ANTIBACTERIAL ACTIVITY OF *BELIMBING WULUH* (*Averrhoa Bilimbi Linn*) EXTRACT ON *Salmonella Typhi* GROWTH

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ABSTRAK

*Demam tifoid adalah penyakit endemik yang masih memiliki tingkat kejadian tinggi terutama di negara berkembang seperti Indonesia. Demam tifoid disebabkan oleh Salmonella typhi. Belimbing (Averrhoa bilimbi Linn) ekstrak daun memiliki kemampuan antibakteri terhadap beberapa strain bakteri. Oleh karena itu, para peneliti mengkaji penggunaan ekstrak daun belimbing terhadap bakteri Salmonella thypi. Ini adalah penelitian eksperimental benar bahwa menguji aktivitas antibakteri belimbing yang (Averrhoa bilimbi Linn) ekstrak daun terhadap pertumbuhan Salmonella typhi. Penelitian ini menggunakan metode difusi pada Muller Hinton Agar Palte. Daun belimbing diekstraksi dengan etanol dan dibagi dalam beberapa konsentrasi. Uji statistik dilakukan dengan uji deskriptif dan uji Kruskal Wallis untuk membandingkan efek antibakteri ekstrak daun belimbing dalam konsentrasi yang berbeda di Salmonella thypi. Berarti diameter penghambatan pada Salmonella typhi uji aktivitas antibakteri adalah sebagai berikut: Pengobatan 1 (0,3 g/ml) = 20.75 mm; Pengobatan 2 (0,4 g/ml) = 21.88 mm; Pengobatan 3 (0, 5 g/ml) = 22.88 mm; Control 1 (kloramfenikol 30 mcg) = 30 mm; Kontrol 2 (5% DMSO) = 0 mm. Berdasarkan hasil yang diperoleh, secara kualitatif dapat disimpulkan bahwa konsentrasi belimbing (Averrhoa bilimbi Linn) ekstrak daun memberikan zona hambatan pertumbuhan Salmonella typhi pada konsentrasi 0,3 g/ml, 0,4 g/ml dan 0,5 g/ml. Berdasarkan standar Clinical Laboratory Standard Institute (2008), semua dari mereka memberikan respon sensitif untuk menghambat pertumbuhan Salmonella typhi serta memberikan kloramfenikol antibiotik sebagai kontrol.**(FMI 2012;48:144-149)***

Kata kunci: *Salmonella typhi, Averrhoa bilimbi Linn leaf, antibakteri, difusi*

ABSTRACT

*Typhoid fever is an endemic disease which still have high incidence rate especially in developing countries like Indonesia. Typhoid fever is caused by Salmonella typhi. Starfruit (Averrhoa bilimbi Linn) leaf extract has antibacterial capability against some strains of bacteria. Therefore, researchers examined the use of starfruit leaf extract against Salmonella thypi bacteria. This was a true experimental research that test the antibacterial activity of the starfruit (Averrhoa bilimbi Linn) leaf extract against Salmonella typhi growth. This research using diffusion method on Muller Hinton Agar Palte. Starfruit leaf extracted with ethanol and divided in several concentrations. Statistical tests is conducted with descriptive test and Kruskal Wallis test to compare antibacterial effect of starfruit leaf extract in different concentrations in Salmonella thypi. Mean diameter of the inhibition on Salmonella typhi antibacterial activity test were as follows : Treatment 1 (0.3 g/ml) = 20.75 mm; Treatment 2 (0.4 g/ml) = 21.88 mm; Treatment 3 (0, 5 g/ml) = 22.88 mm; Control 1 (chloramphenicol 30 mcg) = 30 mm; Control 2 (5% DMSO) = 0 mm. Based on the obtained results, qualitatively it can be concluded that the concentration of starfruit (Averrhoa bilimbi Linn) leaf extract gave growth inhibition zone in Salmonella typhi at concentrations of 0.3 g/ml, 0.4 g/ml and 0.5 g/ml. Based on the standard Clinical Laboratory Standard Institute (2008), all of them provide a sensitive response to inhibit the growth of Salmonella typhi as well as giving antibiotic chloramphenicol as control. **(FMI 2012;48:144-149)***

Keywords: *Salmonella typhi, Averrhoa bilimbi Linn leaf, antibacterial, diffusion*

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INTRODUCTION

Typhoid fever is a systemic disease caused by the bacteria *Salmonella typhi*. Until now, typhoid fever remains a public health problem, and is closely related to poor sanitation, especially in developing countries. In developing countries the estimation of typhoid fever incidence varies from 10 to 540 per 100,000 population. Although the incidence of typhoid fever decreases with the improvement of sanitary disposal in many developing countries, it is estimated that each year there are 35 million cases with 500,000 deaths worldwide. In Indonesia, typhoid fever is an endemic disease with a high incidence, an estimated 350-810 cases per 100,000 population per year; or approximately 600.000- 1.5 million cases annually. Among the diseases classified as

infectious intestinal disease, typhoid fever ranks second after gastroenteritis (Ahmar 2010).

Typhoid fever is one type of bacterial infectious diseases that are found in our country, both in adults and children. In the last five years, clinicians in several countries observe any cases of typhoid fever are severe or even fatal child, which was caused by *Salmonella typhi* strains that were resistant to antibiotics commonly used for the treatment of typhoid fever. *Salmonella typhi* strains that were resistant to two or more types of antibiotics that are commonly used, namely ampicillin, chloramphenicol, and cotrimoxazole, is called strains of multi-drug resistance (MDR) *Salmonella typhi* (Hadinegoro 1999).

The cause of MDR in typhoid fever is suspected because; (1) Excessive use of antibiotics (over-use), (2) use of antibiotics is wrong (misuse), and (3) the lack of appropriate antibiotics (in-NAMAs), in addition to (4) The presence of intrinsic microbiological factor, the plasmid mediated. TB in children with typhoid fever have occurred in China (1987), Pakistan (1988), India (1990), Bahrain (1990), Malaysia (1991), Vietnam, and Egypt (1993). Girgis of Egypt reported in 1990-1993, there has been a MDR in more than 75% of cases of typhoid fever in children. In Pakistan since 1988 have also reported the presence of MDR against *Salmonella typhi.* The incidence is increasing from year to year. In 1995, the in-vitro in cultured MDR *Salmonella typhi* found in 50% of which was later increased to 78% in 1997 (Hadinegoro 1999). With the discovery of MDR *Salmonella typhi*, the selection of appropriate antibiotics will be a problem, including the cost constraints. Therefore, we need a lot of rethinking alternative medicine or complementary treatments that are effective, efficient, safe and reasonably priced. Alternative therapies that researchers propose are to use natural ingredient, the leaves of starfruit (*Averrhoa bilimbi Linn*).

Starfruit (*Averrhoa bilimbi Linn*) is one kind of plant that is often used as a traditional medicine. This plant is widely used to overcome various diseases such as cough, diabetes, rheumatism, mumps, canker sores, toothache, bleeding gums, acne, diarrhea to high blood pressure. Part of the plant is often used as a medicine is the fruit and leaves (Wijayakusuma 2006). The content of the active compounds in the starfruit leaves are tannins, sulfur, formic acid, and flavonoids. Secondary metabolites in plants are flavonoids, tannins and saponins. Based on some research results starfruit has the ability to inhibit the growth of bacteria. Starfruit leaves contain secondary metabolites, the flavonoids and tannins, so that it can be presumed that the active compounds can be used as an antibacterial (Wijayakusuma 2006).

According to research conducted by Masripah (2009), the results showed that ethanol extract of starfruit leaves of (*Averrhoa bilimbi Linn*) have inhibitory effect to bacteria *E. coli* and *S. aureus*. MIC values obtained for *E. coli* bacteria was 400 mg/ml (400,000 ppm) and for *S. aureus* was 900 mg/ml (900,000 ppm). Potential starfruit leaf ethanol extract obtained was 400 mg/ml (400,000 ppm) of ethanol extract of leaves of starfruit equivalent to 0.04 mg/ml (40 ppm) against *E. coli* amoxicillin and 900 mg/ml (900,000 ppm) of ethanol extract starfruit leaves equivalent to 0.04 mg/ml (40 ppm) of amoxicillin against *S. aureus*. The ability of starfruit (*Averrhoa bilimbi Linn*) leaf extract to have antibacterial activity needs to be researched further regarding their effectiveness in inhibiting *Salmonella typhi*. The use of starfruit (*Averrhoa bilimbi Linn*) leaf as antibacterial is highly efficient due to its abundance. The plant is also very easy to obtain. The purpose of this study was to prove that the leaf extract of starfruit (*Averrhoa bilimbi Linn*) has antibacterial properties against *Salmonella typhi*.

MATERIALS AND METHODS

This study was an experimental study, the research to determine the antibacterial activity of leaf extract starfruit (*Averrhoa bilimbi Linn*) against *Salmonella typhi* bacteria. The study was conducted with diffusion test on *Salmonella typhi* on Mueller Hinton agar medium plate will then be given a leaf extract starfruit (*Averrhoa bilimbi Linn*) in concentrations specified in the pre-study. Evaluation is done by calculating the diameter of the inhibition of each treatment after 24 hours of treatment administration in the garden of *Salmonella typhi* in Mueller Hinton agar plates.

This study will be conducted in the laboratory of Microbiology, Faculty of Medicine, University of Airlangga and will be implemented within a period of approximately 6 months. The population of this study is the bacterium *Salmonella typhi*. The sample used in this study was 0.5 McFarland *Salmonella typhi* from the laboratory of Microbiology, Faculty of Medicine, Airlangga in Surabaya. Samples were cultured or grown in Mueller Hinton agar plate. The treatment will be given in the sample (*Salmonella typhi*) of five, namely the provision of three types of leaf extract concentration starfruit, antibiotics chloramphenicol and 5% DMSO. So that the corresponding formula Federer, repetitions performed on samples of the first kind totaling at least 5 times on each treatment. Samples were taken by random sampling. Data collection was performed 24 hours after

administration of treatment by measuring the diameter of inhibition on nutrient agar plate cultures of bacteria.

Leaves starfruit old enough taken from branches and beyond the fresh green. Starfruit leaves are used dried, pulverized and sieved to then extracted by maceration using ethanol as the solvent. Ethanol is used as a solvent because ethanol is polar, universal, easy to obtain, and not toxic (Sampurno 2000). From the extraction, the filtrate obtained is then separated from the solvent by using a vacuum evaporator and evaporated in a desiccator to obtain a dry extract.

For cultivation of *Salmonella typhi* bacteria on Mueller Hinton Medium To Plate, cultured bacterial suspension is applied with a sterile swab on Mueller Hinton To Plate. Germs left incubated at 37o C for 24 hours. After 24 hours the development of *Salmonella typhi* bacteria on Mueller Hinton To Plate, do antibiotics chloramphenicol and leaf extracts starfruit (*Averrhoa bilimbi Linn*) with konsentarasi according to the method of bacterial culture medium. C1 treatment control group is a group of *Salmonella typhi* were given chloramphenicol 30 mcg, while the control group was treated C2 *Salmonella typhi* group given 5% DMSO. The treatment group is a group P1, P2 and P3 are given a group of *Salmonella typhi* ekstak leaves starfruit (*Averrhoa bilimbi Linn*) 0.3 g/ml, 0.4 g/ml and 0.5 g/ml.

The analysis was done by observing and measuring the diameter of growth inhibition in the culture medium after 24 hours of treatment. Data on antibacterial activity test ekstak leaves starfruit (*Averrhoa bilimbi Linn*) against *Salmonella typhi* with diffusion method for determining the diameter of the inhibition zone is analyzed descriptively and statistically using the Kruskal-Wallis test with the help of Windows software SPSS.

RESULTS

Research to determine the diameter of inhibition zone antibacterial activity of leaf extract starfruit (*Averrhoa bilimbi Linn*) performed on Muller Hinton medium plate order, using the diffusion method with the bacterium *Salmonella typhi*. Antibacterial activity test leaves starfruit (*Averrhoa bilimbi Linn*) is done with three different concentrations, ie 0.3 g/ml, 0.4 g/ml, and 0.5 g/ml. Each treatment is done as much as 8 times the experiment replication. Diluent used to dilute extract of leaves of starfruit (*Averrhoa bilimbi Linn*) is 5% DMSO.

Antibacterial test results leaf extract starfruit (*Averrhoa bilimbi Linn*), qualitatively expressed in the inhibition zone diameters were measured after administration of the infusion agar medium for 24 hours. Infusion of leaves extract starfruit (*Averrhoa bilimbi Linn*) appeared to have antibacterial activity against gram-negative bacteria *Salmonella typhi*, which is indicated by the formation of inhibition zone diameter. The resulting activity against bacteria inhibition zone test showed different results depending on the concentration of leaf extract starfruit (*Averrhoa bilimbi Linn*).

Based on Clinical Laboratory Standards Institute (2008) with the use of chloramphenicol-resistant bacteria if the diameter is said resistor is less than or equal to 13 mm, intermediates if between 14-17 mm and sensitive if the inhibitory zone diameter of more than or equal to 18 mm.

Table 1. Diamaeter inhibition zone test results of antibacterial activity in leaf extracts of *Salmonella typhi* starfruit (*Averrhoa bilimbi Linn*).

NO	P1	P2	P3	C1	C2
1.	22	23	24	26	0
2.	20	22	24	28	0
3.	20	22	22	30	0
4.	21	21	22	30	0
5.	20	21	22	31	0
6.	20	21	22	31	0
7.	21	22	23	32	0
8.	22	23	24	32	0

Units in centimeters P1 = Treatment 1 (0.3 g/ml) P2 = Treatment 2 (0.4 g/ml) P3 = Treatment 3 (0.5 g/ml)
C1 = Control (chloramphenicol 30 mcg) C2 = control (5% DMSO)
No = Indicates repetitions performed

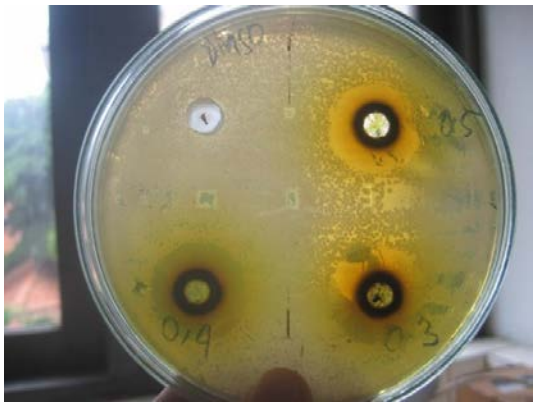


Figure 1. The result of the antimicrobial activity of the leaf extract of starfruit (*Averrhoa bilimbi Linn*) at a concentration of 0.3 g/ml, 0.4 g/ml, 0.5 g/ml, and 5% DMSO control the growth of *Salmonella typhi*. Description: P1: treatment 1 (0.3 g/ml) P2: Treatment 2 (0.4 g/ml) P3: Treatment 3 (0.5 g/ml) C2: Control (DMSO5%)

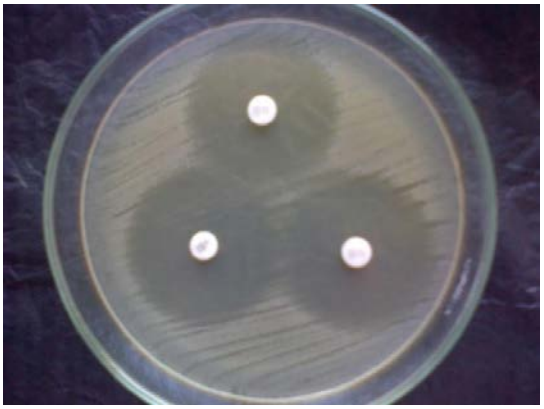


Figure 2. The result of the antimicrobial activity of chloramphenicol (antibiotic control) on the growth of *Salmonella typhi*.

Table 2. Table mean diameter of the inhibition of leaf extracts starfruit (*Averrhoa bilimbi Linn*) on Salmonella bacteria thypi.

Treatment	Mean of Inhibitory Diameter
P1	20.75
P2	21.88
P3	22.88
C1	30
C2	0

The test results of descriptive data is done by calculating the mean of each treatment and control. In experiments testing the antibacterial activity of the leaf extract of starfruit (*Averrhoa bilimbi Linn*) in cultures of *Salmonella typhi*, showed the mean of each treatment, are as follows: mean diameter of inhibition in the treatment with 0.3 g/ml was 20.75 mm, with 0.4 g/ml of 21.88 mm, and 0.5 g/ml of 22.88 mm. Mean diameter of inhibition in control receiving 30 mcg of chloramphenicol was 30 mm and which receives 5% DMSO at 0 mm.

Analysis of variance (Annova) requires the fulfillment of an assumption that the constant error variance of the observation that one observation to another. Testing homogeneity using levene test. From the results obtained it can be seen from lavene test p-value of 0.002, it can be concluded that the experimental data along with the error variance heterogeneity.

Table 3. Results The p-value of each treatment on Antibacterial Activity Test Experiment ekstak leaves starfruit (*Averrhoa bilimbi Linn*) against *Salmonella typhi*.

Groups	p-value
P1	0,462
P2	0,801
P3	0,420
C1	0,699
C2	Uncountable

Based on the results of p-value of each treatment and control is obtained p-value >? = 0.05 except in the C2 group, it can be concluded that the P1 to P3 and C1 group had a normal distribution. Therefore, not all groups have the normal distribution does not meet the assumptions.

Table 4. Results The p-value Kruskal-Wallis Test on Antibacterial Activity Test Experiment ekstak leaves starfruit (*Averrhoa bilimbi Linn*) against *Salmonella typhi*

Groups	N	Mean ± SD	p Kruskall-wallis
P1	8	20.75 ± 0.886	
P2	8	21.88 ± 0.835	
P3	8	22.88 ± 0.991	0.000
C1	8	30 ± 2.070	
C2	8	0	

Based on the test results of Kruskal-Wallis test hypotheses listed in the above table was obtained p-value = 0.000 <? = 0.05. This means that the treatment effect of leaf ekstak starfruit (*Averrhoa bilimbi Linn*) against *Salmonella typhi* with 3 different concentrations of real and giving control 1 (chloramphenicol) and control 2 (5% DMSO) give different results to the response given. Test T-2 free samples conducted to determine whether or not a significant difference between the groups except the control group 2 (C2) by

comparing the average of the two groups were not related to each other. From the test results of the T-2 free samples obtained the following results.

Table 5. Results The p-value T-2 Test Results Free Sample Experiment Antibacterial Activity Test ekstak leaves starfruit (*Averrhoa bilimbi Linn*) against *Salmonella typhi*

Comparison	p-value
P1 and P2	0.020
P1 and P3	0.000
P1 and C1	0.000
P2 and P3	0.047
P2 and C1	0.000
P3 and C1	0.000

In the test T-2 free samples can not be compared between treatment 1 and treatment 2 3 by granting control (C2: 5% DMSO) for C2 has a mean and standard deviation of 0. Therefore to compare between treatment 1 and treatment 3 by giving control 2 (C2: 5% DMSO) was used Mann Whitney Test. From the Mann Whitney Test results obtained the following results.

Table 6. Results The p-value Mann Whitney Test on Antibacterial Activity Test Experiment ekstak leaves starfruit (*Averrhoa bilimbi Linn*) against *Salmonella typhi*

Comparison	p-value
P1 and C2	0.000
P2 and C2	0.000
P3 and C2	0.000
C1 and C2	0.000

DISCUSSION

In accordance with the formulation of the problem that has been stated earlier, the yield of this study was to examine whether there is effect of leaf extract starfruit (*Averrhoa bilimbi Linn*) in a concentration by *Salmonella typhi* bacteria. The study was conducted by using a diffusion method. Concentrations were used to test the antibacterial activity of the leaf extract of starfruit (*Averrhoa bilimbi Linn*) in culture media of *Salmonella typhi* in Muller Hinton Agar, ie 0.3 g/ml, 0.4 g/ml, and 0.5 g/ml. The concentration of each - each weight measurement results obtained from extracts of leaves starfruit (*Averrhoa bilimbi Linn*) were diluted with 5% DMSO up to 100 ml. For dilution diffusion test used in the implementation of 5% DMSO diluent.

The study was conducted as many as eight times in the Laboratory of Microbiology, Faculty of Medicine, University of Airlangga. Based on the research that has been presented in Chapter 5, proved leaf extract starfruit (*Averrhoa bilimbi Linn*) gives effect on growth inhibition of *Salmonella typhi* bacteria with leaf extract concentration starfruit different. Inhibition zone diameter measurement is by measuring the diameter of a circular zone that does not exist the growth of germs in it. Based on the descriptive test, calculated the mean (average) of each treatment and control. The highest mean results obtained in the control group 1 (chloramphenicol 30 mcg) which is 30 mm while the second control (5% DMSO) were 0 mm which means no inhibition zone formed. While on treatment, the best antibacterial activity found in treatment 3 (extract concentration of 0.5 g/ml) is 22.88 mm, then the treatment 2 (extract concentration of 0.4 g/ml) with a mean of 21.88 mm, and the last treatment 1 (extract concentration of 0.3 g/ml) gives a mean diameter of 20.75 mm inhibition zone. In addition to descriptive test, also carried out Kruskal-Wallis statistical test comparing each treatment significant difference. The reason for choosing the Kruskal-Wallis statistical test for normally distributed data and is accompanied by a wide heterogeneity tool. From the Kruskal-Wallis test results, obtained p-value = 0.000 <? = 0.05. Mean treatment effect of leaf extract starfruit with three different concentrations of chloramphenicol control and administration as well as the provision of 5% DMSO control produce different results on the responses given.

To find the treatment that gives a different effect to test the T-2 free samples and Mann Whitney test. From these results it can be concluded that the treatment effect of leaf extract starfruit with three different concentrations and control the provision of antibiotics chloramphenicol and 5% DMSO control giving effect relatively different from one treatment to another treatment. Based on Clinical Laboratory Standards Institute (2007) by using chloramphenicol resistant bacteria if the diameter of the inhibitory say less than or equal to 13 mm, intermediates if between 14-17 mm and sensitive if the inhibitory zone diameter of more than or equal to 18 mm. Based on the results obtained from this study, it appears that administration of starfruit leaf extract concentrations ranging from 0.3 g/ml to 0.5 g/ml antibacterial response is sensitive, as well as chloramphenicol.

Several studies exist to support this research. According to research conducted by Masripah (2009), the results showed that the ethanol extract of leaves of starfruit (*Averrhoa bilimbi Linn*) have inhibitory to bacteria *E. coli* and *S. aureus*. MIC values obtained for *E. coli* bacteria was 400 mg/ml (400,000 ppm) and for *S.*

aureus was 900 mg/ml (900,000 ppm). Potential starfruit leaf ethanol extract obtained was 400 mg/ml (400,000 ppm) of ethanol extract of leaves of starfruit equivalent to 0.04 mg/ml (40 ppm) against *E. coli* amoxicillin and 900 mg/ml (900,000 ppm) of ethanol extract starfruit leaves equivalent to 0.04 mg/ml (40 ppm) of amoxicillin against *S. aureus*. The content of the active compounds in the leaves starfruit are tannins, sulfur, formic acid, and flavonoids. In the leaves starfruit contain secondary metabolites are flavonoids and tannins that can be expected of the active compound can be used as an antibacterial. Phenol and derivatives (flavonoids) is one of antibacterial that works by interfering with the function of the cytoplasmic membrane. At low concentrations can damage the cytoplasmic membrane which causes leakage of important metabolites that inactivate the bacterial enzyme system, whereas at high concentrations could damage the cytoplasmic membrane and cell protein precipitate (Volk & Wheeler 1993). While the mechanism of action of tannins as antibacterial is a reverse transcriptase enzyme and inhibit DNA topoisomerase that bacterial cells can not form (Robinson 1991). This is what allows the cause starfruit leaf extract can inhibit the growth of *Salmonella typhi* bacteria. In this study, the control variables are kept to the same at each treatment, but there is still a possible human errors in the conduct of research and extraction. To improve the accuracy of this antibacterial research, more research is needed to support the weakness of this study. Also needed is more research stage and in vivo toxicity tests to obtain more meaningful results for its use as an antibacterial that can be consumed by humans.

CONCLUSION

Based on data analysis, qualitatively it can be concluded that the concentration of leaf extract starfruit (*Averrhoa bilimbi Linn*) provide growth inhibition zones on *Salmonella typhi* bacteria at a concentration of 0.3 g/ml, 0.4 g/ml, and 0.5 g/ml . Third leaf extract concentration starfruit sensitive response as well as the administration of antibiotics chloramphenicol control. Increasing the diameter of inhibition zone is directly proportional to the increase in the concentration of leaf extract starfruit.

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