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Research Article

# The comparison of chemical content and bioactive capacity of domestic and import *Hypericum perforatum* aqueous extracts

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## ABSTRACT

*Hypericum perforatum*, known as St. John's Wort and most used ethnaformacolgically, belongs to the *Hypericaceae* family. In this work, we aimed to evaluate the antioxidant, antimicrobial, DNA protective activities, and enzyme inhibitor properties of domestic and import *H. perforatum* aqueous extracts. The total phenolic and flavonoid contents were also determined for both samples. Results exhibited a high phenolic content for Domestic *H. perforatum* aqueous extract (DHPE) and Import *H. perforatum* aqueous extract (IHPE) samples. Meanwhile, inhibition activity levels for α-glucosidase were remarkable, the $IC_{50}$ value of DHPE was 16.35±0.07 µg/mL, and the $IC_{50}$ value of IHPE was 15.05±1.36 µg/mL, both samples demonstrated almost twice of the standard inhibitor effect ($IC_{50}$: 30.62±2.07 µg/mL). Moreover, the inhibition activity of both samples against α-amylase was more effective than acarbose. The antibacterial results were as follows; the highest MIC value detected by IHPE was 64 µg/mL against *B. cereus*. In addition, IHPE was shown to be more effective than the domestic sample against all microorganisms. Furthermore, DHPE exhibited good protective activity from oxidative $H_2O_2$-induced DNA damage. Both tested samples had an abundance of phenolic content and were high in inhibitory activity against diabetic enzymes. Generally, bioactivity tests' results appeared quite effective for both samples compared to the standards.

**Keywords:** *H. perforatum*, aqueous extract, phytochemical content, bioactivity.

## Yerli ve ithal *Hypericum perforatum* sulu ekstraktlarının kimyasal içeriği ve biyoaktif kapasitesinin karşılaştırılması

## ÖZ

Sarı kantaron olarak bilinen ve etnaformakoljik olarak en çok kullanılan *Hypericum perforatum*, Hypericaceae familyasına ait bir bitkidir. Bu çalışmada yerli ve ithal *H. perforatum* sulu ekstraktlarının antioksidan, antimikrobiyal, DNA koruyucu aktiviteleri ve enzim inhibitör özelliklerini değerlendirmeyi amaçladık. Her iki örnek için de toplam fenolik ve flavonoid içerikleri belirlendi. Analiz sonuçları, Yerli *H. perforatum* sulu özüt (DHPE) ve İthal H. perforatum sulu özüt (IHPE) numuneleri için yüksek fenolik içerikti. Numunelerin diyabetle ilgili temel enzimlere karşı inhibisyon aktivitesi seviyeleri çarpıcıydı; α-glukosidaza gelince, DHPE'nin $IC_{50}$ değeri 16.35±0.07 µg/mL idi ve IHPE'nin $IC_{50}$ değeri 15.05±1.36 µg/mL idi, bu da sırasıyla enzimin standart inhibitörünün etkinliğinin neredeyse iki katını temsil ediyor. Özellikle, DHPE'nin α-amilaz ve α-glukosidaz üzerindeki inhibisyon aktivitelerinin akarboza göre daha etkili olduğu belirlendi ($IC_{50}$: 30.62±2.07 µg/mL). Antibakteriyel test şu şekildeydi; en yüksek MİK değeri IHPE tarafından *B. cereus'a* karşı 64 µg/mL olarak tespit edildi. Ayrıca, IHPE yerli örnekten daha etkiliydi. DHPE, oksidatif $H_2O_2$ ile indüklenen DNA hasarından iyi koruyucu aktivite sergiledi. Sonuç olarak, test edilen her iki numune de bol miktarda fenolik içeriğe sahipti ve diyabetik enzimlere karşı inhibitör aktivitelerinde yüksekti. Ayrıca, diğer biyoaktivite testleri, standartlara göre oldukça etkili olduğu gözlendi.

**Anahtar Kelimeler:** *H. perforatum*, sulu ekstrakt, fitokimyasal içerik, biyoaktivite.

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## 1. INTRODUCTION

*Hypericum perforatum* is an enduring species distributed worldwide, also recognized as St. John's wort. It is classified under folk medicines due to its curative abilities for many diseases. The Cherokee, Iroquois, and Montagnais all left behind records of using *H. perforatum*. All of these tribes appeared to have utilized the kinds as a febrifuge or cough remedy, but the Cherokee employed it extensively. The use of the herb's distilled oil as a treatment for cuts and bruises was one of the first, most popular, and widespread pharmacological uses of this plant in Europe after the 16th century.[1] *H. perforatum* contains many bioactive molecules with various pharmacologic effects, such as antioxidant activity.[2] Due to the rising need for raw materials as a source of organic bioactive compounds, this plant species has drawn a lot of attention recently.[3] It has naturalized many regions, most notably North America and Australia. It is indigenous to Europe, West Asia, North Africa, Madeira, and the Azores. The plant can infiltrate meadows, disturbed places, dirt roads, the sides of roads and highways, and sparse woodlands and spreads quickly through runners or prolific seed production. *H. perforatum* is currently one of the world's most widely used medicinal plants due to the rapid rise in the consumption of goods derived from it in recent years.[4] In previous studies, plants' importance has been evaluated for their biological and chemical properties and their use in treating infectious diseases. *H. perforatum* contains many bioactive molecules with various pharmacologic effects, such as antioxidant activity.[2] Due to ecological considerations, genetic differences within the species, cutting time, sample preparation and processing, and storage circumstances like light exposure, these constituents' concentrations frequently change. Buds, flowers, and the tips of twigs contain concentrated amounts of significant bioactive substances. Regardless of this diversity, it is understood that about 20% of the plant extract is made up of bioactive substances.[5-7] Recent interest has evaluated its antimicrobial activity against several bacterial and fungal strains.[1]

The plant has many therapeutic uses, including treating burns, eczema, psychiatric disorders, intestinal illnesses, and skin wounds.[8] In addition, its extract's chemical compositions have various pharmaceutic attributes that are related to anti-inflammatory, antiviral, antitumor, and wound-healing activities.[3] In this study, using total phenol and flavonoid content testing, we attempted to create a comprehensive picture of the bioactive chemical components for both *H. perforatum* extracts. Additionally, the number of biological activities is investigated as antioxidant, antibacterial, and DNA-protective activities, in addition to determining the inhibitory effects of the extracts for the enzymes connected to diabetes, α–glucosidase and α–amylase.

## 2. MATERIALS AND METHODS

### 2.1. Reagents and solutions

The domestic (D) and import (I) samples of *H. perforatum* (HP) were provided by ERSAG Company (Denizli, Türkiye). DHP and IHP aqueous extracts (aerial parts) were obtained using the extraction method as follows. 10 kg of DHP and IHP were placed in a heat-resistant net inside the extraction device and mixed with 50 L of distilled water at 80 degrees for 2 hours in a particular-made extraction system. After that, the liquid part was removed from the extraction mixture using the vacuum pump. The next step was applying the spray-drying to produce the dark brown dry extract powder (1 kg, 10%). Then, dried samples of DHP and IHP aqueous extracts (E) were obtained and stored at -20 ºC.

### 2.2. Analysis of chemical content

Analysis of total phenol content (TPC) was obtained using the Folin & Ciocalteu method.[9] The entire total flavonoid content (TFC) was estimated using the aluminum chloride method with slight modification.[10]

### 2.3. Determination of antioxidant capacity

We have applied three theories known and widely used in the literature to determine the antioxidant capacity of the samples. Firstly, the ammonium molybdenum method was applied to detect the total antioxidant activity of samples, based on forming green phosphate/Mo (V) complex within 695 nm.[11,12] Then, the free radical scavenging capacities for samples were spectrophotometrically evaluated using 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\bullet}$)[13] and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$).[14]

### 2.4. Determination of antimicrobial capacity

The antimicrobial effect of samples was detected using six different bacteria gram-positive (*Staphylococcus aureus*; ATCC 25213, *Enterococcus faecalis*; ATCC 29212, and *Bacillus cereus*; CCM 99) and gram-negative (*Pseudomonas aeruginosa*; ATCC 15442, *Klebsiella pneumoniae*; ATCC 10031 and *Escherichia coli*; ATCC 25922). Samples and standard antibiotics were prepared with a concentration of 8.192 mg/mL, and the microdilution method was applied to find the minimum inhibition concentration (MIC).[15] Samples were diluted by mixing with cationic MHB medium (Mueller Hinton II Broth + $CaCl_2$ + $MgCl_2$) in a sterile 96-well plate. Then, 10 µL of 0.5 McFarland bacterial solution was added to each well. After that, the plates were incubated at + 4 °C followed by incubation at 37 °C (*B. cereus* incubation at 30 °C) for 120 minutes. MIC has been read after 16-18 hours of incubation.

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### 2.5. Inhibition of α–amylase and α–glucosidase

α-amylase inhibition activity of samples was determined spectrophotometrically depending on the hydrolysis of starch in an acidic medium.[16] α-glucosidase inhibition also was determined spectrophotometrically by measuring the formation of yellow-colored p-nitrophenol.[17, 18].

### 2.6. DNA protective activity

DNA protection activity of samples was determined using the agarose gel electrophoresis method.[19] The experiment was applied by preparing a mixture of 4 µL of glycerol, 5 µL of the sample, 3 µL of pBR322 plasmid DNA (172 ng/µL), and 1 µL of 30 % $H_2O_2$. The mixture was submitted to UV for 5. Then 2 µL of bromophenol blue was added, and the mixture was loaded to the 1.5 % agarose gel wells (1X TBE buffer + 2 µL ethidium bromide). Then electrophoresis was applied for 120 minutes at 90 volts. Finally, the % DNA protection activity was determined using the ImageJ program.

### 2.7. Statistical analysis

Experimental data were recorded using Microsoft Excel and statistically analyzed by SPSS 22.0. Data like survival rate, hatchability, and malformation rate parameters were analyzed using the one-way analysis of variance (ANOVA) test and Tukey test when the variance data were homogeneous. A rank sum test was used for graded data such as urine analysis indexes. P values lower than 0.05 were considered significant.

## 3. RESULTS AND DISCUSSION

### 3.1. Total phenolics and total flavonoids

The chemical composition analysis was performed to detect the total phenol and flavonoid content, as Gallic acid for phenolics and quercetin for flavonoids. Results revealed that DHPE with 155 mg GAE/g extract had more phenolics content than the other sample, which also was not far from this number, 142 mg GAE/g extract. While the flavonoid content of IHPE came in first place in quantity with 46 mg QE/g extract, as shown (Table 1). This highly content may be behind the vast bioactive effects of these samples. In order to compare our results with literature data, we found that in another study that the total phenolic content of five extracts of *H. perforatum* in different parts using two solvents (EtOH–water and water) was between 191 ± 5 to 257 ± 4 mg of gallic acid/g of dry extract for the organic extracts and between 162 ± 3 to 228 ± 2 mg of gallic acid/g of dry extract for the water extracts, however, our DHPE and IHPE samples have a 155 and 142 mg GAE/g extract, respectively, which is almost the same range with this study results from.[20] In addition, in another study, total

**Table 1.** Chemical contents for *H. perforatum* extracts

| Sample | [a]Total phenol content (TPC) | [b]Total flavonoid content (TFC) |
|---|---|---|
| DHPE | 155.87±0.83 | 35.66±0.03 |
| IHPE | 142.96±0.76 | 46.02±0.67 |

**[a]TPC:** mg gallic acid equivalent/g extract
**[b]TFC:** mg quercetin equivalent/g extract
**DHPE:** Domestic *H. perforatum* aquous extract
**IHPE:** Import *H. perforatum* aquous extract

phenolic and flavonoid content of Turkish *H. perforatum* extracts (ethanol, methanol, and water) were determined to have the highest phenolic content as gallic acid equivalent (355.01 ± 0.43 mg/g ethanol extract). In comparison, the ethyl acetate extract had the highest quantity of total flavonoids as quercetin equivalent (167.37 ± 0.88 mg/g methanol extract). However, both phenolic and flavonoid contents were lower than our aqueous extract contents.[21] The phytochemical analysis of methanol extract of aerial parts of *H. perforatum* showed that the total phenolics content was (21.90 ± 0.9 mg/g sample) and the total flavonoids content was (17.10 ± 0.02 mg/g sample). These results confirm that our tested samples both the phenolic and flavonoid content of the extracts are high relative to their peers.[22] Furthermore, *H. perforatum* methanol aerial part extract showed a total phenolic content of 15.01 ± 1.54 mg/g and total flavonoids of 4.67 ± 0.22 mg/g, and both contents were lower than our plant contents.[23]

### 3.2. Antioxidant activity

The results showed that the antioxidant activity of both samples was very close to the standard antioxidants in general. Further, DHPE showed a higher effect than E vitamin and almost was same to BHA as $IC_{50}$ value 72.68±0.41 µg/mL for the total antioxidant test. Furthermore, both samples give nearly the same activity for the DPPH$^{\bullet}$ sacavenging test as $IC_{50}$ value of 4.35±0.24 µg/mL for DHPE and 4.63±1.27 µg/mL for IHPE. Also, there was a slight difference in the $IC_{50}$ value with the standard antioxidants. For the last antioxidant test, both samples had higher effects than the E vitamin, Table 2.

**Table 2.** Antioxidant acttivity results for *H. perforatum* extracts.

| Sample | Total antioxidant | DPPH$^{\bullet}$ scavenging | ABTS$^{\bullet+}$ scavenging |
|---|---|---|---|
| DHPE | 72.68±0.41$^{a}$ | 4.35±0.24$^{a}$ | 16.16±2.05$^{b}$ |
| IHPE | 110.05±0.15$^{c}$ | 4.63±1.27$^{a}$ | 9.14±0.28$^{a}$ |
| BHA | 72.49±2.59$^{a}$ | 3.41±0.16$^{a}$ | 8.93±0.82$^{a}$ |
| Vitamin E | 86.025±0.40$^{b}$ | 4.05±0.32$^{a}$ | 19.50±0.83$^{b}$ |

**Note:** Data are means of three repetitions ± standard deviation (SD), variance analysis: $p<0.05$
**$IC_{50}$ value, µg/mL:** DPPH$^{\bullet}$, ABTS$^{\bullet+}$
**$A_{0.5}$ value, µg/mL:** Total antioxidant
**DHPE:** Domestic *H. perforatum* aquous extract
**IHPE:** Import *H. perforatum* aquous extract

By comparing what we found to other literature research, we found that the radical scavenging activity of both extracts was higher than the methanolic extracts of *H.*

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*perforatum,* which gave an $IC_{50}$ value of 8.7± 0.23 µg/mL against DPPH.[24] Also, in another study, where they determined a high flavonoid content for *H. perforatum* ethanol extract, the DPPH· scavenging test results were $IC_{50}$ value of 10.63 µg/mL, lower than our aqueous extract.[25] Another research investigated the antioxidant properties of Turkish *H. perforatum* species and found that ethyl acetate, methanol, and water extracts are lower than gallic acid as a standard antioxidant; however, our extracts give an activity almost the same as the standards.[21] In another study, the DPPH· scavenging activity of *H. perforatum*' raw extract was $IC_{50}$ of 10.63 µg/mL, which is almost half the scavenging activity value of our extracts.[25]

### 3.3. α–glucosidase and α–amylase inhibitory effect

The α-glucosidase is one of the critical enzymes of the human digestive system, located in the small intestine. Its role is to process and break down complex carbohydrates into small, simple, and absorbable carbohydrates. Inhibiting this enzyme represents a solid solution to delaying glucose absorption and preventing the postprandial rise in blood glucose levels, which may prohibit the development of diabetes. Table 3 clarifies the α–glucosidase inhibition activity of HPE samples and the acarbose. IHPE exhibited the highest inhibition activity with an $IC_{50}$ value of 15.05±1.36 µg/mL; however, both samples showed an inhibition activity higher than the standard inhibitor, which gave an $IC_{50}$ value of 30.62±2.07 µg/mL. Because of its capacity to break down polysaccharide molecules, α-amylase is also considered one of the most important enzymes of the human digestive system, so it also may prohibit the development of diabetes. Results show that (IHPE) has the highest inhibition effect, with an $IC_{50}$ value of 22.24±1.79 µg/mL; this effect was better than the inhibition effect of both (IHPE) and acarbose, Table 3

**Table 3.** Enzyme inhibition acttivity results for *H. perferatum* extracts.

| Sample | α-Glucosidase | α-Amylase |
|---|---|---|
| DHPE | 16.35±0.07[a] | 22.24±1.79[a] |
| IHPE | 15.05±1.36[a] | 54.35±0.35[b] |
| Acarbose | 30.62±2.07[b] | 59.01±1.04[b] |

**Note:** Data are means of three repetitions ± standard deviation (SD), variance analysis: $p<0.05$
**$IC_{50}$ value (µg/mL):** α-Glucosidase, α-Amylase
**DHPE:** Domestic *H. perferatum* aquous extract
**IHPE:** Import *H. perferatum* aquous extract

Moreover, in the literature review that we had done, we found that α-amylase and α-glucosidase inhibition activity for the roots, non-flower shoots, and flower shoots methanolic extracts of *H. perforatum* were lower than acarbose inhibition for α-glucosidase and lower than half the value of acarbose inhibition for the α-amylase enzyme, on the other hand, our extracts were higher in their inhibition activity than acarbose for both enzymes.[26] In another study, the inhibition activity of *H. perforatum* water-alcoholic extract against α-amylase and α-glucosidase enzymes was found to be lower than acarbose (reference inhibitor); by comparing these results to our result, we conclude that *H. perforatum* aqueous extract was more effective against both enzymes.[27]

### 3.4. Determination of antimicrobial capacity

The antibacterial potential of *H. perforatum* samples differed when tested against three-gram positive and three-gram negative bacteria, including *K. pneumoniae, P. aeruginosa, E. coli,* and *B. cereus, E. faecalis,* and *S. aureus*. *H. perforatum* sample antibacterial activities were represented as Minimum Inhibitory Concentration (MIC, g/mL) and MIC values in Table 4.

**Table 4.** Antimicrobial capacity results for *H. perforatum* extracts and antibiotics.

| Samples | Gram-staining-negative | | |
|---|---|---|---|
| | ***E. coli*** | ***P. aeruginosa*** | ***K. pneumoniae*** |
| DHPE | 4096 | 4096 | 4096 |
| IHPE | 256 | 1024 | 1024 |
| Amoxicillin | >1024 | >1024 | >1024 |
| Tetracycline | 4 | 8 | 8 |
| **Samples** | **Gram-staining-positive** | | |
| | ***E. faecalis*** | ***B. cereus*** | ***S. aureus*** |
| DHPE | 4096 | 2048 | 4096 |
| IHPE | 1024 | 64 | 512 |
| Amoxicillin | >1024 | 0.5< | >1024 |
| Tetracycline | 4 | 0.5< | 4 |

**DHPE:** Domestic *H. perferatum* aquous extract
**IHPE:** Import *H. perferatum* aquous extract

The highest antibacterial activity was IHPE against *B. cereus* bacteria, with a MIC value of 64 µg/mL. However, IHPE has shown low antibacterial activity against almost all bacteria, with a MIC value of 2048 µg/mL against *B. cereus* and 2096 µg/mL against the other bacteria. Also, we used Amoxicillin and Tetracycline as positive control in our research; the results showed that IHPE was nearest in its activity to Amoxicillin; on the other hand, Tetracycline was higher than both samples and Amoxicillin. Moreover, in the literature review that we had done, *H. perforatum* extracts had shown a significant effect on *S. aureus.*[28] Another study found that the gram-positive bacterial strains were more sensitive to the methanolic extract of *H. perforatum* in their minimum inhibitory concentration than gram-negative bacterial strains.[22]

### 3.5. DNA protective activity

DNA protection capability for the *H. perforatum* samples was performed using the plasmid DNA derived from pBR322 in the presence of ultraviolet and hydrogen peroxide. The addition of *H. perforatum* samples to the reaction mix prohibits modification of the formation of line DNA. It helps to protect the native formation of DNA (Figure 1, a-b). Our results showed that IHPE had the highest effect on protecting the DNA supercoiled and

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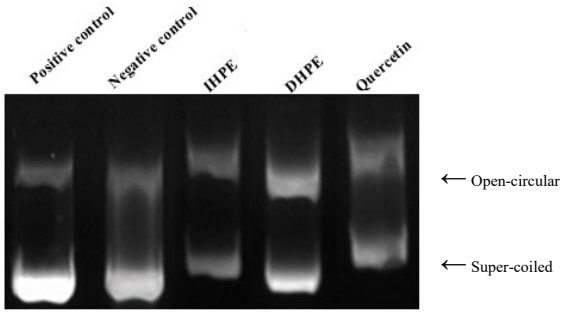
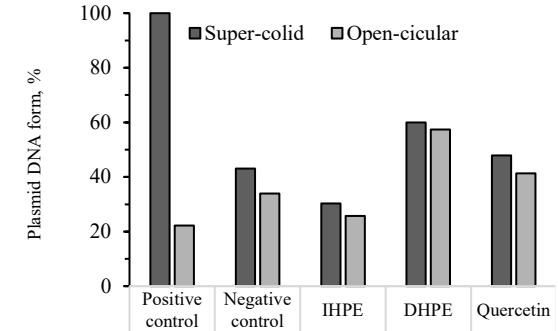
open-circular forms compared to the other tested samples and the quercetin (as a positive control too). However, IHPE gave a 30.31 protection percentage, IHPE gave a 60.01 protection percentage, and quercetin gave a 41.31 protection percentage for the supercoiled form. On the other hand, for the open-circular, the protection percentages were 25.78 for the IHPE, 57.33 for IHPE, and 47.85 for the quercetin. In the previous study made by,[29] they also tested the DNA protection activity for water and ethanol extracts of the *H. perforatum*; it has been proven beyond a doubt that all *H. perforatum* extracts have the ability to offer DNA protection potentials against UV rays when hydrogen peroxide is present. *H. perforatum* seed, flower, fruit methanol, and water extracts exhibit substantial DNA protection activity. However, our extracts also exhibit a high protection activity for plasmid DNA forms.[30]




**Lane 1:** plasmid DNA as a positive control,
**Lane 2:** plasmid DNA with $H_2O_2$ and UV as a negative control,
**Lane 3:** plasmid DNA + $H_2O_2$ + UV + IHPE (Import *H. perforatum* aquous extract)
**Lane 4:** plasmid DNA + $H_2O_2$ + UV + DHPE (Domestic *H. perforatum* aquous extract)
**Lane 5:** plasmid DNA + $H_2O_2$ + UV + Quercetin

**(a)** Agarose gel electrophoresis image




| | Positive control | Negative control | IHPE | DHPE | Quercetin |
|---|---|---|---|---|---|
| Super-colid | 100,000 | 43,058 | 30,310 | 60,010 | 47,850 |
| Open-cicular | 22,178 | 33,913 | 25,780 | 57,330 | 41,310 |

**(b)** Comparing chart of % density of the open-circular and supercoiled forms of plasmid DNA.

**DHPE:** Domestic *H. perforatum* aquous extract
**IHPE:** Import *H. perforatum* aquous extract

**Figure 1.** DNA damage protection potential results for *H. perforatum* samples.

## CONCLUSION

This work represents a cohesive academic base of standard biologic activities for Turkish *H. perforatum* extract for further pharmacological investigation and application. In addition to matching the test results of the domestic extract with the reference materials, we also tested imported *H. perforatum* extract to compare the bioactivity of both extracts. However, we have observed the reflection of the high phenolic content for the domestic extract on the different biological activity as an antioxidant and the inhibitory effect of key enzymes linked to type 2 diabetes mellitus (α-amylase and α-glucosidase), which could be a clue to the existence of potential antidiabetic contents. On the other hand, the imported extract showed a high ability to protect the DNA structure and high antibacterial activity compared to the domestic extract and the reference materials. This is evidence of the difference in the environment of plants in changing the chemical composition.

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## Conflict of interests

*I declare that there is no a conflict of interest with any person, institute, company, etc.*

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