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The Antioxidant Capacities and Consumption Per Capita of Edible Wild Species and Local Varieties Collected from Turkey within the GEF-Funded Biodiversity for Food and Nutrition (BFN) Project

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ABSTRACT: *Biodiversity for Food and Nutrition (BFN) Project enhancing global knowledge of biodiversity for food and nutrition by providing nutrition information about wild species that are currently underutilized or are disappearing from local diets. Wild edible plants are eaten raw, cooked, dried and processed depending on the region where they grow. Wild edible foods are collected from particularly in rural areas and many households village and used for home consumption or sold in local markets. The antioxidant capacity analyses of wild species collected from Turkey within the BFN Project were done by two different methods, DPPH (2,2-diphenyl-1-picrylhydrazyl radical scavenging effect) and TEAC (Trolox Equivalent Antioxidant Capacity/ABTS Method). The antioxidant capacity results of the 42 plant species collected from Turkey were compared to the results of the Butylated hydroxyl toluene (BHT) which is a reference synthetic antioxidant compound.*

The radical scavenging effect values of ***Rhus coriaria*** *(1825.3) and* ***Berberis crataegina*** *(1380.6) are the closest of BHT value according to the DPPH method. The antioxidant capacity values (µM trolox equivalent / g sample) of* ***Rhus coriaria*** *(3055.6) and* ***Berberis crataegina*** *(2362.1) are the closest of BHT antioxidant value (16651.9) according to the TEAC method. When the results of the two methods are compared, the antioxidant capacity results have paralells in most of the species. The annual intake of mM trolox equivalent antioxidant capacity was calculated by multiplying the consumption per capita of TEAC results. This process has led to a different ranking than above.*

Keywords: *Wild edibles, BFN, antioxidant capacity, DPPH, TEAC.*

Beslenme ve Gıda için Biyoçeşitlilik (BGB) Projesi Kapsamında Türkiye'den Toplanan Yenilebilir Yabani Tür ve Yerel Çeşitlerin Antioksidan Kapasiteleri ve Kişi Başı Tüketimleri

ÖZ: *Gıda ve Beslenme için Biyolojik Çeşitlilik (BFN) Projesi ile şu anda yeterince kullanılmayan ya da yerel diyetlerden kaybolan vahşi türler hakkında beslenme bilgisi sağlayarak küresel gıda ve beslenme biyolojik çeşitliliğin önemini arttırmak amaçlanmıştır. Yabani yenilebilir bitkiler, yetiştiği bölgeye bağlı olarak çiğ, pişirilmiş, kurutulmuş ve işlenmiş olarak tüketilirler. Yabani yenilebilir gıdalar, özellikle kırsal alanlardan ve birçok evin bahçesinden toplanır ve ev tüketiminde kullanılır*

veya yerel pazarlarda satılırlar. BFN Projesi kapsamında Türkiye'den toplanan yabani türlerin antioksidan aktivite analizleri, DPPH (2,2-difenil-1-pikrilhidrazil radikal temizleme etkisi) ve TEAC (Trolox Eşdeğeri Antioksidan Kapasitesi / ABTS Yöntemi) olmak üzere 2 farklı yöntemle gerçekleştirildi. Türkiye'den toplanan 42 bitki türünün antioksidan aktivite sonuçları, bir referans sentetik antioksidan bileşik olan Bütilledirilmiş Hidroksi Toluen'in (BHT) sonuçları ile karşılaştırılmıştır.

DPPH yöntemine göre, ***Rhus coriaria*** *(1825.3) ve* ***Berberis crataegina*** *'un (1380.6) radikal temizleme etkisi değerleri BHT'nin sonucuna en yakın değerlerdir. TEAC yöntemine göre,* ***Rhus coriaria*** *(3055.6) ve* ***Berberis crataegina*** *(2362.1) antioksidan kapasite değerleri (μM trolox eşdeğeri / g örneği) BHT değerine (16651.9) en yakın değerlere sahiptirler. İki yöntemin sonuçları karşılaştırıldığında, antioksidan aktivite sonuçları türlerin büyük çoğunluğunda benzer değerlere sahip bulunmuştur. mM trolox eşdeğer antioksidan kapasitesinin yıllık alımı, türlerin kişi başına düşen yıllık tüketimin TEAC sonuçları ile çarpımı sonucu hesaplanmıştır. Bu işlem yukarıda belirtilenlerden farklı bir sıralamaya neden olmuştur.*

Anahtar Sözcükler: *Yenilebilir yabani bitkiler, BFN, antioksidan kapasite, DPPH, TEAC.*

INTRODUCTION

In 2012, the Global Environment Facility (GEF) fund a new project called "Mainstreaming Biodiversity Conservation and Sustainable Use for Improved Human Nutrition and Well-being (BFN project)" which operates in Kenya, Sri Lanka, Turkey, and Brazil. Coordinated by Bioversity International and co-implemented by FAO and the United Nations Environment Program (UNEP).

In Turkey, Biodiversity for Food and Nutrition (BFN) project tries to mainstream biodiversity conservation and sustainable use for improved nutrition into national food and livelihood security strategies formed or strengthened. The BFN project is enhancing global knowledge of biodiversity for food and nutrition by providing nutrition information about wild species that are currently underutilized or are disappearing from local diets. Wild edible foods are collected from particularly in rural areas and many households village and used for home consumption or sold in local markets. Wild edible plants are eaten raw, cooked, dried and processed depending on the region where they grow.

According to the World Health Organization (WHO) (Anonymous, 1991), using edible and/or medicinal plants about three-quarters of the world population rely upon traditional remedies for their health care. In fact, herbs/plants are the oldest friends of mankind. They not only provided food but also served the humanity to cure common ailments. Edible plants have provided the modern medicine with numerous plant derived therapeutic agents (Hudaib *et al*., 2008; Arasimowicz *et al*., 2009; Caylak, 2011). Some phytochemicals (phenolics, flavanoids, vitamin E, vitamin C, lycopene, beta-carotene etc.) which are herbal and identified as beneficial chemicals are evidence of the antioxidant activity of the plant. The antioxidant activity values changes according to the amounts of this beneficial phytochemicals containing in inspected species (Bahorun *et al*., 2006; Isbilir, 2008; Arasimowicz *et al*., 2009). It is known that red-purple colored plant species which are rich as anthocyanin show antioxidant activity sweeping the DPPH and ABTS radicals.

The food we eat, the drugs and medicines we take, the air we breathe, and the water we drink include fried foods, alcohol, tobacco smoke, pesticides, air pollutants, and many more which are cause free radicals (Burt, 2004). Free radicals are atoms or molecules that are highly reactive with other cellular structures because they contain unpaired electrons. Free radicals are natural by-products of ongoing biochemical reactions in the body, including ordinary metabolic processes and immune system responses. Antioxidants, as "free radical scavengers", are compounds that either reduce the formation of free radicals or react with and neutralize them. Antioxidants often work by donating an electron to the free radical before it

can oxidize other cell components. Once the electrons of the free radical are paired, the free radical is stabilized and becomes non-toxic to cells (Burt, 2004; Caylak *et al*., 2007; Bahorun *et al*., 2006; Isbilir, 2008). They are used for the stabilization of polymeric products, of petrochemicals, foodstuffs, cosmetics and pharmaceuticals (Isbilir, 2008).

MATERIALS AND METHODS

The antioxidant capacity and radical scavenging effect results of the 42 plant species collected from the Black Sea, Mediterranean and Aegean Region pilot sites in Turkey were compared to the results of the Butylhydroxyltoluene (BHT) which is a reference synthetic antioxidant compound and available commercially.

Extraction

Antioxidant compounds of the sample are extracted firstly if the sample is in the completely texture (fruit, vegetable, herb) form. For the edible plants which are in the fresh herb form: 9.5 mL methanol with 80% purity is added to the 0.5 g sample and extraction is made in the orbital shaker during 1 hour. The tube is centrifuged in 5204 g during 10 minutes. Later, the liquid phase in the tube is collected. 9.5 mL methanol with 80% purity added to the residual part in the tube and the same procedures are repeated 3 times. After this procedure, the extracts are taken to the 50 mL volumetric flask and it is diluted to the volume of the volumetric flask (Cemeroglu, 2010; Ayas *et al*., 2014, 2017).

Radical Scavenging Effect with Using DPPH (2,2-diphenyl-1-picrilhydrazil)

After extraction, analysis may be done directly or diluting with methanol. Preparation of the DPPH* radical solution (1mM): It should be prepared depending to the number of samples. 600 µl DPPH is taken to the each of the test tubes. Different volumes (20-40-60-80-100 µl, these values may be changed according to the sample) of the sample extracts are added to the test tubes. The total volume of the each tube is completed to the 6 mL with methanol. The tubes are vortexed; the incubation procedure is made during 15 minutes in a dark place in the room temperature. 5400 µl MeOH is added to the 600 µl DPPH for using as a replicate sample. The incubation of the replicate sample is made during 15 minutes. The absorbance values of the samples are read in the spectrophotometer with 517 nm at the end of the incubation procedure.

Calculation: the percentage inhibition values corresponding to each volume of the sample are calculated according to the equation below.

% Inhibition=[(ADPPH–Aextract)/ADPPH] x 100

The determined inhibition values are graphed with the volume values of the samples, linear regression analysis is made, the curve related to the sample and linear equation of the curve are determined. EC50 value is calculated with using this equation. Conclusion of DPPH value (1/IC50) was showed the reverse of the fresh plant value in terms of mg which inhibits 50% of the 1g DPPH radical (Cemeroglu, 2010; Ayas *et al*., 2014, 2017).

Trolox equivalent antioxidant capacity (TEAC) method/ABTS radical cation decolorization assay

7 mM ABTS solution containing 2,45 mM potassium persulfate is waited during 12-16 hours at room temperature and dark place for ensuring ABTS radical solution, phosphate buffer saline (pH:7.4). The radical solution is diluted to the 0.700±0.02 absorbance value at 734 nm wavelength in the spectrophotometer (Obón *et al*., 2005; Papandreou *et al*., 2006; Apak *et al*., 2007; El Rayess *et al*., 2014). 0.2 mL is taken from the ABTS radical solution diluted in microcuvette and the starting absorbance is recorded. The different volumes (as 1, 2, 3, 4, 5 µl) of extract are added to the radical solution in the microcuvette and then the absorbance values are read during 6 minutes in every 1 minute by Thermo Scientific™ Multiskan™ GO microplate reader, the values are

recorded. The inhibition values determined in the end of the 6 minutes are plotted in a graph against to the sample amounts and linear regression analysis is made (Cemeroglu, 2010; Ayas *et al*., 2014; 2017). The slope of the extract curve is rated to the slope of the trolox curve and TEAC value is calculated and expressed as Trolox equivalents in μM (Table 1) or mM (Table 2).

Consumption Per Capita

In order to determine the consumption per capita (kg/year) of plant species, research has been done with monograph technique. The data were compiled by survey using question-answer method.

RESULTS AND DISCUSSION

The antioxidant capacity analyses of wild species collected from Turkey within the BFN Project were done by two different methods, DPPH (2,2-diphenyl-1-picrylhydrazyl radical scavenging effect) and TEAC (Trolox Equivalent Antioxidant Capacity/ABTS radical cation decolorization assay Method). The antioxidant capacity results (Table 1) of the 42 plant species collected from Turkey were compared to the results of the Butylated hydroxyl toluene (BHT) which is a reference synthetic antioxidant compound and available commercially.

The radical scavenging effect values of *Rhus coriaria* (1825.3) and *Berberis crataegina* (1380.6) are the closest of BHT value (2101.2) according to the DPPH method, this shows that these plant species radical scavenging effect are higher than the others and they have natural antioxidant characteristic. Koşar *et al*., (2004) and Charehsaz *et al*., (2015) found similar results. They found that radical scavenging effect values of different extracts from *Rhus coriaria* and *Berberis crataegina* are closest of BHT value according to the DPPH method.

The antioxidant capacity values (μM trolox equivalent/g sample) of *Rhus coriaria* (3055.6) and *Berberis crataegina* (2362.1) are the closest of BHT antioxidant value (16651.9) according to the TEAC method. Charehsaz et al. (2015) were found similarly, antioxidant capacity values of *Berberis crataegina* (2152) are closest of this study value according to the TEAC method. If the results of the two methods are compared, the antioxidant capacity results have paralells in most of the species. This validates the reliability of the study. It is known that red-purple colored plant species which are rich as anthocyanin show antioxidant activity sweeping the DPPH and ABTS radicals, this case were seen in the analyses results.

The consumption per capita (kg/year) results of the 42 plant species collected from Turkey were showed in Table (2). These results showed that *Triticum monococcum* (14.2 kg/year), *Colocasia esculenta* (9.2 kg/year), *Vignaun guiculata* (6,3 kg/year) and *Trachystemon orientalis* (L.) G. Don (6.2 kg/year) are the best consumption per capita in plant species collected from Turkey.

The annual intake of mM trolox equivalent antioxidant capacity (Table 2) was calculated by multiplying the consumption per capita (kg/year) of TEAC (μM trolox equivalent/g sample) results. This process has led to a different ranking than Table 1.

Table 1. The antioxidant capacity values of samples according to the DPPH and TEAC method.
Çizelge 1. DPPH ve TEAC metoduna göre örneklerin antioksidan kapasite değerleri.

Sample Number Örnek No	English name İngilizce ismi	Scientific name Latince ismi	Turkish name Türkçe ismi	1/IC50* (DPPH)	STD	µM trolox equivalent/g sample (TEAC) µM troloks eşdeğeri/g örnek	STD
1	Elm-leaved sumach	*Rhus coriaria*	Sumak	1825.3	76.1	3055.6	20.4
2	Berberis	*Berberis crataegina*	Karamuk	1380.6	14.2	2362.1	2.7
3	Curly dock	*Rumex crispus* L.	Labada	593.1	10.8	921.3	11.2
4	Watercress	*Nasturtium officinale*	Suteresi	420.0	16.1	903.0	17.7
5	Prickly ivy	*Smilax excelsa L.*	*Kırçan*	453.7	19.7	808.8	15.0
6	Rush skeletonweed	*Chondrilla juncea*	Karakavuk	434.2	6.6	690.0	2.2
7	Ground elder	Aegopodium podagraria L.	Keçi ayağı	356.8	27.8	652.6	9.8
8	Knotgrass or knotweed	*Polygonumcognatum*	Madımak	346.7	19.4	611.3	6.6
9	Eastern borage or oriental borage	*Trachystemon orientalis* (L.) G. Don	Kaldirik	347.8	15.6	572.8	13.7
10	Common chicory	*Cichorium intybus*	Hindiba (Akdeniz)	274.2	11.4	539.0	6.4
11	Salsify	*Scorzonera cana*	Tekesakalı	275.6	7.4	522.6	18.0
12	Alexanders	*Smyrnium olusatrum*	Deli kereviz	201.4	1.9	492.5	32.8
13	Caper bush	*Capparis spinosa*	Kapari	85.6	3.9	465.8	10.6
14	N/A	*Ferulago trachycarpa*	Kuzukemirdi	245.4	3.7	315.8	19.7
15	Black bryony	*Dioscorea communis*	Dolanbaç	47.0	0.7	303.5	11.6
16	Gundelia or Galgal	*Gundelia tournefortii*	Kenger	145.1	11.0	284.1	4.1
17	Rock samphire	*Crithmum maritimum*	Denizteresi	173.5	0.9	280.4	13.0
18	Serik crab	*Pyrus serikensis*	Zingit	93.4	6.7	244.4	13.1
19	Sea beet	*Beta maritima*	Kıyı pancarı	50.3	0.2	241.4	7.1
20	Purple salsify	*Tragopogon porrifolius* subsp. *longirostris*	Helevan (Yemlik)	192.6	11.9	220.7	7.5
21	Glasswort or Samphire	*Salicornia emericii Duval-Jouve*	Deniz börülcesi	47.8	0.9	210.7	27.1
22	Syrian juniper	*Juniperus drupacea*	Andız (Enek)	122.4	4.9	208.8	0.1
23	Yellow Herb	*Opopanax hispudus*	Kaymacık	44.1	2.6	208.0	4.4
24	Lamb's quarters	*Chenopodium album* L.	Ak sirken, Sirken	39.8	1.4	206.6	1.8
25	Crown Daisy	*Glebionis coronaria* (L.) Spach	Alagömeç	161.0	7.8	188.9	3.3
26	Wild Radish	*Raphanus raphanistrum*	Eşekturpu	23.5	3.6	180.5	7.6
27	Foxtail lily	*Eremurus spectabilis*	Çiriş	170.0	2.5	168.9	5.1
28	Sorrel	*Rumex acetosella*	Kuzukulağı	26.9	0.5	167.2	13.5
29	Shepherd's-purse	*Capsella bursa-pastoris* (L.) Medik	Çoban çantası	34.8	0.1	158.3	0.8
30	Cowpea	*Vignaun guiculata*	Börülce	17.9	1.7	142.7	5.3
31	Common chicory	*Cichorium intybus*	Hindiba (Ege)	28.3	0.6	139.0	1.6
32	Fennel	*Foeniculum vulgare*	Arapsaçı-rezene	31.7	2.2	122.6	5.3
33	Calamus or Sweet flag	*Acorus calamus*	Eğir	21.0	0.1	117.9	0.1
34	Bladder Campion	*Silene vulgaris* (Moench) Garcke	Ecibücü (Gıvışkan)	81.0	2.5	114.3	8.0
35	Golden thistle	*Scolymus hispanicus*	Şevketi bostan	18.3	1.1	95.4	0.6
36	Star of Bethlehem	*Ornithogalum umbellatum* L	Sunbala, Sakarcık	7.1	0.2	60.5	2.2
37	Ferula or giant fennel	*Ferula elaeochytris*	Çağ (Çakşır kök)	5.5	0.5	38.9	1.8
38	Crab apple	*Eriolobus trilobatus*	Atelması	4.4	0.0	38.3	1.5
39	Arrost's baby's-breath	*Gypsophila arrostii* subsp. *nebulosa*	Çöğen	4.0	0.1	31.2	0.5
40	Taro, Elephant ear	*Colocasia esculenta*	Göleviz	12.9	0.2	29.8	1.5
41	Einkorn wheat	*Triticum monococcum*	Siyez	1.6	0.0	9.3	0.4
42	White lupin	*Lupinus albus*	Termiye	undetected		undetected	
			BHT**	2101.2	277	16651.9	59

*the reverse of the fresh plant value in terms of mg which inhibits 50% of the 1g DPPH radical.
**Butylated hydroxyl toluene.

Table 2. The consumption per capita (kg/year) and the annual intake of mM trolox equivalent of the 42 plant species.
Çizelge 2. Çalışmada kullanılan 42 bitki türüne ait yıllık tüketim miktarları ve bu türlerle yıllık olarak alınan antioksidan kapasitesinin mM troloks eşdeğeri.

Sample Number Örnek No	English name İngilizce ismi	Scientific name Latince ismi	Turkish name Türkçe ismi	Consumption Per Household (kg/year) Ev Başına Yıllık Tüketim (kg/yıl)	Consumption Per Capita (kg/year) Kişi Başına Yıllık Tüketim (kg/yıl)	mM trolox equivalent/year (TEAC) mM troloks eşdeğeri/yıl
9	Eastern borage or oriental borage	*Trachystemon orientalis* (L.) G. Don	Kaldirik	25.9	6.2	3551.4
2	Berberis	*Berberis crataegina*	Karamuk	3.5	1.2	2834.5
3	Curly dock	*Rumex crispus* L	Labada	8.3	2.2	2026.9
12	Alexanders	*Smyrnium olusatrum*	Deli kereviz	11.5	3.5	1723.8
7	Ground elder	Aegopodium podagraria	Keçi ayağı	7.6	2.0	1305.2
5	Prickly ivy	*Smilax excelsa L.*	Kırçan	7.3	1.6	1294.1
4	Watercress	*Nasturtium officinale*	Suteresi	5.0	1.4	1264.2
17	Rock samphire	*Crithmum maritimum*	Denizteresi	11.0	3.5	981.4
18	Serik crab	*Pyrus serikensis*	Zingit	13.5	3.9	953.2
1	Elm-leaved sumach	*Rhus coriaria*	Sumak	1.2	0.3	916.7
30	Cowpea	*Vignaun guiculata*	Börülce	21.9	6.3	899.0
10	Common chicory	*Cichorium intybus*	Hindiba (Akdeniz)	4.6	1.6	862.4
21	Glasswort or Samphire	*Salicornia emericii Duval-Jouve*	Deniz börülcesi	13.9	3.8	800.7
19	Sea beet	*Beta maritima*	Kıyı pancarı	11.1	3.0	724.2
26	Wild Radish	*Raphanus raphanistrum*	Eşekturpu	15.0	4.0	722.0
6	Rush skeletonweed	*Chondrilla juncea*	Karakavuk	2.2	1.0	690.0
11	Salsify	*Scorzonera cana*	Tekesakalı	4.0	1.3	679.4
8	Knotgrass or knotweed	*Polygonumcognatum*	Madımak	5.8	1.1	672.4
23	Yellow Herb	*Opopanax hispudus*	Kaymacık	7.4	2.2	457.6
24	Lamb's quarters	*Chenopodium album* L.	Ak sirken,	8.7	2.0	413.2
15	Black bryony	*Dioscorea communis*	Dolanbaç	4.0	1.3	394.6
22	Syrian juniper	*Juniperus drupacea*	Andız (Enek)	5.8	1.8	375.8
31	Common chicory	*Cichorium intybus*	Hindiba (Ege)	9.4	2.6	361.4
20	Purple salsify	*Tragopogon porrifolius* subsp. *longirostris*	Helevan (Yemlik)	5.1	1.6	353.1
32	Fennel	*Foeniculum vulgare*	Arapsaçı-rezene	10.2	2.7	331.0
28	Sorrel	*Rumex acetosella*	Kuzukulağı	7.1	1.9	317.7
29	Shepherd's-purse	*Capsella bursa-pastoris* (L.) Medik	Çoban çantası	7.5	1.9	300.8
40	Taro, Elephant ear	*Colocasia esculenta*	Göleviz	29.3	9.2	274.2
25	Crown Daisy	*Glebionis coronaria* (L.) Spach	Alagömeç	4.2	1.4	264.5
35	Golden thistle	*Scolymus hispanicus*	Şevketi bostan	10.0	2.7	257.6
27	Foxtail lily	*Eremurus spectabilis*	Çiriş	4.5	1.5	253.4
16	Gundelia or Galgal	*Gundelia tournefortii*	Kenger	2.7	0.8	227.3
41	Einkorn wheat	*Triticum monococcum*	Siyez	84.0	14.2	132.1
14	N/A	*Ferulago trachycarpa*	Kuzukemirdi	1.5	0.4	126.3
34	Bladder Campion	*Silene vulgaris* (Moench) Garcke	Ecibücü (Gıvışkan)	5.3	1.1	125.7
36	Star of Bethlehem	*Ornithogalum umbellatum*	Sunbala, Sakarcık	5.4	1.3	78.7
13	Caper bush	*Capparis spinosa*	Kapari	0.3	0.1	46.6
33	Calamus or Sweet flag	*Acorus calamus*	Eğir	0.4	0.2	23.6
38	Crab apple	*Eriolobus trilobatus*	Atelması	2.2	0.6	23.0
39	Arrost's baby's-breath	*Gypsophila arrostii* subsp. *nebulosa*	Çöğen	2.1	0.7	21.8
37	Ferula or giant fennel	*Ferula elaeochytris*	Çağ (Çakşırkök)	1.4	0.4	15.6
42	White lupin	*Lupinus albus*	Termiye	4.2	1.4	-

The results of multiplying above 1000 mM trolox equivalent / year are respectively: *Trachystemon orientalis* (L.) G. Don (3551.4), *Berberis crataegina* (2834.5), *Rumex crispus* (2026.9), *Smyrnium olusatrum* (1723.8), *Aegopodium podagraria* (1305.2), *Smilax excelsa* (1294.1) and *Nasturtium officinale* (1264.2).

CONCLUSION

It is known that red-purple colored plant species which are rich in anthocyanins show antioxidant activity sweeping the DPPH and ABTS radicals, this case were seen in the analyses results. Nevertheless, the annual consumption of the plant should also be evaluated. In addition, the greater the diversity of species you eat, the more likely you are to cover all your nutritional bases including complementarity effects. Antioxidant capacity and consumption per capita of plants data generated by the BFN Project in Turkey have been scattered across databases in various government agencies and sources for being evaluated. BFN Turkey which was formed three geographically distinct locations in Turkey: the Black Sea, Mediterranean and Aegean Region pilot sites has excelled in raising awareness of the conservation and sustainable use of wild edibles.

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