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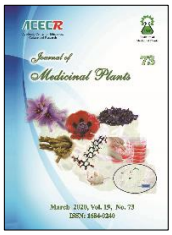

Research Article

Capparis spinosa: a comparative study of raw and processed fruits

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ABSTRACT

Background: *Capparis spinosa* is used in different countries for prevention and treatment of various diseases. Its fruits are usually used in processed form especially with vinegar. **Objective:** The aim of the present study was investigating the effect of processing on some phytochemical constituents and biological activities of *C. spinosa*. **Methods:** The fruits were processed with grape vinegar. Total phenolics and alkaloids contents of the raw and processed fruits were determined by Folin-Ciocalteu and titration methods, respectively. For quantitation of rutin, quercetin and α-tocopherol, R-HPLC was used. Cytotoxic activity of the fruits was determined by MTT assay. Antioxidant properties were evaluated by DPPH and FRAP techniques. Fingerprinting of raw and processed fruits were prepared using TLC and evaluated by TLC densitometry instrument. **Results:** The results demonstrated that while total phenolics, total alkaloids and quercetin contents were decreased in the processed fruits, rutin concentration remained unchanged. Antioxidant activities of processed fruits increased using both DPPH and FRAP methods. Raw and processed fruits showed no cytotoxic effect on MCF-7, HepG-2 and MDBK cell lines up to the concentration of 100 µg/ml. The fingerprints of the fruits were different which admitted the change in the fruit constituents due to processing. **Conclusion:** It seems that processing with vinegar lessens the unpleasant taste of the plant due to alkaloids and increased the antioxidant effects; therefore, it would be more suitable for use in some diseases such as diabetes and hepatitis as it is used in folklore and traditional medicine.

Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing ability of plasma, R-HPLC, reverse phase high performance liquid chromatography; TLC, thin layer chromatography; MDBK, madin-darby bovine kidney cells, HepG-2, hepatocellular carcinoma cells; MCF-7, michigan cancer foundation-7 breast cancer cells; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TPTZ, (2,4,6-tri-pyridyl-s-triazine); BHT, Butylated hydroxytoluene; ITM, Iranian traditional medicine

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1. Introduction

Medicinal plants have been used as effective and almost safe therapeutic agents from ancient times. Many of them have multiple properties and use for diverse diseases. Moreover, they have shown fewer side effects than chemical drugs [1]. Nowadays, medicinal plants and their products are considered as an important source of bioactive constituents such as phenolics and flavonoids which are important in prevention and treatment of many diseases like cardiovascular and neurodegenerative diseases [2-4]. *Capparis spinosa* L., caper, (Capparidaceae) is one of the economical plants widely distributed in different region of the world [5] and is used in traditional medicine of many countries for diverse illnesses [1]. Several countries such as Greece, Italy, Spain and Turkey widely produce caper and export its different products to another countries which is used especially in food industry [6]. During the past two decades, more attention has been paid to phytochemistry and biological effects of *C. spinosa*. Phytochemical analysis demonstrated that the plant is rich in poly phenolics compounds which have been considered as the most responsible agents for health promoting effects of the species [7-9]. In addition, the plant contains alkaloids, glucosinolates, terpenoids and tocopherols [1]. During different studies, it has been proved that *C. spinosa* possesses various biological effects including antioxidant, anti-diabetes, antimicrobial, anticancer and hepatoprotective properties [10-15]. The fruits which are widely used, have bitter taste. Different methods are used to make fruits suitable for use such as fermentation [16] and pickle preparation. In Iran, especially in Iranian traditional medicine (ITM), fruits are used in pickle form for treatment of CNS and liver diseases [17]. Since, processing may change the plant constituents, in this research, the effect of vinegar processing on phenolics, alkaloids, rutin, quercetin and α-tocopherol contents, antioxidant and cytotoxic effects of caper have been investigated. Moreover, TLC fingerprint of raw and processed capers have been prepared.

2. Materials and Methods

2.1. Plant Material

Caper (ripped fruits) were collected in June 2016 from Pars Abad Moghan (Ardabil Province) and identified in Herbarium of Traditional Medicine and Materia Medica Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran (voucher no. 3969 TMRC).

2.2. Chemicals

Folin Cio-calteu and pyrogallol were purchased from Merck Co. (Germany). Rutin, quercetin, α-tocopherol, DPPH, BHT and MTT were prepared from Sigma-Aldrich Co. (Germany). TPTZ was from Fluka Co. (UK) and grape vinegar was from Varda Co. (Iran). Thin layer chromatography was performed by using silica gel plates (Merck, Germany). All other solvents were in analytical grade (Merck, Germany). HepG-2, MCF-7 and MDBK cell lines were purchased from Pasteur Institute of Iran (Iran).

2.3. Pickle preparation

One thousand grams of intact fresh fruits was macerated in water (1:10) for 24 h. The mixture was sieved and again macerated in water for further 24 h. This process was repeated for 10 times and then the fruits were decocted in water for 1 min (three times after refreshing the water). Finally, the fruits were mixed with grape vinegar 30% and deposited for 30 days at room temperature. After that, the fruits separated from vinegar, dried in the shade and powdered.

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2.4. Total phenolics contents

Total phenolics contents of caper were determined by Folin cio-calteu method by using pyrogallol as the standard material [18].

2.5. Rutin and quercetin contents

HPLC method was used for quantification of rutin and quercetin in the caper fruits [19].

Test solution: One gram of the powdered fruits was placed in a 5 ml conical flask and 4 ml methanol was added to the sample. The mixture was treated in an ultrasonic bath for 30 min and the volume adjusted with methanol to 5 ml. A portion of this solution was filtered through a membrane filter.

Standard solution: Quercetin and rutin (5 mg each one) were dissolved in methanol and diluted to 5 ml with the same solvent (stock solution). A diluted solution (5 ppm) was prepared from stock solution.

HPLC condition was as following: AQ C18, ACE, 4.6×250 mm, 5µm column, formic acid 1% in water: methanol with gradient mode (Table 1) as mobile phase, flow rate 0.6 ml/min in wave length of 257 nm, column temperature 25 °C, injection volume 100 µl.

2.6. Determination of total alkaloids as hyoscyamine

Total alkaloids of raw and processed fruits were determined according to Iranian Herbal Pharmacopoeia [20]. In this method, at first alkaloids were extracted by using mixture of ammonia/ethanol/diethyl ether, then the extract was acidified and alkaloids were transferred to aqueous phase. This solution was again alkalized by ammonia and extracted with chloroform. In the final process, chloroform fraction acidified and aqueous phase was titrated by NaOH. The percentage of total alkaloids was determined as hyoscyamine.

2.7. α-Tocopherol content

HPLC was used for α-Tocopherol determination in the fruits. Sample and standard material were dissolved in methanol and injected to HPLC with following condition: C_{18} AQ, 250×4.6 mm, 5µm column in 25°C, methanol:H_2O 96:4 as mobile phase, flow rate of 1.5 ml/min, 20µL injection volume in wave length of 284 nm. The content of α-Tocopherol in the sample was determined by comparing to the standard.

2.8. Fingerprinting of raw and processed caper

Fingerprinting of caper was performed by using silica gel plates and *n*-butanol: acetic acid: H_2O 40:1:10 as stationary and mobile phase, respectively. For preparation of test solution, 1 g of powdered fruits was added to 5 ml conical flask and methanol was added. Sulfuric acid 10% in methanol was used as reagent. The plates were scanned under 366 and 700 nm by Camag TLC scanner 3.

Table 1. Gradient condition for separation of rutin and quercetin in *Capparis spinosa* fruits

Time (min)	Methanol (%)	Formic acid 1% (%)
0	5	95
10	15	85
25	30	70
40	65	35
45	98	2
55	98	2
60	5	95

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2.9. Antioxidant effect of the caper by Ferric Reducing Ability of Plasma (FRAP) method

The antioxidant activity of raw and processed fruits was determined by FRAP method [21]. Briefly, the FRAP reagent contained 2.5 mL of a 10 mmol/L TPTZ solution in 40 mmol/L HCl plus 2.5 mL of 20 mmol/L $FeCl_3.6H_2O$ and 25 mL of 0.3 mol/L acetate buffer (pH 3.6) which was prepared freshly and warmed at 37 ºC. Alliquots of 40 µL of sample (methanolic extract) were mixed with 0.2 mL distilled water and 1.8 mL FRAP reagent and were incubated at 37 ºC for 10 min. The absorbance of the reaction mixture was measured at 593 nm. Calibration curve of $FeSO_4.7H_2O$ was plotted by using different concentrations. FRAP value was determined for each solution and expressed as mmol $FeSO_4.7H_2O$/100 g extract. Butylated hydroxytoluene (BHT) was used as positive control.

2.10. Determination of 2,2-Diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay

DPPH radical scavenging activity of samples was measured according to previous works [21, 22] as following: 100 µL of 100 µM DPPH methanol solution was added to 100 µL of various concentrations of the methanolic extracts. The mixture was shaken and left at room temperature for 30 min. Then, the absorbance of the solutions was measured at 517 nm by an ELISA reader and antioxidant activity was calculated. IC_{50} was calculated from the plot of inhibition percentage against extract concentration. BHT was used as positive control.

Table 2. The results of analysis of raw and processed *Capparis spinosa* fruits

Test	Raw caper	Processed caper
Total phenolics content as pyrogallol (%)	0.19±0.01	0.02±0.001
Rutin content (mg/100g fruits)	3.10±0.03	3.13±0.06
Quercetin content (mg/100g fruits)	1.66±0.01	0.35±0.002
Total alkaloids content (%)	1.87±0.01	0.62±0.001
α-Tocopherol content	0.0	0.0
FRAP value as mmol $FeSO_4.7H_2O$/100g extract	16.05±0.36	65.71±4.33
DPPH radical scavenging activity (IC_{50}, µg/ml)	556.66±26.73	67.13±8.60
Cytotoxic activity on HepG-2 (IC_{50}, µg/ml)	>100 µg/ml	>100 µg/ml
Cytotoxic activity on MCF-7 (IC_{50}, µg/ml)	>100 µg/ml	>100 µg/ml
Cytotoxic activity on MDBK (IC_{50}, µg/ml)	>100 µg/ml	>100 µg/ml

2.11. Evaluation of cytotoxic activity

2.11.1. Cell lines: The cell lines were obtained from the Pasteur Institute, Tehran, Iran: HepG-2 (human hepatocellular carcinoma), MCF-7 (human breast adenocarcinoma) and MDBK (bovine kidney cells). MCF-7 cells were cultured in DMEM medium with 5% FBS, while the other two cell lines were maintained in RPMI 1640 medium with 10% FBS to obtain the desired growth.

2.11.2. MTT assay: Assessing the viability of the cells was carried out in a micro culture tetrazolium/formazan assay (MTT assay). 96-well plates were used and HepG-2 cells 15×10^3, MCF-7 cells 8×10^3 and MDBK cells 10×10^3 were seeded in each well. They were then incubated at 37°C. After 24 h the medium was replaced with fresh medium containing different concentrations of the methanolic extracts to be tested. After 72h exposure of cells at 37°C to each sample, the medium was replaced with fresh medium containing MTT, with a final concentration of 0.5 mg/mL. The cells were incubated for another 4 h in a humidified

atmosphere at 37°C, then the medium containing MTT was removed and the remaining formazan crystals were dissolved in DMSO. The absorbance was recorded at 570 nm with an ELISA reader (TECAN). Tamoxifen was used as positive control. The relative cell viability (%) related to control wells containing cells, cell culture medium and DMSO 1% was calculated by [A] samples /[A] control × 100. Where [A] samples is the absorbance of test sample and [A] control is the absorbance of wells containing cells + medium + DMSO, 1%. To calculate IC_{50} values, viability (%) versus concentrations was graphed using the Microsoft Excel program [23, 24].

3. Results

The results of the comparison between raw and processed caper have been demonstratd in Table 2. As it is obvious, total phenolics content was decreased during processing because of the vinegar effect on phenolic compounds and probably destroying them. Quantization of rutin and quercetin as two major flavonoids in the fruits demonstrated that rutin was unchanged but quercetin decreased which caused lower concentration of total phenolics in processed fruits (Fig. 1). No α-tocopherol was found in the fruits. During fruit processing, total alkaloids content were decreased about 70%, however, antioxidant activity have increased. Both raw and processed fruits showed no cytotoxicity on the studied cell lines up to 100 µg/ml.

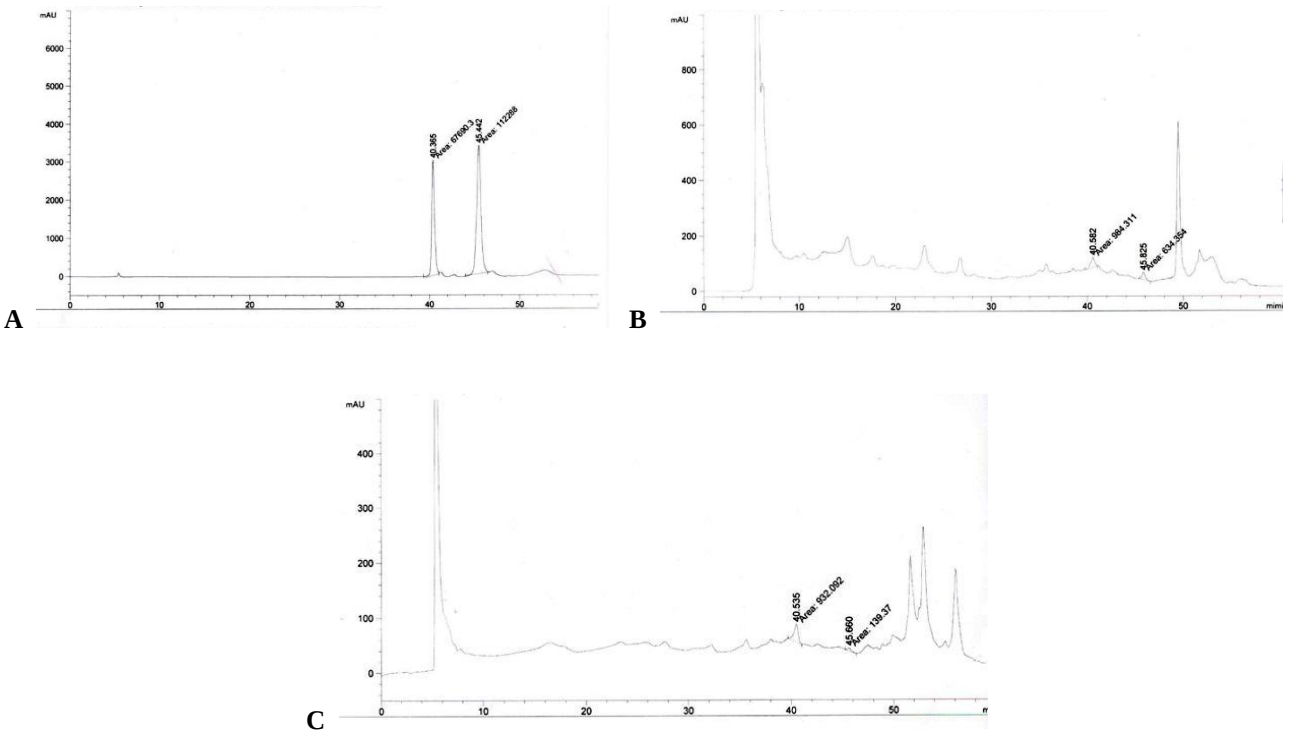

Fig. 1. HPLC chromatogram rutin and quercetin (A), raw caper (B) and processed caper (C)

The chromatograms of raw and processed caper were completely different (Fig. 2). Indeed, in 700 nm, three brown spots were observed in lower half part of the raw fruits chromatogram but no spot was in this part of processed caper chromatogram. In both chromatograms, several spots were obvious in the upper parts of the silica gel plate with different intensities. In 366

nm, there were blue-violet spots in middle parts of the chromatogram in processed caper but these spots were very weak in the raw fruit chromatogram.

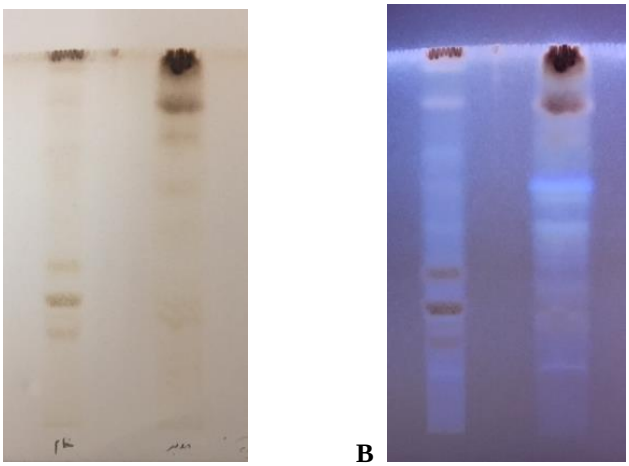

Fig. 2. TLC fingerprint of raw and processed caper under day light (A) and UV 366 nm (B).

4. Discussion

Capparis spinosa is used in traditional and folklore medicine of many countries [1]. It is consumed as pickle form. The findings of the present study demonstrated that vinegar has certainly destructive effect on some phenolic components which cause decreasing of total phenolics content but for determining the vinegar effect on specific constituents, quantitative determination of each component should be performed. In a study on caper collected from Tafresh, Iran, quercetin content was found 9.6 mg/g which is more than our study (1.66 mg/100g) [25]. While other studies demonstrated high concentration of α-tocopherol in caper [26], no α-tocopherol was found in the fruits in our study which demonstrate the effect of different climatic situation on the plant metabolites.

Alkaloids as secondary metabolites play different roles in plants and usually induce a bitter taste. It seems that one of the reasons for several washing of the fruits with water before adding vinegar is omitting the bitter taste. Raw caper is very bitter and many of water soluble alkaloids omit during processing and their concentrations decrease after processing. In fact, processing improves the caper taste. The vinegar effect on caper components were established in fingerprinting study as well with distinct differences between TLC chromatograms of raw and processed fruits.

No cytotoxic effect was seen on HepG-2, MCF-7 and MDBK cell lines up to 100µg/ml. Other investigations have shown cytotoxic effects of different parts of *C. spinosa* on various cell lines. Al-daraji *et al*. demonstrated toxic properties of *C. spinosa* leaves on Hep-2 and HeLa cells in concentration of 125-1000 µg/ml in dose-dependent manner [27]. It has proven that seeds contain 38 kDa protein which is similar to imidazole glycerol phosphate synthase and it inhibits MCF-7, HT-29 and HepG-2 proliferation [28]. In another study, the effect of different extracts of the plant fruits on Hep-2 and HeLa cells have been investigated. The results showed polyphenolics extract were cytotoxic in concentration of 10000 µg/ml. Other extracts showed less cytotoxicity [29]. It is obvious that caper is cytotoxic only in high concentrations and in the recent study it established no toxic effect up to 100µg/ml

which is common concentration for considering an extract to be toxic.

The results of antioxidant activity demonstrated that vinegar induced more radical scavenging and antioxidant activity. Different investigations have been performed on vinegars such as apple and grape vinegars and high antioxidant activity was established [30, 31]. In the other hand, vinegar has antioxidant activity which causes more antioxidant effect in processed caper. In a research on antioxidant properties of grape and its processed products, it has been proved that high effects of wine and vinegar rather than juice [30]. Regarding role of antioxidant compounds in prevention and treatment of many diseases such as cancer, hepatitis, diabetes, Alzheimer, Parkinson it may be concluded that the reason of usage of processed caper is its high antioxidant effect. Although better taste of processed caper is other reason for its processing.

5. Conclusion

It seems that processing with vinegar changes some components in the caper as it is obvious in finger printing study. Total phenolics and among them quercetin decreased during processing but antioxidant effect increased significantly due to vinegar constituents. Moreover, processing ameliorated the unpleasant taste of the plant through decreasing alkaloids; It should be noticed that the raw and processed caper showed no toxicity effect on normal cell line (MDBK); therefore, it could be consider safe for use. In general, regarding the mentioned changes in chemical constituents and biological activities of caper after processing, it would be more suitable for use in some diseases such as diabetes, hepatitis and neurodegenerative disorders as it is used in folklore and traditional medicine.

Author contributions

Homa Hajimehdipoor and Mahnaz Khanavi designed and supervised the study. Narjes Khavassi prepared processed fruits and Leila Ara has performed experimental part.

Conflict of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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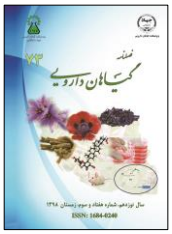

مقاله تحقیقاتی

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اطلاعات مقاله

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مقدمه: میوه گیاه کبر در کشورهای مختلف برای پیشگیری و درمان بسیاری از بیماری‌ها مصرف می‌شود. این میوه‌ها معمولاً به صورت تدبیر شده با سرکه مورد استفاده قرار می‌گیرند. **هدف:** هدف از این مطالعه، بررسی اثر تدبیر با سرکه بر بعضی از ترکیبات میوه گیاه و نیز برخی خواص بیولوژیک آن بوده است. **روش بررسی:** میوه‌های گیاه به صورت ترشی با استفاده از سرکه انگوری آماده‌سازی شدند. میزان ترکیبات فنلی و آلکالوئیدهای تام نمونه‌های خام و تدبیر شده به ترتیب با استفاده از روش فولین سیوکالتو و تیتراسیون اندازه‌گیری شدند. مقدار ترکیبات روتین، کوئرستین و آلفاتوکوفرول با استفاده از HPLC تعیین شد. اثر سایتوتوکسیک دو نمونه توسط روش MTT ارزیابی شد. خواص آنتی‌اکسیدانی میوه‌های خام و مدبر با روش DPPH و FRAP تعیین شدند و فینگرپرینت آنها نیز با روش TLC تهیه شد. **نتایج:** نتایج نشان دادند که میزان ترکیبات فنلی تام، آلکالوئیدها و کوئرستین در میوه‌های مدبر کاهش یافته ولی میزان ماده روتین بدون تغییر باقی مانده بود. اثرات آنتی‌اکسیدانی میوه‌ها طی عمل تدبیر افزایش یافته بود. میوه‌های خام و مدبر فاقد اثر سایتوتوکسیک روی رده‌های سلولی MCF-7, HepG-2 و MDBK تا غلظت ۱۰۰ میکروگرم در میلی‌لیتر بودند. فینگرپرینت دو نمونه نیز تفاوت‌های آشکاری را با یکدیگر نشان داد که نشان دهنده تغییر ترکیبات ناشی از عمل تدبیر است. **نتیجه‌گیری:** به نظر می‌رسد تدبیر با سرکه طعم بد گیاه ناشی از آلکالوئیدها را کاسته و اثرات آنتی‌اکسیدانی را افزایش می‌دهد. بنابراین میوه‌های مدبر می‌تواند انتخاب مناسبی در درمان بسیاری از بیماری‌ها مانند دیابت و هپاتیت با توجه به مصرف آن در طب سنتی باشد.

مخففها: (DPPH) 2,2-diphenyl-1-picrylhydrazyl; (FRAP) ferric reducing ability of plasma, (R-HPLC) reverse phase high performance liquid chromatography; (TLC) thin layer chromatography; (MDBK) madin-darby bovine kidney cells, (HepG-2) hepatocellular carcinoma cells; (MCF-7) michigan cancer foundation-7 (breast cancer cells); (MTT) 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; (TPTZ) (2,4,6-tri-pyridyl-s-triazine); (BHT) Butylated hydroxytoluene; (ITM) Iranian traditional medicine

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