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Phytochemical, GC-MS Analysis, and Antibacterial Activity of Ethanol Extract Coir and Shell U Groh (*Cocos nucifera* L.)

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ABSTRACT

U Groh is a coconut (*Cocos nucifera* L.) in which the meat has not been formed yet, and its shell is consumed as a salad by the Acehnese people. This study aims to determine the phytochemicals, GC-MS analysis, and activity of coir and shell U Groh against *S. aureus* and *E. coli* bacteria. Chemical compounds were analyzed using reagents and GC-MS. Antibacterial activity was tested using well diffusion with concentrations of 5, 10, 20, and 40%. Coir and shells contain flavonoids, tannins, and saponins. GC-MS analysis shows the coir contains 20 compounds, among others Alpha. - Bisabololoxide B-Acetate, Hexadecanoic acid, Hexadecanoic acid methyl ester, 9 octadecenoic acid methyl ester, Stigmasterol, and gamma. -Sitosterol, whereas 16 compounds for the shell Hexadecanoic acid, Hexadecanoic acid methyl ester, and Stigmast-5-en-3-ol. The highest activity of coir and shell U Groh against *E. coli* and *S. aureus* was a concentration of 40%. The antibacterial activity of U Groh coir and shell extract was more effective in inhibiting *S. aureus* than *E. coli*. Coir ethanol extract and its shell formed inhibition zones with diameters of 13.7 mm and 13.4 mm, respectively, against *S. aureus*, and the diameters against *E. coli* were 11.3 mm and 11.03 mm, respectively. The extract ethanol of coir and shell U Groh has good potential as an antibacterial.
Keywords: Antibacterial activity; U Groh (*Cocos nucifera*); coir; shell; phytochemical; GC-MS analysis.

INTRODUCTION

Cocos nucifera is a plant that has value as a material in the industry, foodstuffs, and medicinal ingredient, which has been empirically used for generations by the community to treat various diseases. U Groh is the term for coconut that has not yet formed a dense endosperm and contains a high-water content.

Generally, coconut is processed into coconut milk to be used as an additional ingredient during the cooking of many dishes. In the Aceh area, besides being processed into coconut milk, coconut meat can be made into Pliek U, which results from fermented coconut meat. Additionally, the U Groh shell can be made into a salad.

Nowadays, the use of plants as medicine has been increasing. Coconut can be used as medicine in applications such as anti-tumor, diuretic, and bactericidal (Singla et al., 2011). All parts of the plant can be used as medicine and have many benefits. Coconut coir, part of the mesocarp layer, contains secondary metabolites that have the potential as antimicrobials. Coconut coir has been reported to contain tannins, phenols, and flavonoids (Fitriah et al., 2019; Wulandari et al., 2018). Apart from that, it also contains alkaloids, glycosides, phenols, saponins, and steroids (Abdu et al., 2019). The results of previous research show that coconut fiber has antibacterial activity against *Escherichia coli* (Verma et al., 2012). The research shows that coconut shells have activity to inhibit Gram-positive and Gram-negative (Prakash et al., 2018). *Escherichia coli* and *Staphylococcus aureus* are bacteria that cause diarrhea (Dewi, 2013). The prevalence rate of diarrhea is still high, especially in developing countries such as Indonesia (Kementerian Kesehatan Republik Indonesia, 2012).

Research to find new medicinal ingredients sources needs to be carried out. Based on the research, U Groh coir and shell have the potential to be developed as new medicinal ingredients treatments for treating diarrhea. This study tested the chemical compound and activity of U Groh coir, and shell ethanol extracts as an antibacterial to inhibit *Escherichia coli* and *Staphylococcus aureus* bacteria.

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METHODOLOGY

Materials

The materials that were used are U Groh obtained from Indrapuri, Aceh Besar, has been determined at Indonesian Institute of Science (No,2654/IPH.1.01/If.07/XII/2018), *E. coli* ATCC 25922 bacteria, *S.aureus* ATCC 25923, Mueller Hinton Agar (Merck, Germany), ethanol 95%, alcohol 70%, sodium chloride 0,9%, gentamicin antibiotic 40 mg/ml, and amoxicillin 125mg/ml.

The equipment that was used in this research is extraction containers, analytical balance (Matrix AJ1002B), autoclave (Hirayama Hiclave HVE-50), Laminar Air Flow Cabinet (Microbiology safety cabinet), rotary evaporator (BUCHI Labortechnik AG 9230 Type R-300 EL), and an incubator (Memmert).

Methods

Preparation of Simplicia

Fresh U Groh coir and shell were washed using distilled water to remove any contaminants. The coir and shell cut into small pieces were placed in a clean area and left to air dry for 36 days. They were then milled using a blender and weighed.

Preparation of Extract

The extraction of the U Groh coir and shell simplicia was done by maceration using ethanol. The ratio of simplicia to ethanol was 1:10. 400 g of the simplicia was macerated using ethanol; 7.5 parts out of 10 parts of the ethanol were used, which is 3 L. The container was closed using aluminum foil and stored in a place protected from the sun. Frequent stirring was done. Maceration was done again for 2 days using 1.25 parts of the ethanol solvent, which is 1 L. The solution was concentrated until a thick extract was formed using a rotary evaporator (Anief, 2010).

Phytochemical Screening of Extract

Qualitative phytochemical screening includes testing for alkaloids, tannins, flavonoids, saponins, steroids, and triterpenoids (Harborne J. B., 1987).

Identification Chemical Compound

The chemical compound coir and shell of U Groh were analyzed using GC- MS. Instrumen Agilent Technologies 7890 Gas Chromatograph with Auto Sampler and 5975 Mass Selective Detector and Chemstation data system. HP Ultra 2 column, capillary column (30 m X 0.20 mm X 0.11 m), and helium as carrier gas.

Characterization Simplicia and Extract

Water level

A total of 2 g of simplicia and U Groh extract were placed in a porcelain dish that had been heated at 105°C for 30 minutes. The porcelain dish containing the simplicia and extract was dried at 105°C for 3 hours. The mixture was weighed and returned to the oven until the weight was constant(Depkes RI, 1995).

Water solubility

A total of 5 g simplicia and extract U Groh were macerated in 100 mL of chloroform for 24 hours and stirred for the first 6 hours. After filtering, 20 ml of the filtrate was placed in a porcelain dish, and the filtrate was evaporated in a 105°C oven. Water solubility was expressed as a percentage. (Depkes RI, 1995).

Ethanol solubility

A total of 5 g simplicia and extract U Groh were macerated in 100 mL of ethanol for 24 hours and stirred for the first 6 hours. Moreover, after filtering, 20 mL of filtrate was placed in a porcelain dish and evaporated using a 105 °C oven. The percentage of ethanol solubility was calculated. (Depkes RI, 1995).

Total ash level

A total of 1 g simplicia and extract U Groh were placed in the porcelain dish and heated at 600°C until white. It was then allowed to cool in a desiccator before being weighed. (WHO, 2011).

Antibacterial Activity

The antibacterial activity of U Groh coir and shell ethanol extracts was tested using the well diffusion method. The inoculation of the tested bacteria on the MHA medium was carried out using the pouring method. The absorbance of the bacteria suspension was measured using a spectrophotometer with a wavelength of 600 nm (OD 600) = 0.5 (0.5 x10^8 CFU). 1 mL of the *E. coli* and *S. aureus* bacteria suspension were placed inside different Erlenmeyer flasks containing 30 mL of MHA, which were already sterilized and cooled until a temperature of 40-45 ºC. The Erlenmeyer flasks were shaken until homogenized. A 4 mm diameter hole was made, and 20 µL of the extract with varying concentrations of 5, 10, 20, and 40% was dripped. The medium was then incubated at a temperature of 37ºC for 24 hours. The clear zone that was formed was then measured using calipers.

RESULT AND DISCUSSION

Simplicia of Coir and Shell

The U Groh coir simplicia obtained was 1734 g with a percentage yield of the simplicia as much as 11.15%. The Simplicia of the shell was 636 g with a percentage yield of 10.84%.

Extraction of Ethanol Coir and Shell

The extraction results by maceration, which used ethanol as a solvent, formed 88.51 g of

Figure 1. The coir simplicia U Groh (a) fresh coir; (b) simplicial; (c) simplicia powder

Figure 2. The shell simplicia U Groh (a) fresh shell; (b) simplicial; (c) simplicia powder

Table I. The phytochemical compound of U Groh coir and shell ethanol extracts

Metabolite Compound	Results	
	Coir	Shell
Alkaloid	Negative	Negative
Flavonoids	Positive	Positive
Tannins	Positive	Positive
Saponins	Positive	Positive
Steroids	Negative	Negative
Triterpenoids	Negative	Negative

U Groh coir extract with a percentage yield of 22.12% and 82.9 g of shell extract with a percentage yield of 20.72%.

Phytochemical Screening of Extract

The results of qualitative phytochemical screening showed that the U Groh coir and shell ethanol extracts contained the secondary metabolites flavonoid, tannin, and saponin. (Table I).

The results of the phytochemical screening of U Groh coir and shell extract in this research contained the compounds flavonoids, tannins, and saponins. Young coconut shell extract was reported to contain the compounds tannins, saponins, and steroids (Mazaya et al., 2020). Conversely, (Wulandari et al., 2019), reported that coir ethanol extract from a young coconut contained the compounds tannins, phenols, and flavonoids. The tannins contained inside the young coconut coir extract are classified as condensed tannins with a percentage of 5.62% (Lisan, 2015). The tannins, flavonoids, and saponins contained within the U Groh coir and shell extracts have antimicrobial properties. The difference in secondary metabolites in a particular plant may be due to factors such as the environment, temperature, and humidity (Ramakrishna & Ravishankar, 2011).

GC-MS Analysis

The result of coir and shell U Groh compounds identified using GC-MS is represented in Table II and Table III.

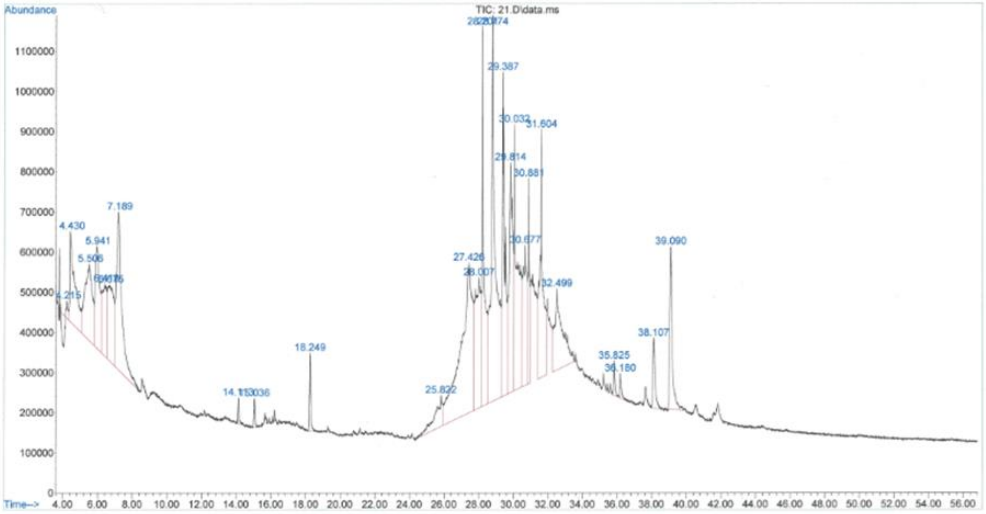


Figure 3. Chromatogram GC-MS results of coir ethanol extract

Table II. Identified result compounds coir ethanol extract using GC-MS

No.	RT	Quality	Senyawa	Area (%)
1	4.428	43	2-BUTANAMNINE, HYDROCHLORIDE	3,25
2	5.504	45	Erythritol	4,08
3	5.938	50	Erythritol	3,44
4	6.421	53	1,2-PROPANEDIOL,3-CHLORO-	2,18
5	6.676	64	GLYCEROL	5,85
6	7.186	81	4H-Pyran-4-one,2,3-dyhydro-3,5-dihydroxy-6-methyl-	6,20
7	25.824	38	2-Amino-5H-pyrrolo[3,4-d} pyrimidine-4,7(3H,7H0-dione	1,59
8	27.424	25	. ALPHA. -BISABOLOLOXIDE B-ACETATE	12,90
9	28.010	50	4-(Dimethylhyrazono-5,5-dimenthyl-hexan-2-ol	5,21
10	28.203	99	HEXADECANOIC ACID, METHYL; ESTER	5,52
11	28.775	99	HEXADECANOIC ACID	11,91
12	29.398	96	Cis-13-Octadecenoic acid,methyl ester	5,56
13	29.817	97	Z, Z-11,13-Hexadecadiene-1-ol acetat	6,09
14	30.030	55	E-3-Petadecen-2-ol	6,61
15	30.678	91	11-Dodecyn-1-ol acetate	4,06
16	30.878	93	HEXANEDIOIC ACID, BIS(2-ETHYLHEXYL) ESTER	2,04
17	31.602	87	1,2-Benzenecarboxylic acid,diisooctyl ester	5,42
18	32.499	95	Cis-13-Octadecenoic acid, methyl ester	4,64
19	38.105	99	Stigmasterol	1,12
20	39.091	99	. *gamma. -Sitosterol*	3,07

Characterization of U Groh Coir and Shell Simplicia

The Characterization of the simplicia and extracts was completed to check the quality of the obtained simplicia and extract (Table IV).

The test results of water content in the U Groh coir and shell extracts are within the water level requirements of the simplicia, which is ≤ 10% (Balai Pengawasan Obat dan Makanan, 2014). The high-water content of the simplicia can become a medium for the growth of microorganisms. On the coir simplicia, water and ethanol soluble levels resulted in 20% and 29.36% respectively, showing that more of the ethanol had better solubility than water. On the shell simplicia, water and ethanol soluble levels resulted in 29.36% and 18.24% respectively. This result shows that the majority of secondary metabolites contained in the U Groh coir simplicia were less polar. Conversely, more of the shell simplicia dissolved in water than ethanol, showing that more of the secondary metabolites within the simplicia were polar. The total ash level

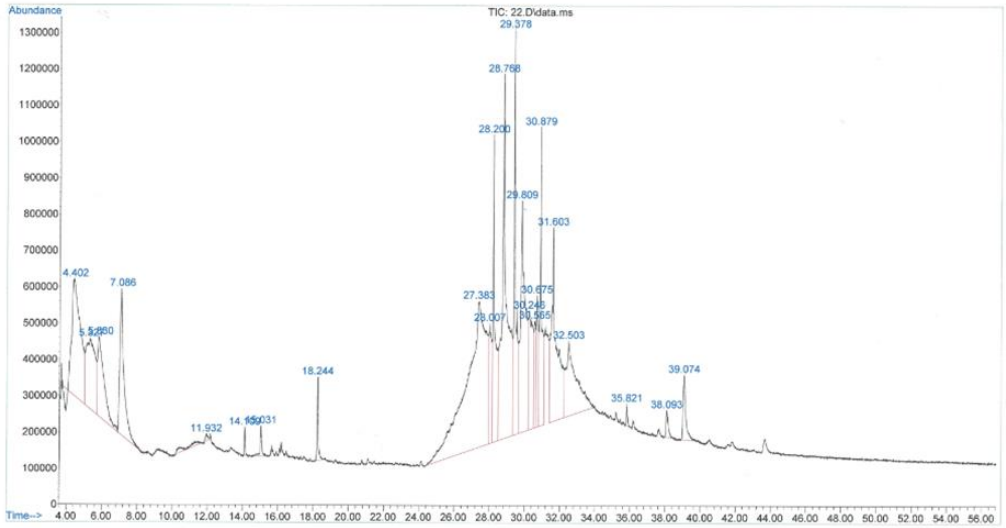


Figure 4. Chromatogram GC-MS results of shell ethanol extract

Table III. Identified result compounds shell ethanol extract using GC-MS

No.	RT	Quality	Senyawa	Area (%)
1	4.400	49	1,4-Pentadiene	7,13
2	5.324	38	Erythritol	4,64
3	5.828	42	(METHYSULFANYL)(METHYLSULFANYL) METHANE	3,72
4	7.090	97	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	4,40
5	27.382	43	. alpha. -Cyano-4-hydroxycinnamic acid	20,85
6	28.010	83	2-ALLYL-1,4-DIMETHOXY-6-METHYLBENZENE	2,45
7	28.196	99	HEXADECANOIC ACID, METHYL; ESTER	4,40
8	28.768	99	HEXADECANOIC ACID	11,84
9	29.375	99	9,12-octadecenoic acid, methyl ester	5,32
10	39.810	99	(9E,2E)-9,12 OCTADECENOIC ACID	8,22
11	30.244	90	Isoaromadendrene apoxide	3,58
12	30.568	95	Cyclopentadecanone,2-hydroxy-	1,44
13	30.878	95	HEXANEDIOIC ACID, BIS(2-ETHYLHEXYL) ESTER	3,75
14	31.602	80	1,2-BENZENEDICARBOXYLIC ACID	7,25
15	32.506	87	9,17-Octadecadienal	5,59
16	39.077	99	STIGMAST-5-EN-3-OL	1,32

Table IV. Characterization of coir and shell simplicial

Test	Percentage (%)	
	Coir	Shell
Water level	9.4	8.5
Water - Soluble level	20	29.36
Ethanol-Soluble Level	25.7	18.24
Total Ash Level	5.3	8

on the coir and shell simplicia were 5.3% and 8% respectively. The total ash level results could be influenced by internal factors such as minerals and external factors such as sand and soil from the environment (Departemen Kesehatan Republik Indonesia, 2009).

Characterization of U Groh coir and shell extracts

The results of the water level for coir extract were 14.13%, whereas, for shell extract, it was 13.56%. The results are within the extract level requirement, which is <30% (Voight,1994).

Table V. Characterization of coir and shell extracts

Test	Percentage (%)	
	Coir	Shell
Water level	14.13	13.56
Water - Soluble level	69.64	74.62
Ethanol-Soluble Level	70.47	76.28
Total Ash Level	5.56	6.5

Table VI. Antibacterial activity of coir and shell extracts

Concentration (%)	Average inhibition zone diameter (mm)			
	Coir extract		Shell extract	
	E. coli ATCC 25922	*S. aureus* ATCC 25923	*E. coli* ATCC 25922	*S. aureus* ATCC 25923
5	5,7 ± 0,38	6,2 ± 0,68	5,6 ± 0,58	6,06 ± 0,05
10	6,3 ± 0,40	6,7 ± 0,15	7,2 ± 0,55	6,4 ± 1,02
20	9,1 ± 0,15	9,1 ± 0,8	8,7±1, 02	8,6 ± 0,15
40	11,3± 0,51	13,7 ± 1,41	11,03 ± 0,11	13,4 ± 0,50
Positive Control [a]	25,4 ± 0,45	22,7 ± 0,85	26,7 ± 0,62	23,0 ± 0,85
Negative Control [b]	0 ± 0	0 ± 0	0 ± 0	0 ± 0

[a] E. coli: gentamicin 4%, S. aureus: amoxicillin 1%; [b] Ethanol solvent

The determination of the water level of an extract was done to limit the water level in an extract as high-water content can be a medium for microorganisms to grow. Besides the determination of water level, the results can also guarantee the quality of the extract so that the extract can be stored for a longer period.

The determination of water-soluble levels for U Groh coir and shell showed a result of 69.64% and 74.62%, respectively, whereas the ethanol-soluble level for coir and shell extracts was 70.47% and 76.28%, respectively. This result shows that the U Groh coir and shell extracts dissolved more in ethanol than in water.

The determination of total ash level results of U Groh coir and shell that were obtained were 5.56% and 6.5%, respectively. The results show that the shell extracts contain more minerals than the coir extract. The total ash level can be affected by several internal factors such as minerals and external factors such as sand and soil from the environment.

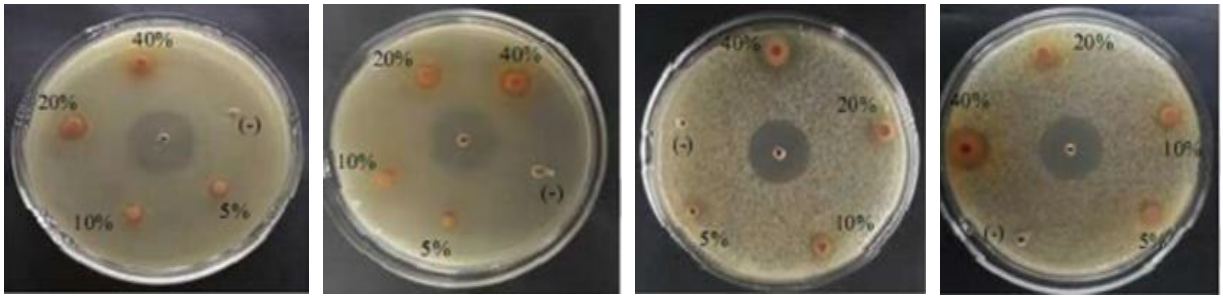
Antibacterial Activity

The inhibition zone test results for U Groh coir and shell ethanol extract are shown in Table VI.

Inhibition zone results of U Groh coir and shell were obtained from the smallest concentration and the bigger zone at a concentration of 40 % for both bacteria. The higher the concentration, the bigger the formed inhibition zone (Lingga et al., 2016). In the inhibition zone that was formed, the diameter was 11.3 mm against *E. coli* and 13.7 mm against *S. aureus,* whereas, for the shell extract, the diameter results were 11.03 mm and 13.4 mm. The inhibition zone results of coir and shell extracts can be seen in Figure 1.

The inhibition zone of U Groh coir and shell ethanol extracts that were obtained was bigger against *S. aureus* in comparison to *E. coli*. The difference in a structure where Gram-positive bacteria is simpler than that of Gram-negative bacteria. Gram-positive bacteria contain a peptidoglycan layer that is thick and teichoic acid. The cell has polar properties to the penetration of antibacterial compounds that are polar to the cell. Conversely, the Gram-negative is complex where it is structured by phospholipids and lipopolysaccharides, so it is harder for antibacterial compounds to penetrate (Pratiwi, 2017). The antibacterial activity of an extract is affected by compounds that are contained within the secondary metabolites

Percentage comparison of the inhibition zone diameters of U Groh coir and shell ethanol extracts against *E. coli* and *S. aureus* at a concentration of 40% against the inhibition zone obtained using the positive control of gentamicin and amoxicillin. The percentage comparison of coir and shell that was obtained was 44.48% and 41.31%, respectively, from the positive control of gentamicin and 60.35% and 58% from the positive control of amoxicillin.



(a) (b) (c) (d)

Figure 5. Activity test results of (a) U Groh coir ethanol extract against *E. coli* (b) U Groh shell ethanol extract against *E. coli* (c) U Groh coir ethanol extract against *S. aureus* (d) U Groh shell ethanol extract against *S. aureus*.

The mechanism of tannins is to slow down the synthesis of peptidoglycan at the formation of the cell wall (Lima et al., 2015). Flavonoids are to disturb the integrity of the bacteria's cell membrane (Egra et al., 2019). In contrast, saponins reduce the surface tension that results in cell leakage (Rahman et al., 2017). Furthermore, the Bioactive compound Hexadecanoic acid, Hexadecanoic acid methyl ester, 9-octadecenoic acid methyl ester, contained in an extract, also contributes as an antibacterial. Antibacterial activity damages bacteria cell walls and membranes (Karunia et al., 2017; Asghar et al., 2011). The steroid compound is stigmasterol, gamma. -Sitosterol, and Stigmast-5-en-3-ol. The mechanism of action of steroids is to inhibit bacterial cell surface protein (Yusuf et al., 2018). Alpha. -Bisabololoxide B-Acetate is terpenoid (Zhandabayeva et al., 2021). Terpenoids work by reacting with porin, reducing the permeability of bacterial cell walls (Wulansari et al., 2020). The metabolite compounds that were obtained have different mechanisms as antibacterial.

CONCLUSION

The conclusion is that U Groh coir and shell contain flavonoids, tannins, and saponins. GC-MS analysis shows the coir contains Alpha. -Bisabololoxide B-Acetate, Hexadecanoic acid, Hexadecanoic acid methyl ester, 9 octadecenoic acid methyl ester, Stigmasterol, and gamma. -Sitosterol, whereas the shell contains Hexadecanoic acid, Hexadecanoic acid methyl ester, and Stigmast-5-en-3-ol. The highest coir and shell U Groh activity against *E. coli* and *S. aureus* were at a concentration of 40%.

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