

## The Evaluation of Total Phenolic, Flavonoid, Sugar Contents and Antioxidant Activity of Tayfi Grape in Turkey

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**Geliş Tarihi/Received:**

03.05.2017

**Kabul Tarihi/Accepted:**

17.11.2017

**Yayın Tarihi/Published:**

27.12.2017

### ABSTRACT

Tayfi (*Vitis vinifera* L.) only grown in the Southeastern Anatolia Region provides a major contribution on the economy of the region people. But, the research related to nutritional content of this species was not found. This study was carried out to determine the total phenolic, flavonoids, sugar content and antioxidant activity of different organs (mature/young seed and leaf) 'Tayfi' grape. The highest amount of total phenolic (380,94 µg GAE/mg extract) was found to be in the young seeds. The high amount of total flavonoid content (107,21 µg QEs/mg extract) was obtained from young leaves. Higher antioxidant activity was observed in young seeds (91,32%) as expected from high contents of total phenolics. In general, as the total phenolics in seed increased, the antioxidant activities also increased. Also, changes in sugar content (glucose, fructose, sucrose and maltose) were determined in leaf from seed (young and mature) of Tayfi grape by HPLC. The highest concentration of sucrose was described in mature seed (9,29 mg/g).

**Keywords:** *Vitis vinifera* L. Tayfi, Total flavonoids, Total phenolics, Antioxidant activity, Sugars.

## Türkiye'de Yetişen Tayfi Üzüm Çeşidinin Toplam Fenolik, Flavonoid, Şeker İçeriği ve Antioksidan Aktivitesinin Değerlendirilmesi

### ÖZET

Tayfi (*Vitis vinifera* L.) ülkemizde sadece Güneydoğu Anadolu Bölgesi'nde yetişen ve bölge halkının ekonomisine önemli katkı sağlayan bir üzüm çeşidi olmasına rağmen, besin içeriği ile ilgili herhangi bir çalışmaya rastlanmamıştır. Bu nedenle çalışmamızda Tayfi üzüm çeşidinin farklı organlarında (genç/olgun tohum ve yaprak) toplam fenolik, flavonoid, şeker içeriği ve antioksidan aktivitesinin belirlenmesi amaçlanmıştır. En yüksek toplam fenolik miktarının (380,94 µg GAE/mg ekstre) genç tohumlardan, toplam flavonoid içeriğinin en yüksek miktarı (107,21 µg QEs/mg ekstre) ise genç yapraklardan elde edilmiştir. Ayrıca yüksek fenolik içeren genç tohumlarda yüksek antioksidan aktivite de (%91,32) gözlenmiştir. Genel olarak tohumdaki toplam fenolik miktar arttıkça, antioksidan aktivite de artmıştır. Bununla birlikte Tayfi üzümünün tohum ve yapraklarındaki (genç ve olgun) şeker (glukoz, fruktoz, sükröz ve maltoz) miktarı değişimleri HPLC ile belirlenmiştir. Sükrözün en yüksek konsantrasyonu, olgun tohumda (9,29 mg/g) bulunmuştur.

**Anahtar Kelimeler:** *Vitis vinifera* L. Tayfi, Toplam flavonoid, Toplam fenolik, Antioksidan aktivite, Şeker.

### 1. INTRODUCTION

Grapes are among the fruits containing the highest content of phenolic substances (Macheix et al., 1990). Antioxidant activity of fruits results mainly from phenolics, particularly flavonoids (Orak, 2007). Total phenolic compounds, anthocyanin contents, antioxidant capacities and phytochemical properties in

grapes vary according to grape varieties, grown in climate and soil conditions, cultural practice and maturity levels (Iland, 1989; Nadal and Arola, 1995; De La Hera et al., 2005).

The different parts of grape have been used because of many biological activities in folk medicine. The leaves of plant are rich in tannins, flavonoids, organic acids, lipids, enzymes and vitamins (Bombardelli and Morazzonni, 1995; Felicio et al., 2001). The leaves of the plant, which have a stringent and haemostatic properties, are used in the treatment of diarrhea, hemorrhage, varicose veins, hemorrhoids, inflammatory disorder, pain, hepatitis, and free radical related diseases and externally for centuries in Anatolia (Baytop, 1999; Lardos and Kreuter, 2000; Orhan et al. 2009). The seed extract has recently been marketed as a dietary supplement and advocated for its beneficial antioxidant effects and free radical-scavenging ability (Singh and Agarwal, 2006).

Sugar content in grape (*Vitis vinifera* L.) berries is important determining berry quality, alcoholic fermentation and also contribute to organoleptic properties (Torija et al., 2003; Conde et al., 2007; Lee and Rennaker, 2011). The grape berry is the major sink organ in the grape vine and requires carbohydrates to support its growth and development. Sugar metabolism plays an important role in berry growth and Development (Xie et al., 2009).

Anatolia has been a land for viticulture and wine making since ancient times all over the world, and is one of the most widely grown fruit crops all over the world. Turkey is known to be the gene center for some types of grapes. According to the archaeological ruins, wine-making and viticulture date back to B.C. 3500, due to very suitable climate conditions for vineyards in Turkey (Çelik et al., 1998; Alp, 1999; Çölkesen et al., 2006).

To our knowledge, only one study has been conducted ampelographical on Tayfi grape variety (Uyak et al., 2011). Thus, more data are needed about the bioactive contents of this grape that may supply important information to the consumer in terms of recognizing a more nutritious fruit.

In this context, the present study was evaluated the total phenolic, flavonoid contents, the antioxidant capacity in Tayfi grapes, separately. This study was carried out to provide sufficient experimental evidence for the antioxidant activity and potential for further development and use of this grape.

## **2. MATERIALS AND METHODS**

### **2.1. Plant Material and Extraction**

In this study was used the seed and leave parts (young and mature) of grape (*Vitis vinifera* L. Tayfi) as a material. ‘Tayfi’ grapes, red-purple color, very tasty, a little-known is variants of a grape that grows only in this region, used for table are major economic contributors to the state of southeastern Anatolia. The mass production of these varieties provides to major economic contribution to southeastern Anatolia

The leaves and seeds of healthy grape were collected at two different times in July and August from Uzundere which is the village of Hasankeyf, a town of Batman province, in Turkey. The seeds and leaves were excised berries and air-dried at the room temperature under shade conditions. They were stored at room temperature until their analysis.

Dry weights of the all plantlets were taken as 3 g pulverized in a mortar. Extract of 3 g dry herb samples was extracted for 2.5 h under using a orbital shaker (v/w). The extract was filtered and evaporated to yield of powder of dry extract.

### **2.2. Determination of Total Phenolic Content (TPC)**

The concentration of total phenolics of metanol extracts were determined by using Folin-Ciocalteu reagent (Slinkard and Singleton, 1977) and external calibration with gallic acid. About 0.1 ml of extract solution, 4.5 ml of distilled water and 0.1 ml of Folin-Ciocalteu reagent were added and the contents mixed vigorously. After shaking 3 min, 0.3 ml of 2% Na<sub>2</sub>CO<sub>3</sub> was added, and finally the mixture was allowed to stand for 2 hours at room temperature. The absorbance was measured at 760 nm using UV-VIS spectrophotometer. The concentration of the total phenolics was estimated as mg of gallic acid equivalent by using an equation obtained from gallic acid calibration curve. The quantification of phenolic compounds in all the fractions were carried out in triplicate and the results were averaged.

$$\text{Absorbance} = 0.0671 \text{ gallic acid } (\mu\text{g}) + 0.0351 \quad (R^2 = 0.9972).$$

### 2.3. Determination of Total Flavonoid Content (TFC)

Total flavonoid contents of leaves and seed (young and mature) of grape (*Vitis vinifera* L. Tayfi) were estimated by using the aluminium nitrate colorimetric method as described by Moreno et al. (2000) with some modifications. Quercetin was used to make the calibration curve. The determination of total flavonoids methanol extract was carried out in triplicate and the results were averaged. The extract (0.5 ml) mixed with 80% ethanol (4.3 ml), 1M potassium acetate (0.1ml) and 10% aluminium nitrate (0.1ml). At room temperature it was incubated for 40 min. The absorbance was measured at 415 nm using UV-VIS spectrophotometer.

$$\text{Absorbance} = 0.0647 \text{ quercetin } (\mu\text{g}) + 0.0383 \quad (R^2 = 0.9989).$$

### 2.4. Determination of the Antioxidant Potential through Free Radical DPPH

The free radical scavenging effects of the metanol extracts were estimated according to the method of Blois (1958) with minor modifications. The DPPH solution was freshly prepared daily, stored in a flask covered with aluminium foil, and kept in the dark at +4 °C. All experiments were carried out in triplicate. 1 ml of each sample, prepared at various concentrations (1, 5, 10, 25, 50 and 100 µg/ml), were added to 4 ml of a 100 µM DPPH radical solution. The mixture was shaken and allowed to stand for 30 min at room temperature in the dark, and then the absorbance was measured at 517 nm with a spectrophotometer. The same procedure was repeated with Ascorbic acid, BHT and BHA as a positive control. The percentage inhibition activity was calculated by the following equation:

$$\text{Scavenging effect } (\%) = [(A_{517} \text{ of control} - A_{517} \text{ of sample}) / A_{517} \text{ of control}] \times 100.$$

where  $A_{517} \text{ of control}$  and  $A_{517} \text{ of sample}$  are the absorbance values of the control sample (containing all reagents except the test compound) and the test sample, at particular times, respectively.

### 2.5. Determination of Free Sugars

Determination of fructose, sucrose, glucose and maltose levels: determined by modified methods of Torije et al. (1998) and Karkacier et al. (2003). The dry plant samples (3 grams) were ground in mortar and pestle with 25 mL of methanol (80%). The mixture was homogenized in an Ultra Tissue Lysis and incubated in magnetic stirrer at 65°C for 30 min. Then, it was centrifuged at 2000 rpm for 15 min. The supernatant was transferred in clean tube. Methanol was removed by rotary evaporator and the residue was dissolved in 5 mL double distilled water. Extracts were passed through Sep-Pak C18 cartridge and 2.5 ml of filtrate was mixed with 7.5 ml acetonitrile. Then it was filtered through 0.45µm membrane filter and

injected into High performance Liquid Chromatography (HPLC). The column was calibrated by fructose, sucrose, glucose and maltose standards.

## 2.6. Statistical Analysis

All the experiments were done in triplicate. Data were reported as mean± standard deviation (SD). The data of sugar content were performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, USA). Significance of differences was tested using one-way analysis of variances (ANOVA) and Duncan tests at a 0.05 level of significance.

## 3. RESULTS

### 3.1 Total Phenolic (TPC) and Flavonoid Content (TFC)

Total phenolic (TPC) and flavonoid contents (TFC) of leaves and seeds of grape (*Vitis vinifera* L. Tayfi) are presented in Table 1. The results indicated that the levels of TPC and TFC changed according to the grape parts.

Among all the grape parts analysed, young seed had the highest total phenolic content (380,94±3,82 µg of gallic acid equivalents per milligram of dry matter of grape), followed by mature seed (206,08±4,04), young leaf (150,94±2,29) and mature leaf (76,17±2,82), as shown in Table 1. In comparisons between extracts of seed and leaf were found significant differences in terms of total phenolic contents; at the same time, significant differences were found between young and mature organs.

**Table 1.** Total phenolic (TPC) and flavonoid (TFC) contents of leaf and seeds (young and mature) of grape (*Vitis vinifera* L. Tayfi).

Explants	TPC (µg GAE/mg extract)	TFC (µg QEs/mg extract)
Young leaf	150,94±2,29	107,21±0,80
Mature leaf	76,17±2,82	66,62±1,01
Young seed	380,94±3,82	5,72±1,17
Mature seed	206,08±4,04	1,75±0,35

µg GAE/mg represent micrograms of gallic acid equivalents and µg QEs/mg represent micrograms of Quercetin equivalents per milligrams of dry matter of grape, respectively. Values are mean ± SD values of three replicates.

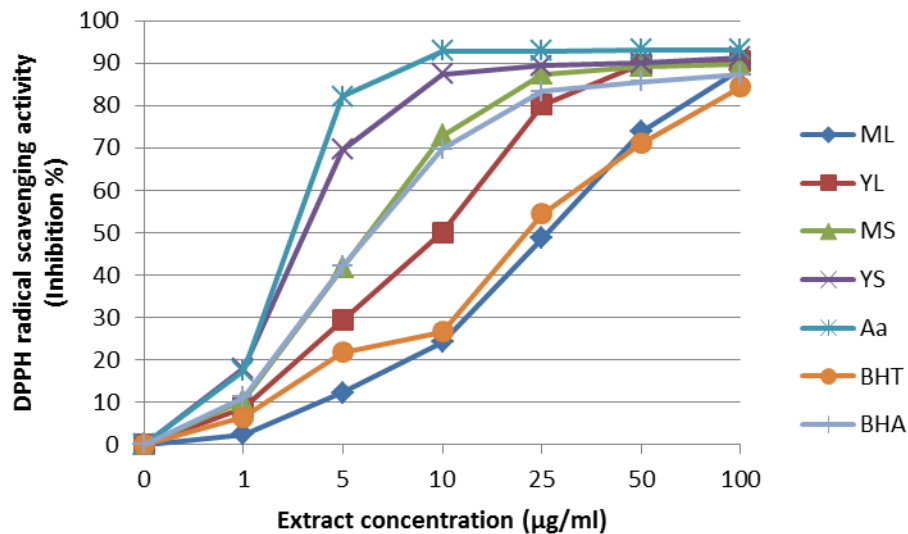
According to results obtained, TPC (total phenolic content) in young leaf and seeds were higher compared to that of mature seed or leaves. Besides, in terms of total phenolic compounds of seeds were found to be better than from the content of leaves. Flavonoid content in leaves and seeds (young and mature) showed significantly differences (Table 1.). The high flavonoid content (107,21±0,80 µg QEs/mg extract) was obtained from the young leaf extracts. It was followed by mature leaf (66,62±1,01), young seed (5,72±1,17), and mature seed (1,75±0,35), respectively. In addition, total flavonoid content was higher in leaves, while, total phenolic content were significantly higher in seed. As the content of phenolic, the levels of total flavonoid content in young organs were found to higher than from mature organs.

### 3.2 Determination of the Antioxidant Potential through Free Radical DPPH

The antioxidant capacity of Tayfi seeds and leaf extracts were evaluated using the DPPH radical scavenging. The results were compared with standard antioxidants as ascorbic acid, BHT and BHA, as shown in Fig 1.

The seeds and leaf extracts and positive controls clearly demonstrated a dose-dependent antioxidant activity against DPPH. At the concentration of 100 µg/ml, all the extracts showed a similar scavenging effect around 90% as shown in Figure 1.

The highest percentage of scavenging activity ( $91,32 \pm 0,07\%$ ) was obtained from the young seed extracts at concentration of 100 µg/ml. The young seed extract showed the strongest free radical scavenging activity than BHT and BHA as a standard antioxidants, at all concentration. The lowest radical scavenging activity was exhibited by mature leaf extracts, which showed activities of 2,39% at 1µg/ml, 12,22% at 5µg/ml, 24,21% at 10 µg/ml and 48,62% at 25 µg/ml concentrations.

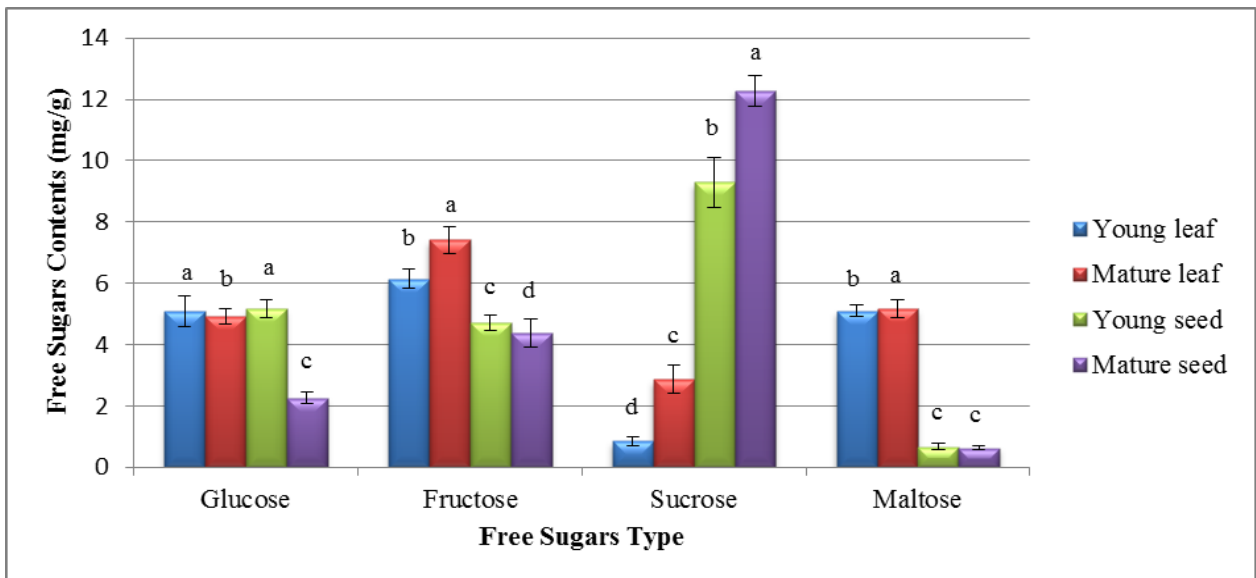


**Figure 1.** DPPH radical scavenging activities of methanolic extracts of leaves and seed (young and mature) of grape (*Vitis vinifera* L. Tayfi) and standard, BHA, BHT, ascorbic acid.(ML:Mature Leaf, YL:Young Leaf, MS:Mature Seed, YS:Young Seed)

### 3.3 Determination of Free Sugars

Sugar (fructose, sucrose, glucose and maltose) levels of leaves and seed of Tayfi grape were determined by High performance Liquid Chromatography (HPLC). Sugar content in leaves and seeds (young and mature) showed significantly differences (Figure 2.).

Glucose concentration in leaves and seeds (young and mature) showed significantly differences between young and mature organs (leaves and seeds) (Figure 2.). The glucose concentration in young leaf was 5,10 mg/g and 5,17 mg/g in young seed, while concentration in mature leaf and seed were 4,94 mg/g and 2,26 mg/g. Glucose content in young organs were found to higher than mature organs. Concentration of fructose in leaves (young and mature) were 6,15 mg/g and 7,41 mg/g, while concentration in seed (young and mature) were 4,70 mg/g and 4,37 mg/g, respectively. Similarly, high maltose concentration was obtained from young leaf (5,10 mg/g) and mature leaves (5,17 mg/g). Fructose and maltose content in leaf was higher than seed and these differences were found to be significantly as statistical.



**Figure 2.** Total free sugar contents of leaf and seeds (young and mature) of grape (*Vitis vinifera* L. Tayfi). (Values are mean  $\pm$  SD values of three replicates, followed by the same letter are not significantly different ( $p \leq 0.05$ ) according to Duncan's Multiple Range tests).

Sucrose content in leaves and seeds (young and mature) showed significantly differences (Figure 2). The high sucrose content (12,28 mg/g) was obtained from the mature seed. It was followed by young seed (9,29 mg/g), mature leaf (2,87 mg/g) and young leaf (0,86 mg/g) respectively. Sucrose content in seed organs were found to higher than leaf organs. In addition, as shown in Figure 2, sucrose was higher in seed (young and mature) compared to all the sugars analysed.

#### 4. DISCUSSION

One important group of secondary metabolites known that the phenolic compounds present in grapevines (*Vitis spp.*). This, play an important role on the quality and nutrition value of grapes. Phenolic compounds are synthesized the early stage of berry development and decline towards ripening (Doshi et al., 2006; Conde et al., 2007). The highest accumulation of phenolic content is occurs in the berry skin and seeds parts (Poudel et al., 2008). Likewise for total phenolic, in this study for total phenolics the best results were obtained from young seeds extracts (380,94 $\pm$ 3,82  $\mu$ g GAE/mg extract) compared with other extracts. The seeds were found to be rich with regard to total phenolic.

In contrast, significant results in terms of total flavonoid contents were obtained from young leaves (107,21 $\pm$ 0,80  $\mu$ g GAE/mg extract). The results obtained in this study showed significant differences among the grape tissues and organs in relation to the phenolic and flavonoid contents. Our results are consistent with the findings of a previous studies Anastasiadi et al. (2010, 2012), concerning the polyphenolic content of different grape tissues and wines, among native *V. vinifera* varieties. It is known that the genetic, agronomic and environmental factors play important role on synthesis or accumulation of phenolic (Matthews and Anderson, 1988; De La Hera et al., 2005; Yang et al., 2009).

Biological activity, total phenolic and flavonoid contents in seeds, stems and leaves of different grape cultivar were studied by many researcher (Yang et al., 2009; Rockenbach et al., 2011; Anastasiadi et al., 2012). However, to our knowledge this grape, phenolic and flavonoid contents of different tissues and organs of has not been studied. This study is the first report about the total phenolic and flavonoid contents of leaves and seed parts (young and mature) of grape (*Vitis vinifera* L. Tayfi).

Phenolics such as flavonoids, stilbenes, and tannins are considered major contributors to the antioxidant activity of vegetables and fruits. Kalt et al. (1999), Wang and Lin (2000) found a strong correlation among antioxidant capacity, total phenols and anthocyanins. Orak (2007), reported that anthocyanin and phenolic compounds either alone or in combination, are responsible for the antioxidant activity in grape cultivars. Doshi et al. (2006) and Orhan et al. (2009) studied that a positive correlation



was found between the phenolic composition and antioxidant potential of different grapevine parts. Similarly, in the study, the analysis of the relationship between the antioxidant activity and phenolic content of the leaf and seeds showed a positive correlation coefficient. Additionally, the highest antioxidant activity was found in young seed extracts (91.32%) which had the highest total phenolic content among all the grape parts analysed. By contrast the lowest antioxidant activity was determined in mature leaves (88,48%) which had the lowest total phenolic content.

Despite having the highest flavonoid content of young leaves showed a lower antioxidant activity according to seeds. There was no relationship between antioxidant activity and flavonoid content. A similar relation was found by Zhao and Hall (2008). The researches found that phenolic acids and low-molecular weight flavonoids, but not high-molecular weight flavonoids, are responsible for the antioxidant activity of raisins.

The total phenol contents, antimicrobial, antiviral, and antioxidant activities of the different fractions of *V. vinifera* leaves of Turkish origin investigated by Orhan et al. (2009). The researchers reported that *V. vinifera* leaves can be used in folk medicine due to the properties antioxidant and antiviral. According to our results, the young leaves of Tayfi grape that have a high antioxidant activity (90,43%) and, therefore, can be used in public health or medicine.

Healthy leaves of *V. vinifera* are free from resveratrol and derivatives which is known as antioxidant (Bombardelli and Morazzonni, 1995). TLC analysis indicated that *V. vinifera* leaves do not contain resveratrol. Şendoğdu et al. (2006) obtained that, the ethanolic extract of *V. vinifera* leaves was found to possess a high antidiabetic and antioxidant activity. The researchers reported that tannins and flavonoids contribution the activities, resveratrol does not the major antioxidant active constituent. In our study, the young leaves of Tayfi grape that have a high antioxidant activity due to high total flavonoid content. The results of the presented study shown that the young leaves of Tayfi grape that have a high antioxidant activity due to high total flavonoid content.

Sugar is imported into the berry from the leaves in the form of sucrose (Swanson and Elshishiny, 1958). After unloading of sucrose in the berry, sucrose is hydrolyzed and glucose and fructose accumulate (Kliwer, 1965). Therefore, the predominant sugars in grape berries are glucose and fructose (Carroll and Marcy, 1982; Shiraishi, 1993). According to results obtained, contents of glucose and fructose in young and mature leaf were higher than these of sucrose. However, the amount of sucrose is higher in young and mature seed than in fructose and glucose. Sugar metabolism plays an important role in berry growth and development (Xie et al., 2009). Liu et al. (2006) also found trace amounts of sucrose in fruit from many grape cultivars during growth and development.

The grape is becoming increasingly popular as a fruit and is a significant source of nutritional antioxidants. Tayfi grape (*Vitis vinifera* L.) only grown in the Southeastern Anatolia Region provides a major contribution on the economy of the region people. But, the reserch related to nutritional contents of this species was not found. Therefore, this study is aimed to evaluate the antioxidant activities, total phenolics and flavonoids. The antioxidant activity results supported the utilization of leaves and seed *V. vinifera* L. Tayfi in folk medicine. The further research should be performed on the active principles of this grape (terpenoids, flavonoids, anthocyanins and other phenolic compounds).

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