* indicate the probability of containing alkaloids, coumarins, flavonoids, phenols, and steroid and triterpenoid glycosides. *T.claveryi* also may contain little of saponins (Table 1). This is the first time that determination of chemical composition to truffle was published.

Table 1. Detection of phytochemical components for two species of truffle: *Terfezia claveryi* and *Tirmania pinoyi*.

Phytochemical Constituents	Tests	Results	
		<i>Terfezia claveryi</i>	<i>Tirmania pinoyi</i>
Alkaloids	Dragendorff	+	+
	Mayer	+	+
	Hager	-	-
Anthraquinones	Borntrager	-	-
Saponins	Foam	+	-
Coumarins	Alcoholic FeCl <sub>3</sub>	+	+
	Fluoresence	+	+
Flavonoids	Shinoda	+	+
	NaOH	+	+
	Ammonia	+	-
Tannins/phenols	Ferric chloride	+	+
	Lead acetate	+	+
	Gelatin deposition	-	-
	Matchstick	+	-
Steroid and triterpenoid glycosides	Salkowski	+	+

Note: - = absent, + = present

The extraction's yields of for both studied species of truffle were in the following arrangement (aqueous > ethanolic 70% > methanolic). The aqueous extracts had the highest yield (44.2%,

43.5%) for *T.claveryi* and *T.pinoyi* respectively. When we compare the yields of two species for each solvent, we observe high similarity (Table 2).

**Table 2. Yields of extraction, phenolic and flavonoid contents, and antioxidant activities of *Terfezia claveryi* and *Tirmania pinoyi* extracts.**

Type of extract	Yield of extraction (%)	Total phenolic content (mg GAE/g dry extract)	Total flavonoid content (mg QE/g dry extract)	DPPH IC <sub>50</sub> (mg/ml)	FRAP (μmol AAE/g dry extract)
AC	44.2±9.4 <sup>a</sup>	43.6±9.6 <sup>a</sup>	3.2 ± 0.4 <sup>a,b</sup>	9.0 ± 1.8 <sup>a</sup>	126.1 ± 25.2 <sup>a,b</sup>
MC	16.7±7.3 <sup>b</sup>	28.7±4.4 <sup>a,b</sup>	7.5 ± 0.4 <sup>a</sup>	8.6 ± 0.6 <sup>a</sup>	136.3 ± 14.8 <sup>a,b</sup>
EC	21.9±3.0 <sup>b</sup>	32.7±0.4 <sup>a,b</sup>	7.7 ± 0.1 <sup>b</sup>	6.1 ± 0.7 <sup>a,b</sup>	153.4 ± 12.4 <sup>a</sup>
AP	43.5±1.4 <sup>a</sup>	25.3±4.6 <sup>b</sup>	7.9 ± 0.7 <sup>b</sup>	8.8 ± 1.4 <sup>a</sup>	90.1 ± 26.6 <sup>b</sup>
MP	11.5±0.9 <sup>b</sup>	30.4±7.1 <sup>a,b</sup>	0.1 ± 0.6 <sup>c</sup>	8.5 ± 0.4 <sup>a,b</sup>	134.8 ± 20.0 <sup>a,b</sup>
EP	23.8±0.7 <sup>b</sup>	33.3±2.8 <sup>a,b</sup>	7.8 ± 0.7 <sup>d</sup>	5.6 ± 0.8 <sup>b</sup>	135.1 ± 14.7 <sup>a,b</sup>

Where AC, MC, and EC: Aqueous, Methanolic, and Ethanolic 70% extracts of *Terfezia claveryi* respectively. AP, MP, and EP: Aqueous, Methanolic, and Ethanolic 70% extracts of *Tirmania pinoyi* respectively.

IC<sub>50</sub>: inhibitory concentration to 50% of substrate.

Values are means ± SD of three measurements. Means within each column with same letters don't differ significantly (P-value > 0.05) according to Tukey's test.

To evaluate the phenolic profile of the truffle's extracts, the total phenolic and flavonoid contents were determined. The total phenolic contents (TPC) for the analyzed truffle extracts which were evaluated by the FC method are shown in (Table 2). TPC values were between 25.3-43.6 mg GAE/g dry extract. Aqueous extract of *T.claveryi* (AC) was found to have the highest phenolic content, on the other hand, the lowest phenolic content was found in aqueous extract of *T.pinoyi* (AP). Statistical study demonstrated that there were no significant differences between three extracts of the same species in TPC values. Boufeldja *et al.* (30) found that TPC for methanolic 80% extract of *T.claveryi* was 15.5 mg GAE/g dry weight, and this value resembles to present finding for aqueous extract. Regarding to *T.pinoyi*, TPC for methanolic extract (30.4 mg GAE/g dry extract) has been obviously above the values of (Stojkovic *et al.*, 2013) (31) and (Gouzi *et al.*, 2013) (27) (13.19 and 2.1 mg GAE/g dry extract) respectively. Depending on previous results, the TPC values for the two studied species of truffle were higher than TPC values for foods which are classified as rich in polyphenols such strawberry and cherries. This high level of polyphenols assists truffle to overcome severe stress condition in deserts (17,32).

Values of total flavonoid contents (TFC) for the analyzed truffle extracts were between 2.5-6.8 mg QE/g dry extract [methanolic extract of *T.claveryi* (MC) and ethanolic 70% extract of *T.pinoyi* (EP) respectively] (Table 2). The flavonoid contents for ethanolic 70% extracts were the highest among other extracts for both species of truffle.

Values of TFC for *T.pinoyi* were approximately two fold the values of *T.claveryi*. Kivrak (6) found that TFC for ethyl acetate extract of *T.claveryi* was 4.71 mg QE/g dry extract, and this value is slightly higher than the value of ethanolic 70% extract in this study (3.74 mg QE/g dry extract). TFC of *T.pinoyi* had not been studied previously.

To extensively characterize the antioxidant potential of truffle extracts, two antioxidant assays were applied. The combination of more than one method helps in understanding the mechanism of action for antioxidants.

With regard to IC<sub>50</sub> values of DPPH (Table 2), they were between 5.6-9.0 mg/ml [ethanolic 70% extract of *T.pinoyi* (EP) and aqueous extract of *T.claveryi* (AC) respectively]. There were no significant differences between two species of truffle in IC<sub>50</sub> values of DPPH. Ethanolic 70% extract of *T.pinoyi* (EP) exhibited the highest scavenging activity with the lowest IC<sub>50</sub> (5.64 mg/ml). The reported scavenging activity for all extracts is too lower than the one showed by ascorbic acid (positive control, IC<sub>50</sub> = 0.11 mg/ml), but the comparison between pure compounds and extracts should be avoided, because they are individual/purified compounds and not mixtures, where the concentration of each individual compound is actually too lower. It seems that the IC<sub>50</sub> value for methanolic extract of *T.claveryi* (8.6 mg/ml) was comparable to that was reported by (Neggaz *et al.*, 2015) (2) (8.6 mg/ml) for an extract which was prepared using Soxhlet, by applying same protocol, but the value of maceration extract was (22.16 mg/ml). Gouzi *et al.* (27) and Stojkovic *et*

*al.*<sup>(31)</sup> found that IC<sub>50</sub> for methanolic extracts of *T.pinoyi* were (2.51 and 6.41 mg/ml respectively), and these values are lower than IC<sub>50</sub> in this study (8.5 mg/ml), by applying same protocol.

The values of FRAP assay for investigated extracts were between 90.1-153.4  $\mu$ mol AAE/g dry extract [aqueous extract of *T.pinoyi* (AP) and ethanolic 70% extract of *T.claveryi* (EC) respectively] (Table 2). There were no significant differences between the extracts of both truffle's species, also between three extracts of the same species in FRAP values. Our results were in agreement with those reported by (Dahham *et al.*, 2018)<sup>(33)</sup> where the values of reducing power for

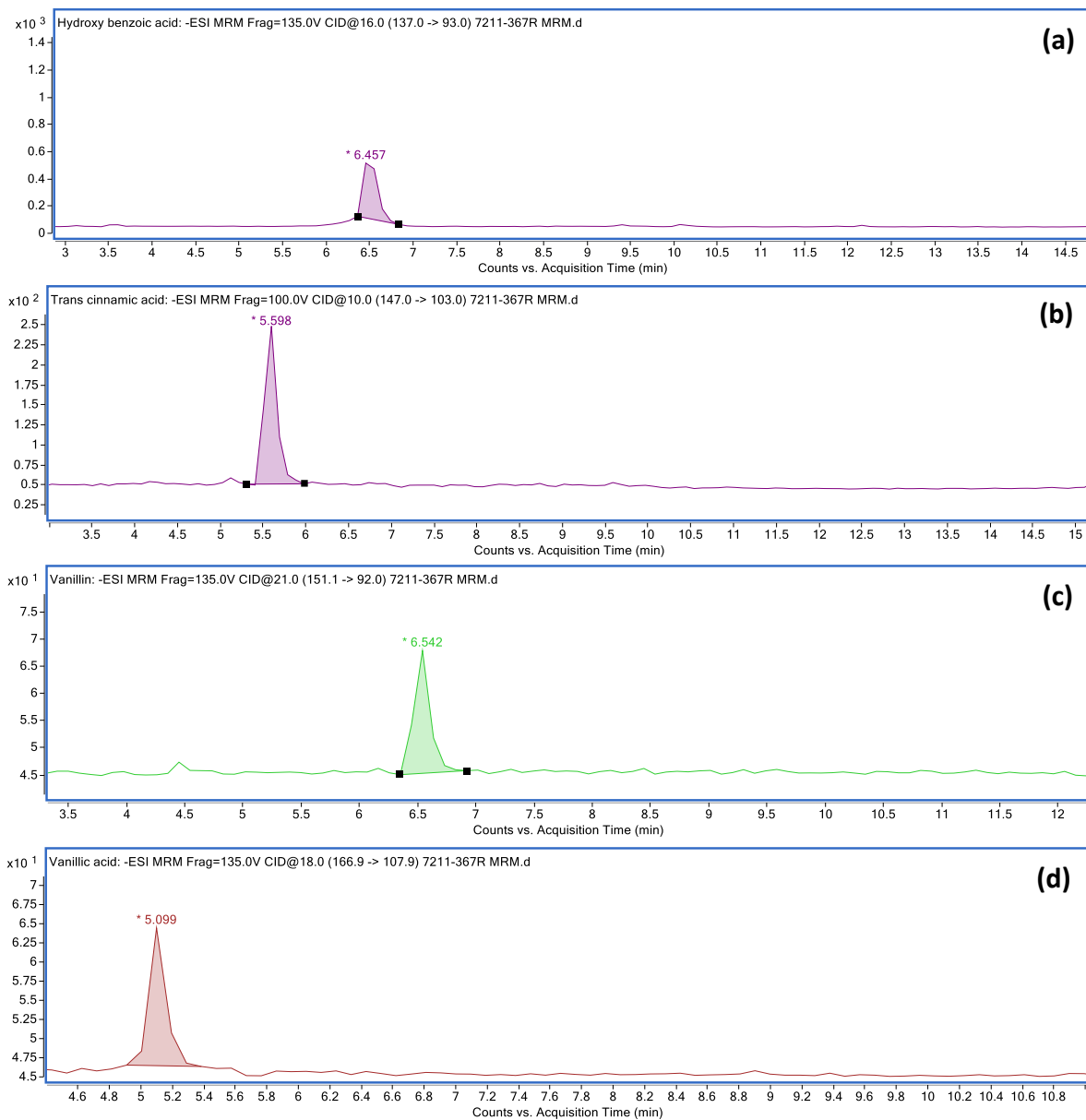
examined extracts of *T.calveryi* were also in this arrangement (ethanolic 70% > methanolic > aqueous). The value of FRAP for methanolic extract of *T.pinoyi* in this study (134.8  $\mu$ mol AAE/g extract) was higher than that in the work of (Gouzi *et al.*, 2013)<sup>(27)</sup> (126.8  $\mu$ mol AAE/g extract). These results demonstrate that the reducing power of truffle was high, and it was among the best foods in this field.

The results of LC-MS/MS revealed the presence of 14 from 19 phenolic compounds which were detected in ethanolic 70% extract of *T.claveryi*, and they are listed in (Table 3) starting by the most intensity. Figure 5 presents the chromatograms of some founded phenolics in the extract.

**Table 3. Phenolic compounds that are founded in ethanolic 70% extract of *T.claveryi* (starting by the most intensity).**

	Compound name	Total MW	Precursor ion [M-H] <sup>-</sup> (m/z)	Product ion [M-H] <sup>-</sup> (m/z)	Rt (min)
1	<i>p</i> -Hydroxy benzoic acid	138.12	137	93	6.457
2	Syringic acid	198.17	199	155.1	8.015
3	<i>trans</i> -Cinnamic acid	148.16	147	103	5.598
4	<i>p</i> -Coumaric acid	164.16	163	119	15.844
5	Gallic acid	170.12	169.1	125	7.275
6	Homogentisic acid	168.15	167	123	3.955
7	Protocatechuic acid	154.12	153	109	2.543
8	Vanillin	152.15	151.1	92	6.542
9	Ferulic acid	194.18	193	134	3.079
10	Rutin	610.5	609	301	6.376
11	Vanillic acid	168.15	166.9	107.9	5.099
12	Apigenin	270.24	269	151	2.115
13	Catechin	290.27	289	245	8.002
14	Hesperidin	610.6	611	303	15.209

Where MW: Molecular Weight, Rt: Retention time.



**Figure 5. Chromatograms of some phenolics in ethanolic 70% extract of *T. claveryi* by LC-MS/MS. Where (a): Hydroxy benzoic acid, (b): *Trans*-cinnamic acid, (c): Vanillin, (d): Vanillic acid**

Kıvrak <sup>(6)</sup> found that *p*-Hydroxy benzoic acid was the most abundance in the same studied truffle, and this resemble present results, But there is no existence of three compounds (Gentisic acid, Pyrocatechol, and 3,4-Dihydroxybenzaldehyde) as a result of this study, also there are no existence of two phenolics (Chlorogenic acid and Eugenol) which are founded by Vahdani *et al* <sup>(34)</sup>. This variation in phenolic profile for studied samples of the same species may be due to variation of synthesis of these compounds as a result to differences in climate conditions, soil and geographical regions, or the difference in the method of extraction and the differences in the parameters of analytical methods. The prediction of the presence of these phenolic compounds supports medical, cosmetic and nutritional applications of truffle.

## Conclusion

The results of this work reveal that two studied species of desert truffle are rich in phenolic compounds, and all of their extracts showed appreciable DPPH free radical scavenging activity and reducing power with FRAP assay, so they can act as antioxidant by donating a hydrogen or an electron. There is great similarity in content and activity between two studied species. The water can be use continually for extracting the phenolic compounds as it is used in traditional medicine, instead of other non-safe polar solvents, because the values of TPC are similar in the two cases. More profound studies of truffle and fungi generally which exist heavily in Syrian environment should be performed, that may provide a great economic and medical benefits.

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