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The Effectiveness of Rome Beauty Apple Peel Extract (*Malus sylvestris Mill*) on the Growth of *Salmonella Typhi*

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Abstract

BACKGROUND: Apple peel extract has more total phenolic compounds than the flesh. One of the active ingredients of phenolic compounds that can be used as antibacterial is flavonoids. *Salmonella typhi* is an illness caused by the bacterium *Salmonella typhi* (abbreviated *S. typhi*). It may affect people of all ages but is most prevalent in young people. At the moment, there is widespread resistance to chloramphenicol as the first-line antibiotic for *S. typhi* infection, necessitating the development of new treatments based on natural components.

AIM: The purpose of this research is to investigate the efficacy of Rome Beauty apple peel extract, which is believed to possess antibacterial properties capable of inhibiting the growth of *S. typhi* bacteria.

MATERIALS AND METHODS: To determine the minimum inhibitory concentration (MIC) and minimum kill concentration (MKC) for antibacterial activity using disc diffusion and tube dilution methods, with nine treatments of Rome beauty apple peel extract at concentrations of 50%, 25%, 12.5 %, 6.25%, 3.125%, 1.56%, and 0.78%, as well as a positive control (chloramphenicol 30 mg/ml) (aquadest). SPSS is used to analyze data.

RESULTS: The findings of this investigation established a 12.5 % MIC with an average diameter of the inhibition zone of 13.67 mm and a 50% MKC with an average number of bacterial colonies of 22.33 CFU/mL. The Kruskal–Wallis test was used to examine the MKC data (significance p = 0.002), and the one-way analysis of variance (ANOVA) test was used to study the MIC data (significance p = 0.001).

CONCLUSION: The research concluded that Rome Beauty apple peel extract inhibited the development of *S. typhi* bacteria. Sixty-seven mm and 50% MKC with an average number of bacterial colonies of 22.33 CFU/mL. The Kruskal–Wallis test was used to examine the MIC data (significance p = 0.002), and the one-way ANOVA test was used to study the MIC data (significance p = 0.001). The research concluded that Rome Beauty apple peel extract inhibited the growth of *S. typhi* bacteria.

Introduction

Typhoid fever is an infection caused by *Salmonella typhi* (abbreviated *S. typhi*). It may affect individuals of any age but is most frequent in children. Each year, it is estimated that 11–20 million individuals worldwide have typhoid fever, resulting in around 128,000–161,000 deaths. Typhoid fever has an incidence of 1,600/100,000 individuals in Indonesia. Typhoid fever was discovered in 1.2% of the 10,966 samples collected in 2007 in Malang City [1], [2], [3], [4].

S. typhi is a member of the Enterobacteriaceae family, which also includes Gram-negative bacilli, and it contains Chloramphenicol Acetyl-Transferase, an enzyme capable of inactivating chloramphenicol, resulting in chloramphenicol resistance, which is the first line of defense against *S. typhi* infection [5], [6], [7]. According to 10-year average data from the National Antimicrobial Resistance Monitoring System, chloramphenicol resistance was 24 % in *S. typhi* [8]. In 2008–2009, Dr. RSU. Saiful Anwar Malang reported a 76.9% incidence of typhoid fever caused by *S. typhi* but resistant to chloramphenicol [9].

Kota Batu is well-known for its apple production in East Java, Indonesia, with Rome Beauty being one of the indigenous varieties. Apple skin has a higher concentration of total phenolic compounds than apple pulp, with one of the components, flavonoids, exhibiting antibacterial activity [10], [11]. Procyanidin, quercetin, floretin, myricetin, and epicatechin are all flavonoids classified under the flavanol (catechin) subclass. Catechins have been proven to increase potassium leakage and destroy bacteria's membranes. Quercetin suppresses the synthesis of the bacterial toxins, the cell envelope, nucleic acid, electron transport chain, and adenosine triphosphate (ATP) [7], [12].

At 477.96 mg/kgd, Rome Beauty apples contained the highest quercetin, followed by three other

apple kinds (Manalagi, Fuji, and Red Delicious) [13]. The goal of this research was to evaluate the minimum inhibitory concentration (MIC) and minimum killing concentration (KBM) of Rome Beauty apple peel extract (Malus sylvestris Mill) against *S. typhi* bacteria growth. Based on the similarity in the content of apple skin with flavonoids as an antibacterial that has been described above, researchers are interested in conducting research on the effectiveness of Rome apple peel extract Beauty (Malus sylvestris Mill) against the growth of *S. typhi* bacteria.

The results of this study are expected to be used for the manufacture of alternative medicines from materials that are easily obtained and reused which have a therapeutic effect so that they can be used for the treatment of typhoid fever.

Materials and Methods

The research's date and location

The research was conducted between December 2019 and February 2020 at the Faculty of Medicine and Health Sciences, Maulana Malik Ibrahim State Islamic University Malang, in the Microbiology and Pharmacy Laboratories.

The apple peel obtained in Malang City and the material with habits: Shrubs; 2–4 m high; well planted in the highlands between 700 and 1200 with dry types. Stem: Woody; round; upright/vertical branching; chocolate. Leaves: Single; long-form slightly folded, pointed ends; blunt leaf base; serrated leaf edges are regularly uneven; dark green leaf color; green young leaves; gray leaf stalks. Flowers: 5 flower crowns; petals 5, pointed; stamens on the pistil; pink color, white after a long time. Fruit: Round to oblong; diameter 5–12 cm; weight 75–300 g; red color if exposed to fruit in the sun, if not green; white flesh with weak aroma; firm and fresh fruit flavor. Seeds: small, oblong, dark brown.

Subjects of research

S. typhi bacteria were isolated from pure isolates and grown for 18–24 h at 37°C on Mueller Hinton agar (MHA) medium.

Instruments and substances

Petri dish, test tube, round loop, bunsen, tweezers, autoclave, Erlenmeyer flask, micropipette, desiccator, sieve, beaker, measuring cup, colony counter, analytical balance, glass jar, ruler, incubator, oven, paper disc, and stirring rod; suspension of *S. typhi* (10^8 CFU/mL) was equated to McFarland turbidity.

Extract dilution

Rome Beauty apple peel extract was prepared using the serial dilution method in a range of concentrations.

I. 50% concentration: 1 mL Rome Beauty apple peel extract at a concentration of 100% is combined with 1 mL sterile distilled water
II. 25% concentration: 1 mL apple peel extract Rome Beauty + 1 mL sterile distilled water
III. Concentration of 12.5 %: 1 mL Rome Beauty apple peel extract in conjunction with 1 mL sterile distilled water
IV. 6.25 % concentration: 1 mL Rome Beauty apple peel extract at a 12 % concentration in 1 mL sterile distilled water, 1 mL Rome Beauty apple peel extract diluted in 1 mL sterile distilled water to a concentration of 3.125 %
V. Concentration of 1.56 %: mixing up to 1 mL Rome Beauty apple peel extract at a concentration of 3.125 % with 1 mL sterile distilled water; and add 1 mL of Rome Beauty apple peel extract produced at a concentration of 1.56 % to 1 mL of sterile distilled water.

Technique process

The technique employed is disc diffusion, which is a multi-step process. To begin, make Rome Beauty apple peel extract (*Malus sylvestris Mill*) at the following concentrations: 50%, 25%, 12.5%, 6.25 %, 3.125 %, 1.56 %, and 0.78 %, as well as a positive control solution (chloramphenicol 30 mg/mL) (aquadest). Positive controls for antimicrobials were chosen using the Clinical Laboratory Standards Institute's criteria. Second, solidify the MHA medium by swabbing it with a sterile cotton swab and streaking it with the *Salmonella typhi* bacterial suspension (10^8 CFU/mL). It is scraped in parallel lines; the cup is then spun 60 times over the whole surface of the medium and left to dry for 4–5 min. Third, soak blank disc paper (6 mm diameter) in Rome Beauty apple peel extract (Malus sylvestris Mill) at the following concentrations for 10–20 min: 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and 0.78%, positive control solution (chloramphenicol 30 mg/mL), and negative control solution (aquadest). Fourth, it was incubated at 37°C for 18–24 hours to determine the extent of the inhibitory zone (clear area formed around the paper disc). Fifth, keep a careful eye on the concentration at which a zone of inhibition develops.

After 10–20 min of soaking blank disc paper (diameter 6 mm) in Rome Beauty apple peel extract (*Malus sylvestris Mill*) at the following concentrations: 50%, 25%, 12.5 %, 6.25 %, 3.125 %, 1.56 %, and 0.78 %, positive control solution (chloramphenicol 30 mg/mL), and negative control solution (aquadest), it was placed on MHA media inoculated with bacteria. Fifth, keep a careful eye on the concentration at which a zone of inhibition develops. For 10–20 min, soak

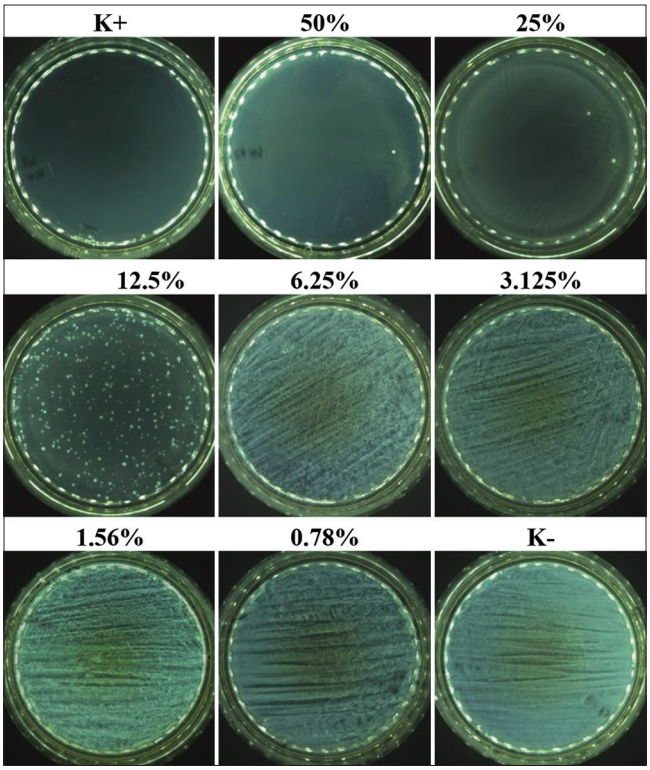

Figure 1*: Bacterial colonies of each extract concentration rome beauty apple skin shown with colony counter source: Research Data, 2020*

blank disc paper (diameter 6 mm) in the following concentrations of Rome Beauty apple peel extract (*Malus sylvestris Mill*): 50%, 25%, 12.5 %, 6.25 %, 3.125 %, 1.56 %, and 0.78 %, as well as a positive control solution (chloramphenicol 30 mg/mL) (aquadest in the Figure 1). Fourth, it was incubated at 37°C for 18–24 h to determine the extent of the inhibitory zone (clear area formed around the paper disc). Fifth, check intensely on the concentration at which a zone of inhibition develops for 10–20 min; and then for 10–20 min with the negative control solution (aquadest) put on bacteria-inoculated MHA medium. Fourth, it was incubated at 37°C for 18–24 h (Table 1), and the width of the inhibition zone (clear region produced around the paper disc) was measured. Subsequently, check the concentration at which a zone of inhibition forms. for 10–20 min; and then place on bacteria-infected MHA medium. Fourth, it was incubated for 18–24 h at 37°C to assess the breadth of the inhibition zone (clear area formed around the paper disc). Fifth, control the concentration at which a zone of inhibition forms.

The inhibitory zone's breadth (Table 2) may be utilized to determine an extract's antibacterial activity (Hapsari, 2015) stated that a diameter of the inhibition zone >20 mm is considered very strong; a diameter of the inhibition zone between 10 and 20 mm is considered strong; a diameter of the inhibition zone between 5 and 10 mm is considered medium; and a diameter of the inhibition zone between 0 and 5 mm is considered weak. On the 1st day, each test tube was filled with 50 %, 25%, 12.5%, 6.25 %, 3.125 %, 1.56 %, and 0.78 % Rome Beauty apple peel extract (*Malus sylvestris Mill*), 1 mL positive control solution (chloramphenicol 30 mg/mL), and 1 mL negative control solution (aquadest), as well as 1 mL *S. typhi* suspension (108 CFU/mL) [14]. Therefore, the mixture was incubated at 37°C for 18–24 h. On the 2nd day, all tubes were removed from the incubator and one swab from each was streaked into the MHA medium in tight, parallel lines; the cup spun 60 times and the same procedure was repeated on the whole surface of the medium.

In addition, Petri plates were inverted in the incubator and incubated for 18–24 h at a temperature of 37°C. Bacteria were counted using a colony counter on the 3rd day. The minimum kill concentration (MKC) is calculated by the concentration at which 99.9 % of bacteria are killed or by the number of bacteria that comprise 0.1 % of the original inoculum.

Data analysis

According to the results of the diameter measurement of the inhibition zone, a 50% concentration resulted in an average diameter of 20.33 mm (very strong), a 25% concentration resulted in an average diameter of 17.33 mm (strong), a 12.5% concentration resulted in an average diameter of 13.67 mm (strong), and a 6.25% concentration resulted in an average diameter of 6.25 mm (strong). As a result, it is possible to assume that the concentration

Results

Minimum inhibitory concentration

From the results of the measurement of the diameter of the inhibition zone, a concentration of 50% resulted in an average diameter of the inhibition zone of 20.33 mm (very strong), a concentration of 25% produced an average diameter of the inhibition zone of 17.33 mm (strong), a concentration of 12.5 % resulted in an average inhibition zone diameter of 13.67 mm (strong), a concentration of 6.25% resulted in an average inhibition zone diameter of 4.00 mm (weak), a concentration of 3.125% produced an average inhibition zone diameter of 3.67 mm (weak), a concentration of 1.56% resulted in an average diameter of the inhibition zone of 3.33 mm (weak), the positive control, namely chloramphenicol, produced an average diameter of the inhibition zone of 28.67 mm (very strong), and negative control, namely aquadest does not produce an inhibition zone. Therefore, it can be concluded that the concentration.

The normality and homogeneity tests were passed, allowing for the performance of a one-way analysis of variance (ANOVA) test, with the findings p =

0.001 (p0.05) indicating significant results. In addition, the least significant difference (LSD) *post hoc* test was used to determine the concentrations at which there was a significant difference in concentrations of 50%, 25%, and 12.5% with positive controls.

The minimal concentration that inhibits the development of *S. typhi* bacteria is 12.5% with an average diameter of the inhibition zone of 13.67 mm across three repetitions, meeting the criterion for high antibacterial strength.

Table 1 : Average inhibitory zone diameter

Rome beauty apple peel extract (malus sylvestris mill)	Repeat 1 (mm)	Repeat 2 (mm)	Repeat 3 (mm)	Average inhibition zone diameter (mm)
50%	18	24	19	20.33
25%	14	19	19	17.33
12.5%	9	18	14	13.67
6.25%	4	4	4	4.00
3.125%	3	4	4	3.67
1.56%	4	3	3	3.33
0.78%	2	3	3	2.67
Positive control (chloramphenicol 30 mg/mL)	28	29	29	28.67
Negative control (aquadest)	0	0	0	0

The normality distribution of the data was determined using the Kolmogorov–Smirnov Test, and the result was p = 0.052, indicating that the data were normally distributed. Then, using the Levene's test, determine if the data are homogenous or not. If the data are not homogeneous, the one-way ANOVA test cannot be performed. As a consequence, the Kruskal–Wallis test was utilized, with a significance level of p = 0.002 (p0.05). In addition, the Mann–Whitney test was used to determine the concentration at which a significant difference existed. The Mann–Whitney test analysis reveals that although the concentration of 50% does not vary significantly from the concentrations of 25% and 12.5%, there are significant differences at the concentrations of 6.25%, 3.125%, 1.56 %, and 0.78%.

Table 2: Average number of colonies

Rome beauty apple peel extract (malus sylvestris mill)	Repeat 1 (CFU/mL)	Repeat 2 (CFU/mL)	Repeat 3 (CFU/mL)	Average number of colonies (CFU/mL)
50%	14	13	40	22.33
25%	45	24	41	36.67
12.5%	350	21	49	140
6.25%	Spreaders	Spreaders	Spreaders	Spreaders
3.125%	Spreaders	Spreaders	Spreaders	Spreaders
1.56%	Spreaders	Spreaders	Spreaders	Spreaders
0.78%	Spreaders	Spreaders	Spreaders	Spreaders
Positive control (chloramphenicol 30 mg/mL)	5	6	3	4.67
Negative control (aquadest)	Spreaders	Spreaders	Spreaders	Spreaders
Original inoculum	Spreaders	Spreaders	Spreaders	Spreaders

Minimum kill concentration

According to the results of the calculation of *S. typhi* bacterial colonies using a colony counter, at a 50% concentration, an average bacterial colony contained 22.33 CFU/mL, at a 25% concentration, an average bacterial colony contained 36.67 CFU/mL, and at a 12.5 % concentration, an average bacterial colony contained 140 CFU/mL; while at 6.25 %, 3.125 %, 1.56 %, 0.78 %, negative control, and spreaders. The spreader is a bacterial colony whose number cannot be estimated because multiple developing colonies create a row, making it impossible to calculate the distance between one colony and another. In addition, there was an uneven distribution of colonies, with some colonies growing on top of others [15].

According to the data generated by the colony counter in the form of numbers indicating the number of bacterial colonies and images indicating that 99.9 % of bacteria are dead or that the number of bacteria 0.1 % of the original inoculum is at a concentration of 50%, MKC occurs at a 50% concentration. At a 25% concentration, it provides a rather clear image of bacterial colony development, however when computed using a colony counter, the findings indicate an average bacterial colony growth of 36.67 CFU/mL, which is much more than the average growth rate. Bacterial colonies at a 50% concentration, so that a 25% concentration is not used as MIC, and the number of bacterial colonies that grow excessively and cannot be determined using colony counters at concentrations of 6.25%, 3.125 %, 1.56%, and 0.78%, as well as a negative control.

Thus, only data on the number of colonies of *S. typhi* bacteria at 50%, 25%, 12.5%, and a positive control were examined using SPSS to establish the data's significance. To begin, the Kolmogorov–Smirnov test was used to determine if the data were regularly distributed. The result was p = 0.196 (p>0.05), indicating that the data were normally distributed. Then, using the Levene's Test, determine if the data are homogeneous, with a value of p = 0.408 (p>0.05) or whether the data have the same variance. The normality and homogeneity tests were passed, allowing for the performance of a One-Way ANOVA test, with the findings p = 0.001 (p value 0.05) indicating significant results. In addition, the LSD *post hoc* test was used to determine the concentrations at which there was a significant difference in concentrations of 50%, 25%, and 12.5% with positive controls.

Discussion

Minimum inhibitory concentration

In this investigation, the MIC was 12.5 % with an average inhibition zone width of 13.67 mm. Three repeats were used to determine the average diameter, and the bacteria were cultured for 18–24 h. The diameter of the inhibition zone was determined by the concentration of Rome Beauty apple peel extract used. The larger the diameter of the inhibition zone, the more antibacterial activity was generated. Antibacterial action is attributed to the Rome Beauty apple peel's phytochemical richness, namely catechins, quercetin, and floretin. Catechins promote potassium leakage and inhibit bacterial cell wall production, acting as an early indicator of microorganism membrane damage [7], [16].

Rome Beauty Apples contain the highest amount of quercetin at 477.96 mg/kg, which is capable of causing damage to bacterial cell membranes, inhibiting Gyrase/Topoisomerase enzymes (enzymes that separate DNA strands during bacterial DNA replication), and inhibiting the hydrolysis of ATP, thereby inhibiting bacterial cell ATP synthesis. [7], [12], [13]. Meanwhile, floretin has been shown to suppress pathogenic strains while being non-toxic to normal flora [7]. The presence of this antibacterial mechanism results in the formation of an inhibitory zone in the Rome Beauty apple peel extract.

Harlambang validated this in 2015 [17] when he used Rome Beauty apple peel extract as an antibacterial *Streptococcus mutans in vitro* and got a MIC of 25% with an average inhibition zone width of 9.425 mm. Octaviany *et al*. (2017) demonstrated that the flavonoids contained in the Rome Beauty apple peel extract at doses of 0.12 mg/kg bw, 0.24 mg/kg bw, and 0.49 mg/kg bw can inhibit the SGPT enzyme in mice (*Rattus norvegicus*) induced with carbon tetrachloride (CCl4); this demonstrates that flavonoids not only have antibacterial activity but can also inhibit lipid peroxid [10].

Pratiwi *et al*. (2013) [18] conducted antibacterial test study on *S. typhi* with the same MIC findings using lime peel extract at a concentration of 12.5 %. Similarly, Noorhamdani *et al*. (2015) [19] showed that ginger rhizome extract was antibacterial against *S. typhi* at a 17.5 % extract concentration with a 13 mm inhibition zone width.

The discrepancy between the MIC and MKC values determined in this study and previous studies could be attributed to a variety of factors, including the type of extract used, the extraction method used, the type of solvent used, the medium used, and the different methods used to determine the MIC and MKC values.

Minimum concentration of killing agents

In this investigation, the MKC was 50%, resulting in an average bacterial growth of 22.33 CFU/mL after three cycles. The more germs that die, the more evidence there is that Rome Beauty apple peel extract has an antibiotic mechanism capable of killing bacteria at a given dosage.

The antibacterial mechanism offered by the phytochemical content of Rome Beauty apple skin is capable of killing bacteria. For example, quercetin may damage bacterial cell membranes and inhibit ATP hydrolysis, preventing bacteria from living and dying. A research previously shown that extracts containing flavonoid extracts from the bark of Kemuning stems were capable of inhibiting the development of *S. typhi* bacteria at concentrations ranging from 25% to 75%. This is also consistent with Noorhamdani *et al.'s* (2015) study on the antibacterial action of flavonoids from ginger rhizome extract, which was shown to be capable of killing *S. typhi* at a 25% concentration [17], [20].

The number of bacterial colonies that formed at concentrations of 6.25%, 3.125%, 1.56%, and 0.78% in this research was so enormous that it could not be counted using a colony counter or referred to as a colony spreader. Numerous variables contributed to the establishment of spreader colonies, including the fact that bacteria were grown on MHA medium using the streak plate technique, which meant that the *S.typhi* bacteria most likely proliferated in great numbers and then followed the groove when the researcher streaked the plate. This allows *S. typhi* to grow in a row, making it impossible to precisely count with the colony counter. In addition, the factor equating the formation of a bacterial suspension to a McFarland turbidity of 0.5 was subjective, affecting the quantity of bacteria put into the MHA medium.

The data is regularly distributed, as shown by the findings of the Kolmogorov–Smirnov test. The Levene's test homogeneity test then revealed homogeneous data, indicating that the data on the number of colonies between concentration groups had the same variance and that the difference in statistical tests in this study was indeed caused by differences in the number of colonies between groups of extract concentrations. The one-way ANOVA test also revealed significant results, so the LSD *post hoc* test was used to determine which variables had significant differences; the results indicated that data on the number of colonies at 50%, 25%, and 12.5% had a significant difference in the number of colonies with positive controls.

Correlation test results indicate that the correlation value (R) = 0.538 is positive, indicating that the Rome Beauty apple peel extract is directly proportional to the growth of *S. typhi* bacteria; or that the higher the concentration of Rome Beaut apple peel extract, the greater the inhibition produced against the growth of *S. Typhi* bacteria.

As a conclusion to this study, based on prior research, it can be inferred that Rome Beauty apple peel extract may be utilized as an antibacterial, particularly against *S. typhi* bacterium.

Conclusion

Rome Beauty apple peel extract (*Malus sylvestris Mill*) inhibited the development of *S. typhi* bacteria at a MIC of 12.5%, i.e., the average inhibition zone width was 13.67 mm, and the MKC was 13.67 mm. At a 50% concentration, the average number of bacterial colonies per milliliter was 72.33 CFU/mL. As such, it is envisaged that it may serve as a substitute for other medications derived from natural substances

that operate as antibacterial agents against *S. typhi* bacterium.

Limitations of the research

Numerous restrictions apply to this investigation. First commence, no phytochemical analysis was performed, making it impossible to determine the active ingredients included in the Rome Beauty apple peel extract. Second, the number of colonies was determined using an auto-count/colony counter, which allowed for the counting of not only bacterial colonies but also detritus on the petri dish. Third, since repetition is limited to four times or KBM and five times for KHM, obtaining the best data remain tough. Fourth, since the width of the inhibitory zone is utilized to determine the MIC, it is vital to examine other approaches such as microdilution.

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