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RESEARCH PAPER

Essential Constituents of Truffle in Kurdistan Region

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A B S T R A C T:

Essential constituents of truffle distributed in three different habitats of Kurdistan Region were investigated. Carbohydrates are the most abundant macronutrient ranged from 24.5% to 37%, the protein content was 4.4-5.0 %, fat 2-2.5 %, ash 3-3.2 % and moisture 73-75 %. High level of Chitin, a water insoluble polysaccharide was determined, (21-25 % of DM). The obtained results show high concentration of macro elements, potassium, Calcium, Magnesium and sodium, lower contents of essential elements iron, zinc, copper and chrome were found. Chemical constituents responsible for the antioxidant actions were examined as antioxidant activity (FRAP values), ascorbic acid, total phenolic, total flavonoids, β-carotene, tannin, lycopene and overall anthocyanins stated on a dry mass basis were (13-17 mmol Fe+2 equivalent/100g), (14-15.6 mg/100g), (14-20 mg/g DM), (6.19-8.41mg/100g), (1.55-2.16 mg/100g DM), (10.9-17.3 mg/g) (0.03-0.06mg/100g) and (10-15mg/100g DM) respectively. Total phenolics were the major antioxidant compounds as compared to β-carotene and ascorbic acid content.

KEY WORDS: Nutrient; Antioxidant Activity; Chitin; Macro Element; Hypogenous Fungi; Truffle; Kurdistan.
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1. INTRODUCTION

Depended on their good nutritious value and characterized agreeable aroma, epigeous macro fungi (mushrooms) and hypogeous macro fungi (truffles) for millennia conceder as expensive food in many nations (El Enshasy et al., 2013). Truffles are seasonal grow wild and the number of truffles generally varies from season to season depending on the amount of precipitation (Al-Shabibi et al., 1982, Dahham et al., 2018). Truffles also known as "black diamonds," they are one of the Tuberaceae family as important underground eatable fungi (Gao et al., 2001).

It is hypogenic fungi and grows between 5 and 10 cm deep underground in combination with plant roots of both angiosperms and gymnosperms (Patel, 2012), forming ectomycorrhizas (ECM) (Mello et al., 2006, Splivallo et al., 2015) that form symbiotic relationships with well-groomed hosts in a variety of ecosystems including subtropical cloud forests, flood plains, boreal forests, forests temperate, tree nurseries, renovation and Mediterranean forests (Bonito et al., 2010). Tree species (beech, birch, hornbeam, hazel, oak , poplar ,and pine) (Segneanu et al., 2012). Truffle's etymological root is from tuber, meaning, and "lump". It is referred to by the Latin as Tuber, derived from the word tumor (to swell) that indicates the shape of the globoid. In scientific term, truffles are micro aerobic (AL-Damegh, 2014), they grow all over the world in

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temperate, humid climates; similar warm, dry summers, cold, wet winters, and alkaline soil (AL-Damegh, 2014, Segneanu et al., 2012), though one of the most expensive mushrooms. Visually, truffles have different physical characteristics which make them very easy to differentiate them from the more popular mushrooms that are eaten every day. Truffles usually don't have stalks and root (Wang and Marcone, 2011). Truffles are very prevalent in Italian and French cooking; summer truffles (Tuber aestivum Vittadini) are the most common (or one of these) truffle types in Middle Europe (Carpathian Basin). Their habitats are wide-spreading; they occur in different European countries (from Great Britain, Russia, Sweden up to Spain), North-Africa (Morocco), Israel, besides the Arab Peninsula countries Like Bahrain, Iraq, Jordan, Syria, Saudi Arabia and Kuwait (Khojasteh et al., 2013, Segneanu et al., 2012). Truffles (Tuber aestivum) occur on different habitats of Hungary mainly in the central chain of mountains but in Middle and South Hungary on the lowlands, too. Soil requirements of summer truffle are relatively wide: best are the loamy soils with pH: 6.1-7.4; content of organic substances 3.1-9.1 %; P_2O_5 and K_2O content are 200, 500 ppm respectively (Vetter, 2007, BRATEK, 2010).

Truffles are known for their biological properties in humans, such as anti-inflammatory, antimicrobial, antioxidant , anti-mutagenic, antiviral, hepatoprotective, immune-modulating and antitumor activity (Gülsen et al., 2018, Segneanu et al., 2012). The studies have shown that certain truffles contain steroids as important components of champignon fragrance as well as volatile organic compounds. In addition to; Tuber magnatum Pico (white truffle) and Tuber melanosporum Vitt (black truffle) are highly regarded for their unique fragrance of sulfur (Gao et al., 2001). The white truffle (Tuber magnatum pico) is a hypogean fungus that lives fully underground; it is most sought after among the roots and those among the oaks. It is renowned for its luscious, heady white truffle which was considered the finest in Italy due to its complicated aroma, and is also the best and most expensive (Segneanu et al., 2012). The black truffle widely known as the "diamond of black cuisine" is considered the most aromatic (Segneanu et al., 2012), dimethyl sulfide is the predominant sulphur compound in black truffle (Gao et al., 2001). For this purpose, a conservation method must be established to maintain the unique aroma and flavor of truffles intact. Black truffles are almost always served raw (they cannot tolerate cooking heat) while white truffles are softer and more perishable (Segneanu et al., 2012).

2. Materials and methods

2.1. Collection of samples

Summer truffle samples of three separate Kurdistan Region ecosystems were collected (sample No.1: truffle of Zumar, sample No.2: truffle of Qaraj and sample No.3: truffle of Shangal) beginning of March 2015. The location of truffles was identified on the soil surface from cracks that appeared above the truffles (Figure 1). The fresh truffles were thoroughly cleaned, sliced, carefully and immediately frozen with lyophilizes (Nohita, Japan) at 50 ˚C in an oven until constant weight (5 days). Then dried Samples were crushed by grinder (IKA Werke, Germany) and sieved to obtain a uniform particle (20 meshes). The finely pulverized powder was capped and stored at room temperature in a dry (in hermetically sealed plastic bags up to and until use in dark.

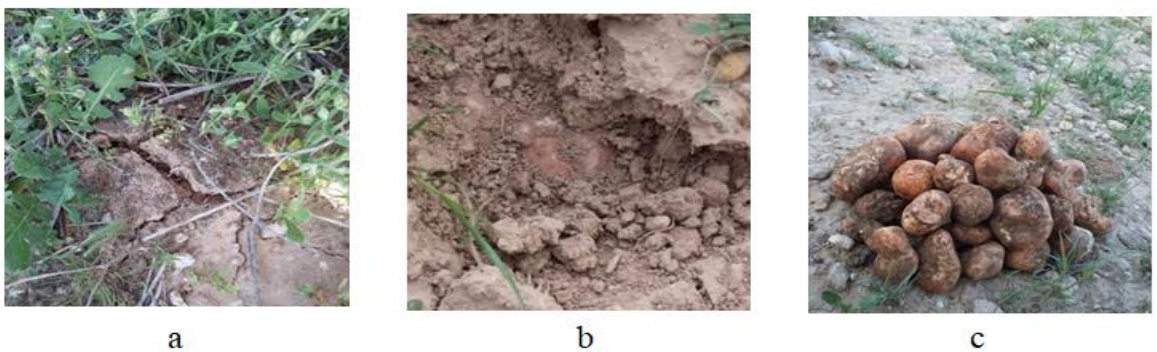

Figure 1. The species of *Truffles* from Kurdistan region of Iraq, a description of the organisms growing deep in the

earth and surrounding vegetation; b, c-Truffle ascocarps and deep soil truffles after removal of the surface soil.

2.2. Determination of primary metabolites

Truffle samples were analyzed according to the AOAC protocol for chemical composition (ash, starch, fat, moisture, and protein)(Horwitz and Latimer Jr, 2006). The humidity content was determined by drying samples in a 105 ˚C oven until constant weight was reached. The amount of ash was measured by burning the samples for 2.0 hours in a furnace at 600 µC in a crucible. The fat content at the solvent boiling point was estimated for 8.0 h with soxhlet extraction using hexane. Row chitin samples were treated at room temperature with 0.5 M HCL with a solid to acid solution ratio of 1:25 g/ml (w/v) for 2 h, and then washed with Distilled water. The acid treated solid was then treated at room temperature with a solution of NaOH in a ratio of 1:25 g/ml chitin to NaOH (0.5 M NaOH) ratio of (w/v) for 2 hours to obtain more pure chitin. Purified chitin was dried overnight at 55 ˚C (Vetter, 2007). The concentration of different mineral constituents was then analyzed separately using Flame Atomic Absorption Spectrometer (FAAS) , After the preparation of the sample solution when the summer truffle samples (0.25-0.26 g) weighed on the analytical balance, the samples were treated with 5.5 ml HNO3 14.6 M and digested for 15 minutes at 160 µC (Segneanu et al., 2012).

2.3. Determination of secondary metabolites

2.3.1. Ethanolic extraction of samples

10 g of each sample was cut into small pieces and then mixed with 100 ml of 50 percent (v/v) ethanol, then shaken at room temperature at 150 rpm for 24 hours, then centrifuged for 15 min at 12000 rpm. Under similar conditions the residue was re-extracted. The obtained extract was concentrated under vacuum at 40 ° C using the rotary evaporator and added 100 mL of 50 percent ethanol, well mixed, transferred to a dark plastic bottle and processed for analysis at -20 ° C (Boonsong et al., 2016).

2.3.2. Determination of Phenolic total content

Folin-Ciocalteu analysis was used, depending on the procedures, to determine total phenolic compounds in sample extracts described by Kaewnarin et al. (2016), (Keleş et al., 2011, Vamanu, 2014). In short, 1.0 ml of sample extracts transferred into volumetric flask containing 5.0 ml Folin-Ciocalteu reagent (1:10 Folin-Ciocalteu reagent: distilled water). After that 4.0 ml of sodium carbonate solution (7.5 percent, w/v) was added to the mixture and then the distilled water volume was completed to 10 ml. The volumetric flask permitted standing at room temperature for 60 min, then absorbance was read at 765 nm against blank. As standard, garlic acid was used in the same condition and standard curve of garlic acid ranging from 10-50 ppm. The quantity of phenolic compounds per gram of the sample extract was expressed as mg of garlic acid equivalent (GAE) (Aali et al., 2018).

2.3.3. Determination of Total Flavonoids Content

The Aluminum trichloride (AlCl3) method was used to estimate the total flavonoid compound of the sample extracts according to the method described by Gan et al. (2013), (Yan et al., 2017) with some modification. 0.75 ml of 10 percent Aluminum Trichloride (AlCl3) solution was applied after 5.0 min at room temperature, after the next 6.0 min 5.0 ml of 1.0 M NaOH was applied, then the volume of deionized water was completed to 25 ml. The mixture was shaken, and estimated at 510 nm against blank. Rutin was selected as a standard, with various concentrations. The results were expressed as mg rutin equivalent per gram of sample extract.

2.3.4. Determination of Vitamin C (Ascorbic acid)

The amount of vitamin C was taken by spectrophotometric process, the absorbance was measured at 515nm that described by Wahiba et al. (2016) with some modification. The collected sample was prepared by combining a appropriate quantity of samples with 10 ml of 1.0 per cent oxalic acid then centrifuged for 15 minutes, 5.0 ml of supernatant was combined with 9.0 ml of 0.2

mM of 2,4-dichlorophenol-indophenol then the amount completed with distilled water to 25 ml.

2.3.5. Determination of Tannin

When 0.05 ml of sample extracts is applied to 1.5 ml of 4.0 percent vanillin solution when prepared in methanol (w / v) and then mixed well, the dosage of tannin was determined. After adding 0.75 ml of hydrochloric acid, the mixture stayed to react for 20 min at room temperature. The absorbance was measured at 550 nm (Julkunen-Tiitto, 1985, Wahiba et al., 2016).

2.3.6. Determination of β-Carotene and Lycopene

The dried ethanol extraction (100 mg) was stirred continuously with 10 mL of a mixture of acetone-hexane (4:6) for 1 min to assess β-carotene and lycopene, and then filtered through whatman filter paper number 1. The contents for β-carotene and lycopene is calculated by the following equations:

Lycopene (mg/100 mL) = − 0.0458 ×A663 + 0.372 × $A505$ − 0.0806 × $A453$.

β-carotene (mg/100 mL)= 0.216 × $A663$ − 0.304 × $A505$ + 0.452 × $A453$.

Where A is absorbance of sample in this wavelength.

The results are expressed as mg of carotenoid/g of extract (Vamanu, 2014).

2.3.7. Determination of total anthocyanin's

To determine total anthocyanins, 0.05 g of dried truffle was mixed with 4.0 ml of distilled water (D.W), 3.0 min. sonicated in water bath, and 15 min. preserved at room temperature, with 10 min. continuous centrifugation mixing. 1.0 ml of pure solution was then mixed with 24 ml of buffer solution (KCl/HCl) pH 1.0. Subsequently 1.0 ml of the sample extract was mixed with 24 ml sodium acetate buffer (pH 4.5). The absorbance was read at 510 and 700 nm as well as the equation (Ab) was used to calculate the anthocyanin absorption as:

$Ab = (A_{510} - A_{700})$ pH 1.0 - $(A_{510} - A_{700})$ pH 4.5

Total anthocyanins (mg/100g) = $\frac{Ab}{\varepsilon} \times MW \times \left(\frac{V}{G}\right) 100$

Where ε is the coefficient of molar extinction taken as 26900, MW is the molecular weight of standard anthocyanins taken as 449,2, V is the final volume, and G is the gram sample weight (Al-Laith, 2010).

2.3.8. Ferric antioxidant capacity reduction assay (FRAP)

The FRAP value was calculated according to the methods described in Benzie and Strain (1996), (Gan et al., 2013, Vamanu, 2014) with some changes. The Ferric antioxidant capacity reduction reagent was prepared freshly by mixing (10:1:1 v/v/v) of 25 ml of 300 mM acetate buffer pH 3.6, 2.5 ml of (10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) was prepared in 40 mM HCl) and 2.5 ml of 20 mM of $FeCl_3$. Then about 2.0 ml of FRAP reagent was applied to the test tube containing 100 µL of standard solution sample extraction or volume, and 900 µL of distilled water. At room temperature at 593 nm, the absorbance was assessed against blank for 30 min after incubation. Ferrous sulfate was used as standard in the same condition at different range (0-100 mM) and ascorbic acid was used as reference. Using the Fe^{+2} calibration curve, the value of FRAP was measured and expressed as mM Fe^{+2} equivalent per 100 g sample or µM Fe^{+2} equivalent / gram extracted.

2.3.9. Total Antioxidant Capability evaluation

The total antioxidant capacity of the extract was assessed using the phosphor molybdenum method. The evaluation is based on the reduction of Mo (VI) to Mo (V) in an acidic medium solution by the extract and subsequent development of a green phosphate / Mo (V) complex. Extract volume (20-100µg / ml) was mixed with a reagent of 1.0 ml (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). After cooling down to room temperature, the amount of deionized water was completed to 25 ml. The mixture was incubated for 90 min in a boiling water bath at 95° C. The blank solution prepared in the same condition without containing sample extraction. Using a spectrophotometer the solution absorbance was measured at 695 nm. Extract antioxidant capacity was compared to standard ascorbic acid within such a range (20-100 µg/ml) (Aguilar Urbano et al., 2013).

3. Results and discussion

Wilderness truffles are a rich source of amino acids, protein, fatty acids, carbohydrates and minerals (Al-Naama et al., 1988, Bokhary and Parvez, 1993, Bokhary et al., 1989, Bokhary et al., 1987). Table 1 illustrated the effects of the composition of the nutrients on a dry foundation. The fresh truffle's average moisture content was

74 % DM, which was in close agreement with those studies (Al- Laith, 2010, Al-Laith, 2014, Hamza et al., 2016b). The truffle ash content was 3.07-3.18 % DM, which was similar to that for other sand truffles such as a Tirmania and Terfezia claveryi (Gan et al., 2013), and lower than what has been reported by (Ahmed et al., 1981, Akyüz, 2013, Yildiz et al., 2006). Tests obtained have shown that truffle protein content (4.40-5 % DM) was low and not comparable with the results obtained for Tirmania nivea truffle rates by (Hamza et al., 2016a, and Truffle Terfezia boudieri from the arid region of Tunisia (Hamza et al., 2016b). Crude fat (2-2.5 percent of DM) is similar to other species of mushrooms (Vetter and Kruzselyi, 2014) but lower than truffle what has been reported. Compared to our results, Nivea truffle had a comparatively great fat content (6.78 g / DM) (Hamza et al., 2016a). The fat content varied between 2.81 to 7.42 percent DM for other desert truffles (Wang and Marcone, 2011). Carbohydrates are the most abundant macronutrients in truffle, in this study were ranged from 24.5 to 37% DM which is less than what has been reported earlier for truffle. Nivea truffle (57.83 g/DM) by Hamza et al. (2016a), and 60% of carbohydrates for other desert truffles (Kagan-Zur and Roth-Bejerano, 2008). Chitin level is high (21.35-24.85% of DM), this is in accordance with what has been earlier reported by Vetter (2007), Chitin level of truffle aestivum is high, higher than the average of other common cultivated mushrooms, also found that chitin content of summer truffle (22.03 % in average) seems to be higher than normally in common mushrooms (for example: in Pleurotus species 11 %) (Del Toro et al., 2006).

Table 1: The concentrations percentage contents of moisture, ash, protein, fat, carbohydrates and chitin in the dried truffle samples. (Sample number 1 is from Zumar, number 2 from Qraraj and number 3 from Shangal.

Sample	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Chitin (%)
1	73.75 ± 1.08	3.18 ± 0.20	4.64 ± 1.18	2.05 ± 0.35	24.53 ± 0.58	22.36 ± 0.59
2	75.12 ± 0.69	3.07 ± 0.42	4.39 ± 2.52	2.48 ± 1.85	36.34 ±0.78	21.35± 0.77
3	73.63 ± 0.13	3.15 ± 0.35	5.16 ± 0.76	1.95 ± 0.98	36.89 ± 0.08	24.85± 0.25

The analytical methods used to characterize the samples indicated that the results obtained showed a high concentration of macroelements, potassium , calcium , magnesium and sodium, lower contents of iron, zinc , copper and chromium critical elements. Mineral spectrum of truffle fruit bodies is similar to composition of other edible mushrooms, i.e. they have high potassium and phosphorus contents, lower but important level from calcium, Magnesium and some microelements (Vetter and Kruzselyi, 2014). No traces of poison elements nickel, lead, cobalt and cadmium were detected; this is in accordance with (Akyüz, 2013). Accumulation (occurrence) of the problematic, "poisonous" elements (as Cd, As, Cr or V) was not established in truffle fruit bodies. According to Vetter and Kruzselyi (2014), several studies have also shown that truffles are a rich source of important minerals, such as Al, Cu, Na, Si, K, Ca, Mg, Mn, Fe and Zn (Darwesh, 2019, Nedelcheva et al., 2007). Truffle consumption contributes the highest percentage of the body's required mineral demands that shown in Table 2.

Table 2: Mineral concentrations in the truffle samples (mg/kg dry mass). (Sample number 1 is from Zumar, number 2 from Qraraj and number 3 from Shangal.

Sample	K	Ca	Mg	Na	Fe	Zn	Cu	Cr	Cd	Ni	Pb	Co
1	2462	1825	1751	1439	228.3	106.06	27.46	0.99	< d.I	< d.I	< d.I	< d.I
2	2525	1726	1600	2271	271.8	81.66	20.56	0.94	< d.I	< d.I	< d.I	< d.I
3	2583	1779	1489	1420	453.8	114.9	26.26	1.02	< d.I	< d.I	< d.I	< d.I

*< d.I: Under detections limit

This study investigated the chemical constituents that contribute to antioxidant activity in dessert truffle. The analyses of ascorbic acid, total phenolic, total flavonoids and total dry-mass anthocyanins were reported in Table 3.

Table 3: The concentration of ascorbic acid, total phenolics, total flavonoids and anthocyanins in total dried desert truffle (Sample number 1 is from Zumar, number 2 from Qraraj and number 3 from Shangal.

Sample	Extraction yield (g/100g dry weight)	Ascorbic acid (mg/100g)	Total phenolics (mg gallic acid/g extract)	Total flavonoids (mg rutin/g extract)	Total anthocyanins (mg/100g extract)
1	2.53	15.6 ± 0.07	14.25 ± 0.72	8.41 ± 1.01	10.02 ± 0.98
2	3.33	14.04 ± 0.62	18.32 ± 1.32	6.25 ± 0.09	15.03 ± 1.05
3	3.23	14.95 ± 0.54	19.95 ± 1.19	6.19 ± 0.81	15.03 ± 1.05

The chemicals responsible for the antioxidant activities have been established as ascorbic acid, anthocyanins, total esterified phenolics, total free phenolics and total flavonoids and total carotenoids (Patel, 2012).

The Results showed that the major antioxidant compounds is phenolics compound (14-20 mg/g DM), as compared to β-carotene (1.55-2.16 mg/100g), and ascorbic acid content (14.04-15.6 mg/100g)), (Table 3 & 4). The highest levels of phenolic compounds in desert truffles could be described by several harsh environmental conditions characterized by their natural habitat (Hamza et al., 2016b).There is a popular belief in Kurdistan about-truffle circumstances; there is a clear connection between thunderclap force and truffle production (number and size) of truffle. Hence, the ability of some plants or wild mushrooms to tolerate severe situations is probably due to their ability to neutralize the reactive oxygen species by increasing the level of antioxidants, specifically phenolic compounds (Al-Laith, 2014).

Table 4: Antioxidant activity (FRAP values), lycopene, of dried desert truffle samples (n=3). (Sample number 1 is from Zumar, number 2 from Qraraj and number 3 from Shangal.

Sample	Antioxidant activity (FRAP values) (mmol Fe^{+2} equivalent/100g)	Lycopene (mg/100g)	β-carotene (mg/100g)	Tannin (mg/g extract)
1	17.32 ± 3.06	0.0585 ± 0.28	2.12 ± 1.56	17.31 ± 2.38
2	13.08 ± 2.38	0.0865 ± 0.78	2.16 ± 1.28	10.94 ± 0.88
3	14.34 ± 2.07	0.0356 ± 0.93	1.55 ± 1.03	11.45 ± 1.58

Flavonoids (6.19-8.41mg/g DM) and anthocyanins (10-15mg/100g DM) (Table 3), they are well known as potentially protective substances, and work as antioxidants, having a scavenging effect on free radicals. Due to the antioxidant activity (Fig.2), it can be concluded that truffles has some medicinal properties. Therefore, it is consumed as food and for medicinal purposes as well. Total flavonoid concentration found in our desert truffle species in this study is higher than those in T. aestivum (0.093 mg/g DM) in Tirmenia nivea truffle. Truffles have a higher content of antioxidants including certain vitamin A, C, β-carotene and phenolic compounds that can scrounge peroxy radicals and chelate ferric ions, thus reducing lipid peroxidation (Al-Laith, 2010). The obtained results showed that ascorbic acid content (14.04-15.6mg /100gDM) is higher than Tirmenia nivea truffle (10.63 mg/100 g), whole carotenoids content (1.55-2.16 mg/100gDM g) is higher than Tirmenia nivea truffle (1.17 mg/100 g), and complete anthocyanins (10-15mg/100g DM) are lower than (29.1 mg/100 g) in Tirmenia nivea truffle reported earlier (Hamza et al., 2016a).

Total phenolic (14-20 mg/g DM) and flavonoid (6.19-8.41mg/g DM) contents of truffle in this study markedly higher than in Tirmenia nivea truffle samples 2.8 mg/g DM and 0.093 mg/g DM, for phenolic and flavonoids respectively (Hamza et al., 2016a). The highest content of tannins was recorded in sample number 1 from Zumar (17.31 mg/g extract), while the lowest content was recorded in sample number 2 that shown in (Table 4). The lycopene showed, respectively, for three samples, quantities of (0.0585 mg/100g), (0.0865 mg/100g) and (0.0356 mg/100g) that shown in (Table 4).

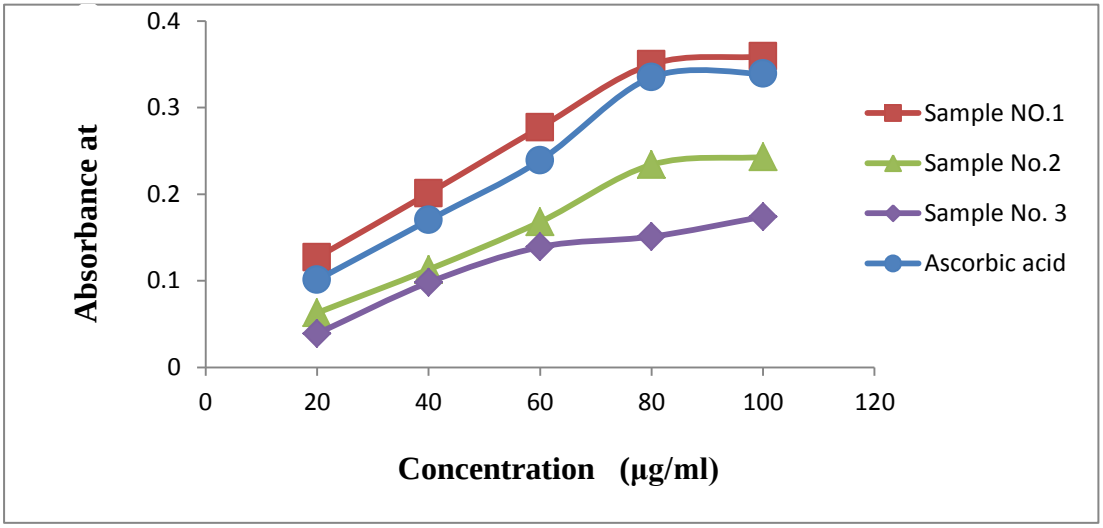

Figure (2): Full antioxidant strength of (●) for Ascorbic acid, (■) for *Truffle Sample No.1*, (▲) for *Truffle Sample No.2*, and (♦) for *Truffle Sample No.3.*

4.Conclusion

The present paper is contribution to the studies on the nutraceutical potential; primary metabolites, minerals and secondary metabolites of a wild edible desert truffle from Kurdistan region of Iraq. The results of this study, it is clearly indicated that the truffle was an excellent source of carbohydrate, protein fat and minerals compered than those of other truffles reported in literature. On the other hand, according to the results obtained from the study the protein content is significantly higher than most vegetables and fungi. The chemicals responsible for antioxidant activities were known as anthocyanins, ascorbic acid (Vitamin C), total esterified phenolic, complete free phe4.nolic, total flavonoids and total carotenoids. Throughout this analysis, the overall truffle content of phenolic and flavonoid was significantly higher than Tirmenia nivea truffle sample. More importantly, truffle seems to be a good source of several important nutrients and phytochemicals, such as phenolics, minerals and proteins. It could provide a healthy meat alternative that satisfied the nutritional requirements, especially for non-meat eaters.

Therefore, these finding provide evidences of truffle health benefits, owing it to it is antioxidant and nutrient properties which may be used nutraceutical or pharmaceutical industries.

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