Phytochemical status and antioxidant capacity of selected hot pepper varieties cultivated in Tanzania

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Abstract

Hot pepper is one of the most important economic fruits with abundant of nutritional qualities. Thus, the consumption of foods of plant origin with high nutritional and bioactive compounds of high antioxidants capacity is a major concern nowadays. A study was conducted to determine and quantify concentrations of important bioactive compounds present in the nine selected hot pepper varieties cultivated in Tanzania. Hot peppers were grown in the field at Botany department research farm, University of Dar es Salaam from March to September, 2021. Then, ripe-fruit extracts were used to determine vitamin C, beta-carotene, lycopene, total phenolic content (TPC) and total flavonoid content (TFC)among the varieties quantitatively. The results indicated that there were very high significant variations on the nutrients and bioactive compound concentrations (p<0.0001) among all varieties of hot pepper evaluated. The highest amount of vitamin C was 948.30 ± 22.6 mg/100g recorded from orange bird eye variety. The high concentration of beta-carotene was 0.62 ± 0.006mg/ 100 ml recorded from orange bird eye. The highest concentration of lycopene was 0.55 ± 0.0003 mg/ 100 ml from Big red cayenne. The highest concentration of TPC was 454.247 ± 20.9mg GAE/ 100g obtained from Big red cayenne. The highest concentration of TFC was 8.92 ± 0.15 mg RE/ 100g obtained from Big red cayenne. High variability was observed among the hot pepper varieties; Big red cayenne variety with the highest concentrations of TPC, TFC and lycopene, showed to have the highest radical scavenging capacity. Big red cayenne can be a cheap source of natural antioxidants, therefore recommended for future development of nutraceuticals or healthy food products and conveying great benefits for food and pharmaceutical industries, consumers, and producers.

Keywords: Hot pepper varieties in Tanzania; phytochemical, bioactive compounds; antioxidant activity

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INTRODUCTION

Hot peppers (chili peppers) are fruits of herbaceous flowering plants which belong to the family Solanaceae and genus Capsicum (Othman et al., 2011; Chakrabarty et al., 2017). All Capsicum species originated from American continents: northern South America, Central America, the Caribbean islands, Mexico and southern part of the United States of America (USA). Hot peppers were used as spices to add spiciness and flavor to dishes and as traditional medicines for many centuries since the beginning of civilization in the Americas 7500 BC (Csillery et al., 2006; Delelegen 2011). The Capsicum species have various names in different countries, while in Tanzania they are collectively called “pili pili”, but universally they are known as “hot” or “chilli” peppers (Gobie et al., 2019).

Hot pepper is one of the most appreciated crops used as vegetables, spices and source of medicines worldwide (Martínez-Ispizua et al., 2021). Currently, people are more careful with the foods they choose to consume. Most people prefer natural foods of plant origin as they are considered as most safe compared to artificial food supplements (Chávez-Mendoza et al., 2015). Studies conducted in different countries have indicated that hot pepper fruits are known to have high concentration of bioactive compounds such as vitamin C, beta-carotene, lycopene, total phenolic and flavonoid contents among others which are powerful antioxidants. But the bioactive contents depend on the variety and the influence of various conditions at the growing area.

Antioxidant compounds help in the protection of body important molecules such as protein, lipid and genetic materials against the effect of free radicals produced during metabolic processes taking place in the body (Phuyal et al., 2020). Thus, consumption of hot pepper with high contents of antioxidant compounds in the diets would provide preventive and therapeutic properties for many diseases such as various types of cancer, rheumatism, stiff joints, bronchitis and chest colds with cough and headache, arthritis and heart diseases among others before their commencement (Saleh et al., 2018). The inclusion of hot pepper in the diets would increase levels of antioxidant in the blood and help to balance the antioxidant with free radicals which are continuously produced during metabolic activities in the body. This would help to reduce the risks of development of many degenerative diseases, as well as aging (Chávez-Mendoza et al., 2015).

Many studies on hot peppers have focused on the determination and quantification of important nutritional and bioactive compounds present in plant food materials. However, the variabilities reported on the amounts of nutritional and non-nutritional bioactive compounds among the hot pepper varieties could be due to variation in genetics and environmental conditions at the growing area (Chakrabarty et al., 2017). A recent study by Flores-Felix et al. (2021) indicated that phenolic compounds present in most plant origin foods have higher capacities to inhibit corona virus to attack host cells and cause infection. Understanding of the variabilities on the amounts of nutrients and antioxidant compounds present among the hot pepper varieties is necessary and might be helpful to hot pepper farmers, processors and consumers in the selection of varieties with desirable chemical qualities. There are several reports about the chemical composition of the divergent hot pepper seeds grown under different ecological conditions in Egypt, United States and Korea (Jarret et al., 2013; Park et al., 2006). However, there is little information available about phytochemicals constituents of hot pepper seeds grown in Tanzania. Therefore, the aim of this study was to determine the content of bioactive compounds and antioxidant activity of selected hot pepper varieties cultivated in Tanzania.

MATERIALS AND METHODS

Study Area

This study was conducted at the Department of Botany Research Farm, University of Dar es Salaam (UDSM), Tanzania. The study was conducted from March to September, 2021. The field is located at latitude 6°46’50” S and longitude 39°12’12” E (www.udsm.ac.tz). The area has tropical climate with high temperatures and relative humidity ranging from 19°C to 32.5°C and 74% to 84%, respectively (https://www.udsm.ac.tz). It normally receives average rainfalls above 1000 mm per year which occurs in short and long rainy seasons from October to December and March to May respectively with some fluctuations.

Experimental Materials

The hot pepper varieties were collected from local farmers at different places; they included: Orange Habanero (OH) variety obtained from Kigamboni, Dar es Salaam and Mbulu, Manyara; Red Habanero (RH) from Mazinde-Ngua in Korogwe district, Tanga; Scotch Bonnet Habanero (SBH) obtained from Ubungo, Dar es Salaam; Big Red Cayenne (BRC) obtained from Kigamboni, Dar es Salaam; African Bird’s Eye (ABE1) was collected from Kigamboni, Dar es Salaam; African Bird’s Eye (ABE 2) from Kwesine – Lushoto, Tanga; Long Red Cayenne (LRC) obtained from Kwesine – Lushoto, Tanga; Orange Bird’s Eye hot pepper (OBE) obtained from Mbelei – Lushoto, Tanga and Chiltepin chili peppers (CCP) obtained from Ubungo, Dar es Salaam (Plate 1). The seeds extracted from the hot pepper fruits collected from farmers were sown in plastic containers containing a mixture of soil, compost manure and sand soil in the ratio of 3:2:1. The seedlings were transplanted to the field when they had 5 to 7 leaves. At the field, they were uniformly supplied with water and nutrients and other agronomic practices. Weeding was done manually whenever required in order to control weed growth.

Experimental Design

The design used in this study was Completely Randomized Design (CRD) with three replications. Two hot pepper plants from the middle row and one plant at the side row of each plot were selected as sample plants. The plants at the side edges of each plot were not selected in order to minimize edge effects (Tesfaw et al. 2013). From each variety, nine sample plants were used to collect samples. The fresh and ripe hot pepper fruits randomly picked from the sample plants were taken to the laboratory and were cleaned pure water in order to remove any dirt. Then, fruit extracts were prepared and used to measure vitamin C, beta-carotene, lycopene, total phenolic and flavonoid contents, and antioxidant capacity.
Bioactive Compounds Determination

Vitamin C

Determination of vitamin content in the hot pepper fruit extracts carried out by using a method described by Adebisi et al. (2014) and Desai et al. (2019) with modification; where extraction was done by using hot distilled water, and ascorbic acid was used as the standard. Before the analysis, 2 ml of each sample extract solution, 2 ml of standard solution and 2 ml of blank solution (distilled water) were measured by using a measuring cylinder and placed into separate 25 ml capacity flasks. Then, 2 ml of sulphuric acid (10% v/v) was added and followed by 5 ml of ammonium molybdate (10% w/v). The mixtures were vortex mixed and allowed to stand for 50 minutes at room temperature for color development. Each mixture was further diluted with distilled water to obtain 25 ml final volume. The absorbances of samples and standard solutions were measured at 450 nm against the blank by using

**Plate 1:** Pictures of hot pepper fruits used to extract seeds used for the study.
a spectrophotometer. Standard absorbances were plotted to obtain a calibration curve and equation. The calibration equation was then used to calculate concentration of vitamin C in each sample extract solution. The results were reported in mg/100 grams of the sample.

**Total Flavonoid Content (TFC)**

The TFC were estimated by using aluminum chloride colorimetric assay described by Rahman et al. (2015). Exactly 0.01 g of each hot pepper fruit extract was dissolved in 50 ml of ethyl acetate. Rutin reagent of 1mg/ml concentration was used as standard for flavonoids stock solution. Then, several concentrations: 0.1, 0.2, 0.4, 0.8 and 1.0 mg/ml were prepared from the stock using proper dilutions. To determine TFC, 10 ml of each extract in ethyl acetate was pipetted into a test tube and mixed with 1 ml of 2% (w/v) AlCl₃ in methanol solution containing 5% acetic acid; then, the contents in the test tubes were agitated using a vortex mixer. Absorbances of both sample and standard solutions were immediately measured at 425 nm using a spectrophotometer against a blank (mixture of ethyl acetate and aluminum chloride) solution. The absorbances of standard solutions were used to plot the calibration curve from which the calibration equation was obtained and used to calculate total flavonoid contents in all sample extracts. Data were reported as mean ± standard deviation of three replications.

**Antioxidant Capacity**

The DPPH free radical scavenging activity assay described by Rahman et al. (2015) and Phuyal et al. (2020) was used to determine antioxidant activity of hot pepper extracts. A series of extract concentrations with different ratios of extracts in methanol: 1:10, 1:102, 1:103, 1:104 and 1:105 were prepared. To 4.9 ml of each hot pepper fruit extract solution, one hundred microliters (100 µl) of 5 mM DPPH in methanol was added. The mixtures were vortex mixed thoroughly and allowed to stand in a dark place at room temperature for 30 minutes. The absorbance of sample extract solutions and that of control (DPPH only) were measured by using a spectrophotometer at 517 nm. Ascorbic acid (AA) was used as standard. The radical scavenging activity (RSA) of each variety expressed as percentage of DPPH inhibition (%DPPH RSA) was calculated by using the following formula:

\[
%\text{DPPH RSA} = \left[ \frac{A_0 - (A_1 - A_S)}{A_0} \right] \times 100
\]

where \(A_0\) is the absorbance of control (DPPH only), \(A_1\) is the absorbance of sample extracts containing DPPH and \(A_S\) is the absorbance of sample extracts dilution without DPPH. Then %DPPH RSA values were plotted against sample extracts concentrations to obtain non-linear sigmoid curve. The curves were then
used to calculated the lowest concentrations of each extract solution required to inhibit DPPH RSA by 50% (LC50).

Statistical Analysis

All analyses were performed in triplicated. The results were analyzed by a One-way analysis of variance (ANOVA) and a comparison of means was made by Tukey’s test with the help of SAS (Statistical Analysis Software of 1999) version 8.0. The means were accepted as significantly different at a 95% confidence interval (p ≤ 0.05).

RESULTS

Vitamin C

In general, high significant differences in vitamin C concentration (p<0.0001) were observed among the hot pepper varieties evaluated (Figure 2). However, when observed at species level, in C. annuum varieties, the concentration of vitamin C ranged from 577.83 ± 10.74 mg/100 g in LRC, 475.93 ± 0.36 mg/100 g in BRC and 204.39 ± 5.3 mg/100 g in CCP. In C. frutescens varieties, vitamin C concentration varied from 948.30 ± 22.6 mg/100 g in OBE, 201.00 ± 7.82 mg/100 g in ABE1 and 20.77 mg/100 g in ABE2. Whereas in C. chinense, the mean values of vitamin C concentration ranged from 388.73 ± 24.33 mg/100g in RH, 388.17 ± 47.87 mg/100g in SBH and 127.05 ± 14.96 mg/100g in OH respectively. On average, the highest concentration of vitamin C was found in C. annuum varieties followed by C. frutescens and C. chinense varieties had the lowest content of vitamin C.

Beta-carotene

The analysis of variance revealed that there were high significant differences in beta-carotene concentration (p<0.0001) among the varieties of hot peppers evaluated (Figure 3). At species level, the content of beta-carotene varied in the decreasing order as follows; in C.frutescens varieties, OBE, ABE2 and ABE1 contained 0.62, 0.56 and 0.54 mg/ 100 ml respectively. In the C. annuum varieties, LRC, BRC and CCP contained 0.41, 0.28 to 0.19 mg/ 100 ml of beta-carotene respectively. Whereas in C. chinense varieties: SBH, OH and RH, have beta-carotene contents varied from 0.30, 0.20 to 0.07 mg/ 100 ml respectively.

Lycopene

The results showed a strong significant difference in the concentration of lycopene in the varieties of hot peppers evaluated (p<0.0001). Observation of the results revealed that the concentration lycopene decreased from deep-red hot pepper fruits to light-red fruits and finally to orange fruits with the lowest concentration (Figure 4). On average, a greater content of lycopene was recorded from C. annuum varieties then followed by the content found in C. frutescens and C. chinense had the lowest content of lycopene.

Total Phenolic Content (TPC)

High variations were observed on the content of TPC (p<0.0001) among the hot pepper varieties evaluated (Figure 5). At species level, the amount of TPC found in the hot pepper fruits extracts decreased in the following manner: in C.annuum, the concentration ranged from 454.247 ± 20.9 (BRC) >447.880 ± 14.6 (CCP)>301. 417 ± 12.08 (LRC)mg GAE/ 100g of extracts. In C. chinense, the content varied from 374.98 ± 23.5 (RH) >296.807 ± 1.84 (OH) > 260.35 ± 1.32 (SBH)mg GAE/ 100g of extracts. In C. frutescens, the concentration varied from 288.13 ± 21.98 (ABE 2) > 271.99 ± 3.79(ABE 1 > 236.197 ± 7.81 (OBE) mg GAE/ 100g of extracts.

Total Flavonoid Content (TFC)

The analysis of variance indicated that there were high significant differences on the TFC content (p<0.0001) among hot pepper varieties evaluated (Figure 6). The observation at species level indicated that C. annuum varieties: BRC, LRC and CCP the TFC content varied from 8.92 ± 0.15 mg RE/ 100g,5.27 ± 0.08 mg RE/ 100g and 4.46 ± 0.08 mg RE/ 100g respectively. In C. chinense varieties: SBH, OH and RH the TFC concentration varied from 7.2 ± 0.06 mg RE/ 100g, 6.25 ± 0.07 mg RE/ 100g and 4.04 ± 0.17 mg RE/ 100g as recorded respectively. In C. frutescens varieties: OBE, ABE 2 and ABE 1 the TFC concentration varied from 6.55 ± 0.3 mg RE/ 100g, 5.95 ± 0.04 mg RE/ 100g to 4.40 ± 0.06 mg RE/ 100g respectively.
Figure 2: The variation in concentration of vitamin C observed in the hot pepper fruit extracts

Figure 3: The variation in beta-carotene contents in hot pepper varieties

Figure 4: Variation in concentration of lycopene in hot pepper extracts

Figure 5: Total phenolic contents in hot pepper fruits extract solutions

DPPH radical scavenging activity

The results obtained from DPPH radical scavenging activity assay were expressed in terms of the lowest concentration of antioxidant required to scavenge 50% of DPPH free radicals (LC$_{50}$). The sample extracts with the lowest LC$_{50}$ value were considered as varieties with the greatest antioxidant capacity. The findings from this study revealed that BRC variety had the smallest LC$_{50}$ value and followed by CCP, OBE, OH, LRC and SRH compared to the ascorbic acid (AA) used as antioxidant standard (Figure 7).

**Figure 6:** Total flavonoid contents in the hot pepper fruits extract solutions

**Figure 7:** Variation in LC$_{50}$ values for hot pepper fruit extracts and AA

DISCUSSION

Vitamin C (Ascorbic acid)

The results obtained from this study suggest that hot peppers are an excellent source of vitamin C. Highest amount of vitamin C was recorded from OBE variety and followed by LRC and BRC varieties. However, all varieties evaluated contained higher amounts indicating that the some of the selected hot pepper varieties cultivated in Tanzania are composed...
of higher amounts of vitamin C. Even the lowest amount (127.05 ± 14.96 mg/100g) recorded from OH variety exceeded the recommended daily amount of 60 mg/100g (Chaves-Mendoza et al. 2015). These results are consistent with the findings reported by Korkutata and Kavaz (2015) and Hamed et al. (2019). Also, the results are in line with findings reported by Palma et al. (2014) who stated that hot peppers contained higher amounts of vitamin C compared to many other types of fruits. Kantar et al. (2016) stated that some hot pepper varieties have higher amount of vitamin C as twice as the concentration found in tomatoes, apples and oranges per gram of fruit weight.

Vitamin C is an essential dietary nutrient for various biological functions in human health and well-being. It is a powerful antioxidant; an excellent free radical scavenger molecule. It can act as a cofactor for several important biosynthesis numerous enzymes involved in plant and human metabolism (Chaves-Mendoza, 2015). Increasing consumption of vitamin C rich foods and spices would help to strengthen body’s immunity and prevent the development of many diseases (Slowiak and Leszczynska 2016; Desai et al., 2019). High intake of vitamin C rich food has been linked with reduced risk of several types of cancer in the laboratory tests in animals (Grosso et al., 2013). Therefore, high concentration of vitamin C found in the selected hot pepper varieties indicated that the crop can be used to improve nutritional qualities of hot pepper consumers in the society.

**Beta-carotene (Pro-vitamin A)**

The results obtained from this study, showed that beta-carotene content in the hot pepper varieties depends strongly on the variety. The varieties of hot peppers evaluated indicated that on average, a greater content of beta-carotene was found in *C. frutescens* varieties and followed by the content found in *C. annuum* and the *C. chinense* varieties had the lowest beta-carotene content. The concentration of beta-carotene was higher in orange and bright red peppers - bird eye varieties. These results conform with findings reported by Aremu and Nweze (2017) and Villa-Rivera et al. (2020) that levels of beta-carotene in hot peppers depends on the variety and environmental conditions at the growing area, experimental conditions, extraction procedures and method used. The main purpose of studying beta-carotene is due to its properties, such as provitamin A, immune improvement agent and powerful antioxidant, which help to neutralize a wide range of free radicals through electron transfer process. Beta-carotene molecules also have been extensively investigated as possible cancer preventive agents; however, the greatest benefits are obtained when these compounds are consumed along with other phytochemicals in whole foods and not from expensive dietary supplements (Chávez-Mendoza et al., 2015).

**Lycopene**

The results showed that dark-red hot pepper fruits contain higher amount of lycopene than medium red fruits and orange hot pepper fruits evaluated (Figure 3). These results are parallel with findings reported by Adebisi et al. (2014) that concentration of lycopene was high in red hot peppers. The compound is mostly present in most red fruits such as tomatoes and contributes to antioxidant activities and maintenance of human health (Martinez-Ispizua et al., 2021). The variation observed in the content of lycopene could be due to genetic differences among the hot pepper varieties, agronomic practices and influence of environmental conditions at the growing area. The knowledge of the variability of bioactive contents among hot pepper cultivated in Tanzania is necessary because it might help farmers, processors and consumers in the identification and selection of high-quality varieties to improve production and nutrition in the society. The increased interest in lycopene, has grown rapidly due to many studies which suggest that the compound has important role in the human health and disease. Lycopene is said to be non-toxic and has antioxidant, anti-inflammatory and chemotherapeutic effects, in cardiovascular or neurodegenerative disease and in some cancer. Lycopene seems to be the most efficient in neutralizing singlet oxygen free radicals compared to the other common carotenoids due to its unique chemical properties. It cannot be converted to vitamin A; therefore, it is completely available for other properties such as antioxidant activities (Chaves-Mendoza et al., 2013).

**Total phenolic content (TPC)**

The results obtained in this study showed that on average, a greater content of TPC was recorded from *C. annuum* varieties then followed by *C. chinense* and the lowest average was recorded from *C. frutescens* varieties. These results agree with the findings reported by Aryal et al. (2019) that TPC content depended on the variety; that is, the variations in the TPC in the hot pepper fruit extracts evaluated could be due to genetic difference of

the varieties. But also, climatic and soil conditions at the growing area could affect the concentration of the bioactive compounds. During preparation, the extraction solvent used and method used for analysis could also contribute to the variations in the quantity obtained from each variety. The amount of TPC in the extract is directly related to the antioxidant properties which allow the extracts to act as antioxidants (Phuyal et al., 2020). Thus, if significant amount of TPC is present, the plant extract might be suitable to be used as herbal medicine (Johari and Khong 2019).

**Total phenolic contents (TFC)**

The TFC amounts obtained from this study showed greater variation among the hot pepper varieties evaluated. On average, a greater content of TFC was recorded in *C. annuum* varieties and followed by the concentration obtained from *C. chinense* varieties and *C. frutescens* varieties had the lowest concentration. These results supported findings by Pavun et al. (2018) and Phuyal et al. (2020) who stated that the variation on the content of bioactive compounds could be due to genetic variability among the varieties; but also, other factors such as soil type, fertilization, climatic conditions, geographic origin, preparation and determination method used. The results revealed that there are fluctuations in the levels of TFC and the levels of TPC are always higher than that of TFC. Maisuthisakul et al. (2005) described these differences in concentrations between TPC and TFC in extracts that flavonoid levels are always lower because they are part or portion of the TPC. Flavonoids are important compounds synthesized by plants as secondary metabolites and have properties such as antioxidant, anticancer, anti-inflammatory, anti-allergic, antiviral, anticarcinogenic and antibacterial, pharmacological, therapeutic and cytotoxic properties among others. They are powerful antioxidants and have free radical scavenging capacities and can prevent coronary heart diseases, inflammatory diseases and various types of cancer. Recently, flavonoid compounds have been proved that they can prevent possible antiviral activities (Annunziata et al., 2020).

**Antioxidant Activity**

The results obtained from DPPH RSA test showed that antioxidant activity of hot pepper fruit extracts depended on the variety and concentration of TPC, TFC and vitamin C. On average, at species level, *C. annuum* varieties have a lowest LC₅₀ value, followed by *C. chinense* varieties and *C. frutescens* varieties have the highest LC₅₀value. Higher LC₅₀ indicate that the extract has lower the antioxidant capacity and lower LC₅₀ value showed that the extract has greater antioxidant capacity. On the other hand, hot pepper fruit extracts contained lower concentrations of both TPC, TFC and vitamin C such as ABE1 had the lowest radical scavenging capacity. Presence of higher amounts of TPC, TFC and vitamin C, directly related with higher antioxidant capacities of hot pepper fruit extracts assessed. These results supported the findings reported by several researchers such as Phuyal et al. (2020) who stated that presence of total phenolic content (TFC) in any plant extract, directly related to their antioxidant properties. Maisuthisakul et al. (2007) and Chavan et al. (2013) also stated that the presence of significant amount of TPC in fruit extracts contributed to high antioxidant activity of the plant materials sample extracts.

**CONCLUSION**

In this study, the results revealed differences in the content of bioactive compounds and antioxidant activity among the nine selected hot pepper varieties cultivated in Tanzania. Vitamin C content was determined in the fresh ripe hot pepper fruit extracts. Beta-carotene and lycopene were determined in dried ripe hot pepper fruit extracts and TPC and TFC were determined in the thick and viscous ripe hot pepper extracts. Big red cayenne presented the highest concentrations of TPC, TFC and lycopene; but also had higher amounts of vitamin C. Orange bird’s eye variety presented the highest concentration of vitamin C (ascorbic acid) and beta-carotene, but had high amount of TFC. Orange habanero presented high amount of TFC and TPC. Therefore, varieties presented the highest content in bioactive compounds also presented the greatest antioxidant activity. BRC > CCP > OBE > OH. The presence of significant amount of TPC and TFC in fruit extracts highly contributed to high antioxidant activity of the sample extracts. However, this study did not involve determination of capsaicin content. Assessment of capsaicin of hot peppers varieties would also be required because capsaicin is one of the most active components of hot peppers. Therefore, future studies on the assessment of capsaicin in hot peppers cultivated in Tanzania and antimicrobial potential, anti-inflammatory, anti-rhinitis, and analgesic properties of Big red cayenne (BRC) is necessary.
Conflict of Interest

The authors declare no conflict of interest.

Author contribution:

APM participated in the field study and laboratory analysis of hot peppers as well as in the preparation and organization of the manuscript and interpretation of relevant literature. SKM initiated the idea of the study and was involved in study design and critical revision of the manuscript for important intellectual content. All authors read and approved the final draft of the manuscript.

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