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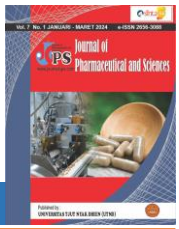

Optimization of aqueous extraction methods for Japanese taro (*Colocasia esculenta* (L) Schott)) tuber: analysis of antioxidant activity, total flavonoid content, and luteolin content

Optimasi metode ekstraksi air umbi talas Jepang (*Colocasia esculenta* (L) Schott)): analisis aktivitas antioksidan, kandungan flavonoid, dan kadar luteolin

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Abstract

Japanese taro (*Colocasia esculenta* (L) Schott)) is one of the edible plants that contain phytochemicals such as flavonoids, phenolics, triterpenoids, tannins, and vitamin C which are beneficial to the health of the human body. The compound is also known to have antioxidant activity. Proper extraction can produce quality extracts. This study aims to optimize the extraction method on Japanese taro tubers. Taro tuber was extracted using water with three different temperatures (room temperature, 40°C, and 60°C). The ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) method was used to determine the antioxidant activity of the aqueous extract, in addition to determining the total flavonoid content by colorimetric test, and also analyzing the luteolin content by Thin Layer Chromatography (TLC) Densitometry. The total flavonoid content of Taro extracts at room temperature (TERT), 40°C (TE 40), and 60°C (TE 60) were 13.97±3.52 mg QE/gr extract; 7.99±2.10 mg QE/gr extract; and 5.98±0.49 mg QE/gr extract, respectively. Luteolin content of TERT, TE40, and TE60 were 0.27%±0.08; 0.25%±0.06; and 0.31%±0.05, respectively. Antioxidant IC_{50} values of TERT, TE40, and TE60 were 120.32, 137.03, and 159.37 µg/mL, respectively. The study shows that the greater the temperature in the extraction process, the less flavonoid and antioxidant compounds content. While there is a slight difference in determining luteolin levels, optimum luteolin content is obtained at 60°C for 6 hours.

Keywords*: *ABTS, Densitometry, Luteolin, water extract

Abstrak

Talas Jepang (*Colocasia esculenta*) merupakan salah satu tanaman yang dapat dimakan dan mengandung senyawa fitokimia seperti flavonoid, fenolat, triterpenoid, tanin, dan vitamin C yang bermanfaat bagi kesehatan tubuh manusia. Senyawa tersebut juga diketahui memiliki aktivitas antioksidan. Ekstraksi yang tepat dapat menghasilkan ekstrak yang berkualitas. Penelitian ini bertujuan untuk mengoptimalkan metode ekstraksi pada umbi talas Jepang. Umbi talas diekstraksi menggunakan air dengan tiga suhu yang berbeda (suhu kamar, 40°C, dan 60°C). Metode ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) digunakan untuk menentukan aktivitas antioksidan dari ekstrak air, selain itu ditentukan juga kandungan flavonoid total dengan uji kolorimetri, dan analisa kandungan luteolin dengan Kromatografi Lapis Tipis (KLT) Densitometri. Kandungan flavonoid total ekstrak talas pada suhu kamar (TERT), 40°C (TE 40), dan 60°C (TE 60) berturut-turut adalah 13,97±3,52 mg QE/gr ekstrak; 7,99±2,10 mg QE/gr ekstrak; dan 5,98±0,49 mg QE/gr ekstrak. Kandungan luteolin dari TERT, TE40, dan TE60 masing-masing adalah 0,27% ± 0,08; 0,25% ± 0,06; dan 0,31% ± 0,05. Nilai IC_{50} antioksidan TERT, TE40, dan TE60 masing-masing adalah 120,32; 137,03; dan 159,37 µg/mL. Penelitian ini menunjukkan bahwa semakin besar suhu dalam proses ekstraksi, maka

kandungan senyawa flavonoid dan antioksidan semakin berkurang. Meskipun terdapat sedikit perbedaan dalam menentukan kadar luteolin, namun kadar luteolin optimum diperoleh pada suhu 60°C selama 6 jam.

Kata Kunci: ABTS, Densitometri, Luteolin, ekstrak air

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Introduction

Aracea is a plant family that is useful worldwide because it has economic value. Aracea is used as an ornamental and food plant. In Vietnam, some species of Araceae act as ornamental plants and others species are used as food plants for humans and animal feed, such as Alocasia, Colocasia, and Xanthosoma [1]. One of the Araceae species known to be edible food is *Colocasia esculenta.* This plant is an essential source of carbohydrates. *C. esculenta* contains 1300 kilo calories per 100 g, twice the carbohydrate content of potatoes. The protein content in *C. esculenta* is higher than in sweet potatoes, cassava, and potatoes. These *C. esculenta* Tubers contain 85-87% starch, minerals, Vitamin C, thiamine, riboflavin, and niacin. Besides tubers, *C. esculenta* leaves are also abundant in protein and minerals such as calcium, iron, phosphorus, and vitamins such as vitamin C, riboflavin, thiamine, and niacin [2]. Besides containing primary metabolites, *C. esculenta* also contains secondary metabolites that are useful for the body. *C. esculenta* tubers are also known to have several beneficial ingredients in their tubers: luteolin-6-C-hexoside-8-C-pentoxide, luteolin- 40-O-glucoside, luteolin-7-O-glucoside, luteolin-3',7-di-O-glucoside, isovitexin, orientin, schaftoside, homoorientin, vitexin, and apigenin-7-O-glucoside [3], and the root also contains flavonoid compounds, namely schaftoside, isoschaftoside, orientin, isovitexin, isoorientin, vitexin, and luteolin 7-O-sophoroside [4].

In Indonesia, *C. esculenta* is known as "talas Jepang" or Japanese taro. Taro contains primary and secondary metabolites widely used as a health plant worldwide. Research to determine the pharmacological activity of taro has been carried out in worldwide. The research was conducted by in vitro method, starch and crude extract from taro are proven to be used as antitumor/antimetastatic agents, and the research was conducted by in vitro and in vivo, taro proven as immunomodulatory, anti-osteoarthritis, antihyperglycemic, anti-hypercholesterolemic or antihyperlipidemic, antimutagenic, and antioxidant agent [5,6].

The pharmacological activity of herbs is influenced by the metabolite content contained therein. Taro contains 70-80% starch per 100 grams of dry taro. Taro starch is in the form of small granules which are very easy to digest and are used as a tablet disintegrator [7]. The content of carbohydrates, potassium, protein, and fat in taro starch makes taro starch have several pharmacological activities, such as immunomodulatory [8] and anti-melanogenic. The content of secondary metabolites from taro tuber, flavonoids, and total phenol supports the activity of taro tuber as an antioxidant agent [9]. Antioxidants play a crucial role in supporting health. Antioxidants are molecules that can inhibit the activity of oxidants that harm the body [10]. In addition, taro is also been reported to contains tarin compounds, a proteo glycan that has many activities such as anti-tumor, antimicrobial, and anti-malarial, etc [11].

The metabolite content is obtained using organic and organic solvent with distilled water by the extraction method. The extraction techniques that have been carried out to get the primary metabolite content

are water extraction ,[7,12], centrifugation process, and wet milling process with 1% sodium metabisulphite solution [7], extraction with 80% ethanol at a temperature of 90°C [13]. The content of secondary metabolites from taro tubers was obtained from the extraction method by maceration using ethanol as a solvent [14] and extraction using the Soxhlet method with methanol as a solvent [15]. An appropriate extraction technique is needed to get the maximum metabolite content. Previous researchers have carried out various types of extraction and solvents. Still, no research has been conducted on temperature variations in the extraction process to get the maximum metabolite content in taro tubers. In this research, we do optimization of the extraction using water as a solvent with three temperature variations, room temperature (RT), temperature of 40℃ (TE40), and temperature of 60℃ (TE60), with a time of 6 hours. Optimization of the extraction method is also related to four parameters, total phenolic content, total flavonoid content, luteolin content, and antioxidant tests with the ABTS method. ABTS free radical scavenging is a test method to measure the amounts of free radicals that antioxidants, known as antioxidant activity, can counteract.

Experimental Section

Materials and Apparatus

Materials

Dried powder of Japanese taro tuber (*Colocasia esculenta* (L) Schott) was obtained from CV. Herbagold, Purwakarta Indonesia. The plant was identified at the Bogoriense Herbarium, Directorate of Scientific Collections Management, BRIN by Dr. Silva Abraham M.Si with No. B-3312/II.6.2/DI.05.07/9/2022.

Chemicals and Reagents

Gallic acid, Quercetin, ABTS, Ascorbic acid, and Potassium peroxodisulfate were obtained from Sigma-Aldrich St. Louis, Missouri, US. Luteolin was obtained from MarkHerb, Bandung, Indonesia.

Extraction of Japanese taro tuber (*Colocasia esculenta* (L) Schott)

Extraction was carried out by maceration at three different temperatures. Each flask contained an amount of 100 grams of dried powder taro and was added with 500 mL of distilled water, then two flasks were heated at 40℃ and 60℃ magnetic for 6 hours. The extracts were evaporated and dried, hereafter called TERT, TE40, and TE60. The yield value and the secondary metabolites were determined using a usual phytochemical screening test.

Total Flavonoid Content

Total flavonoid content was carried out by using a colorimetric assay in microplate 96-well [16]. 50 µL of extract solution (5 mg extracts diluted in 10 mL of ethanol) and 50 µL of 2% $AlCl_3$ were added into microplate 96-well. Quercetin (50 µL) in several concentrations (10, 20, 40, 80, and 160 µg/mL) was used as the standard solution. The blank solution was prepared from 50 µL of extract and 50 µL of distilled water. The plate was incubated in the dark at room temperature for 15 min. Determine the absorbance using a microplate reader at a wavelength of 435 nm. Total flavonoid content was described as mg equivalent of quercetin QE/g extract and was calculated using the following formula (eq.1). Each assay was repeated three times.

$$\text{Total flavonoid content} = Cp \times V \times \text{f/g extract} \quad \text{(eq. 1)}$$

Cp = reference solution (mg/mL)

V = volume of test solution before dilution (mL)

F = dilution factor of the test solution

Luteolin Content with TLC-Densitometry

Luteolin as the standard solution was prepared in several concentrations (4, 8, 12, 16, 20, and 24 µg/mL). The standard solutions (10 µL) and the test solutions (TERT, TE40, and TE60) were applied on a TLC plate (Silica gel 60 GF_{254}). The mobile phase was toluene-ethyl acetate-formic acid-methanol (3:3:0.8:0.2). The TLC plate was scanned using a CAMAG TLC Scanner at 335 nm after elution. The area under the luteolin curve in several concentrations was used as curve calibration. The calibration curve determined the Luteolin content of extracts. Each test was replicated three times.

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Antioxidant Activity by ABTS Method

The antioxidant activity carried out by ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) method referred to Chatatikun and Chiabchalard with slight modification[16]. Pipetted 2 mL solutions of ABTS (40 mg/5 mL of distilled water) and 2 mL $K_2S_2O_8$ (33 mg/5 mL of distilled water) and mixed to make ABTS a working solution and incubated for 18 hours. The working solution (1 mL) was diluted with 5 mL of ethanol, called ABTS working solution. Each extract (20 µL) was added with 180 µL ABTS, a working solution as the first concentration (175 µg/mL), then diluted the concentration of 175 µg/mL to 150, 125, 100, and 75 µg/mL into microplate 96-well. Incubated for 45 min and measured the absorbance at a wavelength of 734 nm. Ascorbic acid was used as the standard solution in concentrations of 4, 6, 8, and 12 µg/mL. Distilled water (20 µL) was used as the control solution. Each test was replicated three times. The IC_{50} value was calculated and correlated with the concentration of the standard solution.

Analysis Data using Statistical analysis (ANOVA)

An analysis was performed using statistics to see a significant difference in the result. The analysis used the statistical method of two-way analysis of variance (ANOVA) using the SPSS version 25 program with a significance 95%.

Results and Discussion

In Indonesia, there are various traditional herbal products known as "jamu" that contain taro with indications for treating knee joints, anti-aging, etc. The preferred extraction method is to utilize water as a solvent rather than other solvents. However, it is very important to research the optimal temperature conditions to produce the best compound content and achieve the highest biological activity. So in this study, extraction was carried out at three different temperatures, room temperature (TERT), 40℃ (TE40), and 60℃ (TE60) with observing antioxidant parameters, TFC, and luteolin levels.

Extraction of taro tuber

The extraction process resulted in three different organoleptics (Table 1). The extract weights obtained after drying were 12.85, 12.24, and 10.79 grams, respectively, in TERT, TE40, and TE60 (Table 2). The result of the phytochemical of dried powder of taro tubers and the extracts are represented in Table 3. The organoleptic characteristics observation of the extract are the color, smell, and texture of the dry extract powder on the Japanese taro tuber. The difference in the color of the Japanese taro tuber extract in this study was due to temperature differences used for extraction treatment. Temperature is one of the factors that accelerates the extraction process, so it will affect the amounts of extracted compounds. The higher the temperature, the color will change due to heating, and the changes also occur in the texture. The TERT powder sample has a high moisture content, so the liquid content in the powder is still a lot and makes the texture smoother and not sticky, unlike TE40 and TE60 which have a rough texture, dry, and sticky. Taro content of carbohydrates is around 85% [17], causing a sticky texture on heating to higher temperatures.

Extraction is done by maceration in three different temperatures using a magnetic stirrer. Stirring and heating can speed up extraction and maximize the extracted compounds. Heating at a temperature of 48-50°C can increase the solubility [18]. Of the three temperature variations, the most significant yield was obtained from extraction at room temperature (Table 2). Extraction at room temperature can represent cold extraction, i.e., kinetic maceration because this method is accompanied by stirring. Extraction without heat (room temperature) makes the solvent constant so that the solubility of the solute is higher. Extraction by heating and without a condenser will cause the solvent to evaporate so that the solvent will decrease and reduce the solubility of the solute.

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Table 1. Organoleptic of taro Extract

Organoleptic	TERT	TE 40℃	TE 60℃
Color	Dark Brown	Light Brown	Brown
Odor	Odorless	Odorless	Odorless
Form	Smooth and dry	rough, dry, sticky	rough, dry, sticky

Note: Taro extract at room temperature (TERT); Taro extract at a temperature of 40℃ (TE40); Taro extract at a temperature of 60℃ (TE60).

Table 2. The percentage yield of extracts

Extracts	Extract yield (%)
TERT	12.85
TE40	12.24
TE60	10.79

Note: Taro extract at room temperature (TERT); Taro extract at temperature of 40℃ (TE40); Taro extract at temperature of 60℃ (TE60).

Table 3. Phytochemical screening of dried powder and extracts of taro tubers

Group	Taro tubers	
	Dried powder	Extract
Alkaloid	-	-
Flavonoid	+	+
Saponin	+	+
Tannin	+	+
Quinone	+	+
Steroid	-	-
Triterpenoid	+	+

(+) = detected, (-) = not detected

Total flavonoid content of taro aquoeus extract

The quercetin regression equation was (y) = 0.0114x - 0.0315, with a correlation coefficient (r^2) of 0.9842. TFC was measured for each extract (Figure 1). Determination of total flavonoid content was measured at a wavelength of 435 nm using a microplate reader so that samples could be tested simultaneously at one time, as well as saving testing time and being able to test many samples. The principle of determining the content of flavonoids in the colorimetric-$AlCl_3$ method is the formation of a complex between aluminum chloride and the keto group on the C-4 atom and the hydroxy group on the C-3 or C-5 atom of flavones and flavonols. The formation of a complex system between aluminum ions and flavonoids will shift the wavelength to the visible direction so that a yellow solution will be formed [19].

The total flavonoid content produced is linear with the amount of yield produced. The result obtained shows that the highest yield is also found at TERT, followed by TE40, and the lowest is TE60 (Figure 1). The higher yield of the three extracts indicates the more bioactive components contained in the extract. The total flavonoid content is expressed in mg quercetin equivalent (QE)/gram extract.

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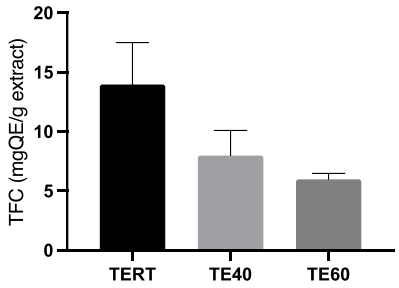
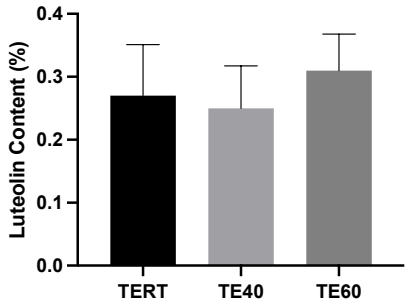


Note: Taro extract at room temperature (TERT); Taro extract at temperature of 40℃ (TE40); Taro extract at temperature of 60℃ (TE60). *Data are expressed as mean values ± SD (n = 3).

Figure 1. The Flavonoid content of TE

Luteolin content of taro aquoeus extract

Determination of luteolin content in taro extract was carried out using a calibration curve between concentrations and the luteolin standard's Area Under Curve (AUC). The Luteolin regression equation was (y) = 102.14x - 426.88, with a correlation coefficient (r^2) of 0.995. The results of determining luteolin content in TERT, TE40, and TE60 are shown in Figure 2. Apart from quercetin, luteolin levels in the extract were also determined. Luteolin is a flavonoid from the flavone class, found in many vegetables, fruits, and herbal plants. Luteolin is a natural antioxidant that has potential as an anti-cancer agent [20]. The results obtained were inversely proportional to the total flavonoid content, in which the flavonoid content measured by the standard was quercetin (mg QE/gram). Determination of luteolin content in extracts using a TLC-Densitometer method. The mobile phase used was toluene-ethyl acetate-formic acid-methanol (3:3:0.8:0.2) with an Rf value of 0.69. From the results obtained, it was found that the most extensive luteolin content was found in TE60 (**Figure 2**). Luteolin and quercetin are different groups of flavonoids based on their molecular structure, Luteolin belongs to the flavones group, and quercetin is a flavonols group. According to research, the solubility of luteolin is the lowest in water compared to other organic solvents [21]. In plants, luteolin is mainly bound to glucose [22], requiring high temperatures to dissolve luteolin. The results obtained in this study are appropriate if there is a lot of luteolin in TE60.



Note: Taro extract at room temperature (TERT); Taro extract at temperature of 40℃ (TE40); Taro extract at temperature of 60℃ (TE60). *Data are expressed as mean values ± SD (n = 3).

Figure 2. The Luteolin content of taro extracts

Antioxidant activity of taro aquoeus extract

Al The Ascorbic acid regression equation was (y) = 13.075x – 10.648, with a correlation coefficient (r^2) of 0.9918. The TERT, TE 40, and TE 60 regression equations were found to be (y) = 0.2738x + 17.054, with a correlation coefficient (r^2) of 0.9784; (y) = 0.2979x + 9.179, with a correlation coefficient (r^2) of 0.9711; and (y) = 0.3322x – 2.943, with a correlation coefficient (r^2) of 0.9901, respectively. The IC_{50} value is presented in **Figure 3**. The IC_{50} value of Ascorbic acid was obtained a 4.63 µg/mL with extreme antioxidant activity. Antioxidant activity of TERT, TE40, and TE60 are 120.32, 137.03, and 159.37 µg/mL, respectively, with very weak activity categories.

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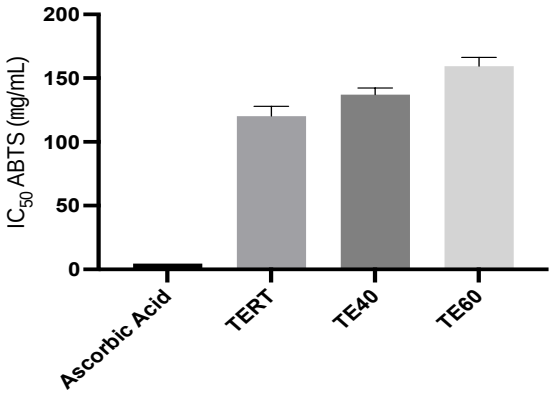

Note: Taro extract at room temperature (TERT); Taro extract at temperature of 40℃ (TE40); Taro extract at temperature of 60℃ (TE60). * Data are expressed as mean values ± SD (n = 3).

Figure 3. The percentage inhibition and IC_{50} of extracts and Ascorbic acid

The antioxidant activity of Japanese taro tuber dry extract with three temperature variations was determined using the ABTS method. The principle of testing antioxidant activity using the ABTS method is removing the color of the ABTS cation to measure the antioxidant capacity, which directly reacts with the ABTS cation radicals. This process is characterized by a change in the color of the ABTS solution from blue to colorless, the absorbance of which can be measured with a Microplate Reader. Determination of antioxidant activity can be determined from the IC_{50} value. The smaller the IC_{50} value, the higher the antioxidant activity. Specifically, a compound is said to be a very strong antioxidant if the IC_{50} value is less than 50 µg/mL, strong antioxidant (50 < IC_{50} < 100 µg/mL), moderate (100 < IC_{50} < 150 µg/mL), weak (150 µg/mL < IC_{50}200 µg/mL), very weak IC_{50} > 200 µg/mL), and no antioxidant activity if the IC_{50} value is > 500 µg/mL [23]. The ABTS method has advantages over other methods, simple testing, ease of calculation, flexibility, more sensitivity, and can be used for test measurements over a reasonably extensive pH range. Ascorbic acid is used as a comparison or positive control compound in antioxidant activity tests using ABTS free radicals. The IC_{50} measurement of Japanese taro tuber dry extract using various concentrations of 75, 100, 125, 150, and 175 µg/mL and ascorbic acid as a standard solution. The samples were incubated for 45 minutes and measured at a wavelength of 734 nm. The results of the antioxidant activity of ascorbic acid is the best antioxidant activity with an IC_{50} value of 4.63. The extract with better antioxidant activity than the other two extracts is TERT, with an IC_{50} value of 120.32 ± 7.66 (**Figure 3**). This result was consistent with the extract with the best yield and highest levels of flavonoids. Quercetin is a flavonoid included in the flavonol group because it has an OH substitution at the C-3 position, one of the characteristics of compounds with antioxidant activity [24]. The ortho-hydroxy substitution of the B ring of flavonoids also affects the formation of chelates with Cu^{2+} ions, affecting antioxidant activity. Luteolin, a flavonoid that does not have a free hydroxy in the C-3 position, has poor antioxidant activity because it is not more easily oxidized in the presence of Cu^{2+} ions than flavonoids with free hydroxy at C-3, such as quercetin [25].

The research of extraction with boiled distilled water by Yadav and others shows the IC_{50} value with the ABTS method of taro tuber was 132.29 ± 14.13 µg/ml [26]. Compared to the result of this study, The IC_{50} values of three different temperatures are 120.32 ± 7.66, 137.03 5.17, and 159.37 ± 6.84, respectively, for TERT, TE 40, and TE60. The best antioxidant activity was shown by extraction at room temperature. The statistical test results using SPSS version 25, the total flavonoid content, total luteolin content, and antioxidant activity of ABTS, which were affected by three different temperatures, were normally distributed with P (0.017), P>0.05. The test results showed significant differences in the three extraction temperature parameters on total flavonoid, luteolin, and antioxidant activity.

Conclusions

Extraction yield are affected by temperature. Extraction at room temperature produces the highest extract rendement. The higher total flavonoid content and antioxidant activity were obtained from extraction at room temperature compared to extraction at 40 and 60℃. The luteolin content obtained from Japanese taro tuber

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extract showed different results from the other three parameters, where the extract at 60°C had the highest luteolin content. This is influenced by the structure and solubility of luteolin. Luteolin is known to be more soluble in hot water, so giving a higher temperature will increase the luteolin content in the extract.

Conflict of Interest

The authors declare no conflict of interest.

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