



## Efectiveness of Shallot Root Extract Local Palu Against *Candida albicans* Fungus and its Utiliation as Learning Media

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*Allium cepa* var aggregatum  
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### **ABSTRACT**

*The potential of Palu local shallot roots as an antifungal agent has not been optimally utilized. The aim of this research is to determine the effectiveness of Palu local shallot root extract against the fungus *Candida albicans*, identify the optimal concentration for inhibiting the growth of *Candida albicans*, and to use the findings as an educational medium. This research employed a laboratory experimental method with a completely randomized design (CRD) consisting of 5 treatments and 4 replications at concentrations of 0% as a negative control, 25%, 50%, 75%, and 100%. The *Candida albicans* fungus was obtained from Paramartha Lab Mojokerto with strain code ATCC-14053. The parameter observed was the diameter of the inhibition zone formed. The data were analyzed using Analysis of Variance (ANOVA), followed by a Least Significant Difference (LSD) test. The results of this study showed that the extract of Palu local shallot roots effectively inhibited the growth of *Candida albicans*\* at a concentration of 100%. This research is feasible to be used as an educational medium in the form of a digital flipbook with an eligibility percentage of 87.08%.*

## INTRODUCTION

Indonesia's abundant biodiversity with 1,300 of them is a plant that can be used as an ingredient for medicine (Siregar et al., 2020). Research on plants as a new medicinal ingredient begins with an empirical study of its medicinal properties. The tradition of Indonesia people who use plants for medicine is the first step to explore the potential of plants as a source of medicine. The main focus in research on plants that are believed to have potential as drugs begins with identifying the chemical composition and effectiveness of the plants (Edy, 2022). One of the plants that is often encountered and has the potential as a medicinal plant is the Palu valley variety shallot (*Allium cepa var aggregatum* L.) This shallot is one of the types of local shallots traditionally produced by farmers, especially in the Palu Valley, Central Sulawesi (Anshar & Ete, 2017). Empirically, people have consumed or used shallots as medicine for colds, coughs, heartburn, flatulence, asthma, nosebleeds, constipation, acne and other diseases.

Shallots contain nutrients and secondary metabolite active compounds that have pharmacological effects, so they are very beneficial for health (Aryanta, 2019). Adding to the scientific evidence for the benefits of shallots, a study by (Simanjuntak & Butar - Butar, 2019) tested the antifungal activity of ethanol extract of onion tubers (*Allium cepa* L.) against the fungus *Candida albicans* which showed that ethanol extract of shallots was effective in inhibiting the fungus *Candida albicans* in the strong category. The existence of an antifungal effect on *Candida albicans* is important because *Candida albicans* is a fungus that lives in the human body, precisely in the digestive tract, mucosal membranes of the oral cavity and the female genital area (Makhfirah et al., 2020).

*Candida albicans* has become the causative agent of a life-threatening invasive disease with a mortality rate of nearly 40% even though it has been treated (Makhfirah et al., 2020). Currently, the treatment of *Candida albicans* infection generally involves the use of antibiotics, in clinical conditions the extensive use of antibiotics unfortunately increases cases of antifungal resistance to *Candida albicans* (Gerald et al., 2022). The increasing cases of antifungal resistance to diyle need other alternative treatments. Consideration of the potential use of the leading commodity of local wisdom of local shallots in Palu in the root part that has secondary metabolites in the form of flavonoids, tannins, alkaloids, and saponins (Faidah et al., 2020) as a source of antifungal drugs *Candida albicans* needs to be done to determine the effectiveness of secondary metabolite compounds in the roots of local shallots in Palu so that the truth of the manifestation can be ascertained and can be Scientific data is obtained by scientific studies.

The research process and the results of this research will be used as scientific information that is poured into learning media in the form of digital *flipbooks*. *Flipbook* media is a visual media in the form of sheets of paper presented in a digital format that makes users more interactive with the media (Saparina et al., 2020). Setiadi et al., (2021) stated that the use of digital *flipbook* learning media can improve learning outcomes and learning activities with a percentage of learning completeness of 81.8% from the previous only 39.4%. This assumes that the use of digital *flipbook* media in the research on the efficacy of local shallot root extract in Palu against *Candida albicans* fungus will be feasible to be used as a learning medium.

## METHOD

This study was conducted by an experimental method using a Complete Random Design (RAL) with four replicates and five concentration treatments, namely 100%, 75%, 50%, 25% and 0% as negative controls. The results of the research were analyzed using Variant Analysis (Anova) and continued with the BNT Test. This research was carried out in the Laboratory of the Biology Education Study Program. The ingredients used are pure isolate of *Candida albicans* fungus from Paramartha Lab Mojokerto with strain code ATCC-14058, Palu local shallot root extract, 96% ethanol, sterile aquades, Potato Dextrose Agar (PDA), NaCl, filter paper, safranin dye, cotton, tissue, label paper. The tools used in are blenders, oisau, petri cups, autoclaves, Erlenmeyer, measuring cups, analytical scales, baths, aluminum foils, beaker cups, stirring rods, test tubes, test tube racks, incubators, rotary evaporators, bunsen, ose needles, hole pipes, micropipettes, wheelbarrows, funnels, microscopes, scissors, cameras and stationery.

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The ingredients used include a pure isolate of *Candida albicans* fungus from Paramartha Lab Mojokerto with strain code ATCC-14058, Palu local shallot root extract, 96% ethanol, sterile aquades, Potato Dextrose Agar (PDA), NaCl, filter paper, safranin dye, cotton, tissue, and label paper. The tools used include blenders, ose needles, petri dishes, autoclaves, Erlenmeyer flasks, measuring cups, analytical scales, water baths, aluminum foil, beakers, stirring rods, test tubes, test tube racks, incubators, rotary evaporators, Bunsen burners, hole punchers, micropipettes, wheelbarrows, funnels, microscopes, scissors, cameras, and stationery.

Sterilization of tools and materials was carried out using an autoclave at a temperature of 121°C for 30 minutes, then left for 15 minutes after turning off the autoclave (Utami et al., 2018). Identification of *Candida albicans* fungus was performed by planting 1 ose of the fungus in a petri dish using a scratch technique and incubating it for 24 hours. The grown *Candida albicans* fungus was then taken as much as 1 ose and placed on a concave object glass, stained with safranin dye, and observed under a microscope with a magnification of 1000x (Juariah & Maritza, 2019).

The process of making local shallot root extract in Palu began with cleaning the shallot roots, drying them by aeration for approximately two days, and grinding them using a blender. The maceration process was then carried out by soaking 250 grams of shallot root powder in 96% ethanol for 3x24 hours. The maceration results were filtered using a filter funnel and then evaporated using a rotary evaporator at a temperature of 50°C to obtain a thick extract (Badaring et al., 2020).

Fungal inoculum preparation was done by taking *Candida albicans* as much as 1 ose, then streaking it onto an oblique medium, followed by incubation at 37°C for 2x24 hours in an incubator (Komala et al., 2020). The fungal suspension was prepared by weighing 0.9 grams of NaCl solution, dissolving it into 100 ml of distilled water, and homogenizing the solution. Then, 1 ose of *Candida albicans* fungus was introduced into a test tube containing 10 ml of 0.9% NaCl solution (Nasution et al., 2021).

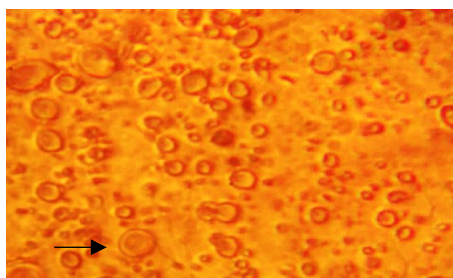
For the preparation of Potato Dextrose Agar (PDA) media, 20.3 grams of PDA powder was weighed and dissolved in 500 ml of distilled water, then heated in a water bath until boiling. A fungal suspension of 1 ml was taken using a micropipette and poured into a petri dish, followed by pouring the preheated PDA into the petri dish while still warm. The mixture was then homogenized in the shape of the number eight (8). Once solidified, well holes with a diameter of 5 cm were created (Mendrofa et al., 2019).

The dilution of shallot root extract was carried out with a final extract volume of 10 ml, preparing different concentrations of 100%, 75%, 50%, 25%, and 0%. The composition was calculated using the dilution formula:  $V_1 \times M_1 = V_2 \times M_2$  (Indah et al., 2017). The testing of shallot root extract was conducted by pipetting 2 ml of the extract into the well holes in the agar medium, ensuring each concentration was placed in its designated well. The medium was then incubated at 37°C for 3x24 hours (Maria Dimova & Stirk, 2019). The inhibition zone parameters were determined by observing the clear areas formed around the well holes. The inhibition zone measurements were performed using a caliper (Agustina et al., 2021).

## RESULTS AND DISCUSSIONS

### Isolation Results of *Candida albicans* Fungus

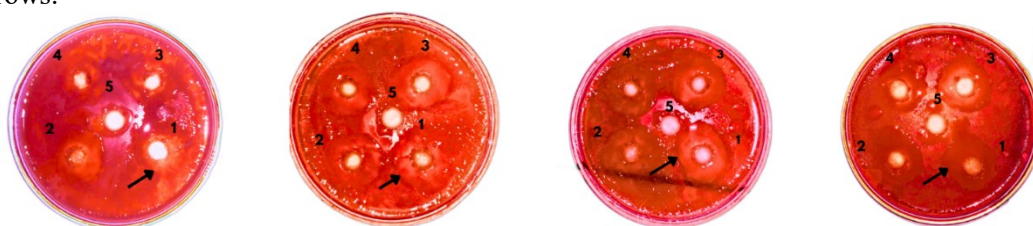
This study used the fungus *Candida albicans* with strain code ATCC-14053 which is a pure isolate obtained from Paramartha Lab Mojokerto. Before being used for research, the mushrooms were first cultured on *Potato Dextrose Agar* (PDA) medium and analyzed using a microscope with a simple dye safranin, so that an oval-shaped blastopore (yeast cell) was obtained and an image of the budding cell of the fungus *Candida albicans* as seen in the arrow in Figure 1.



**Fig 1.** Cells of *Candida albicans*

### Testing Antifungal Effectiveness Using the Well Technique

Anti-fungal testing of shallot root extract on the growth of *Candida albicans* fungus using the well technique as presented in Figure 2 shows the existence of an inhibition zone formed as indicated by the arrows.

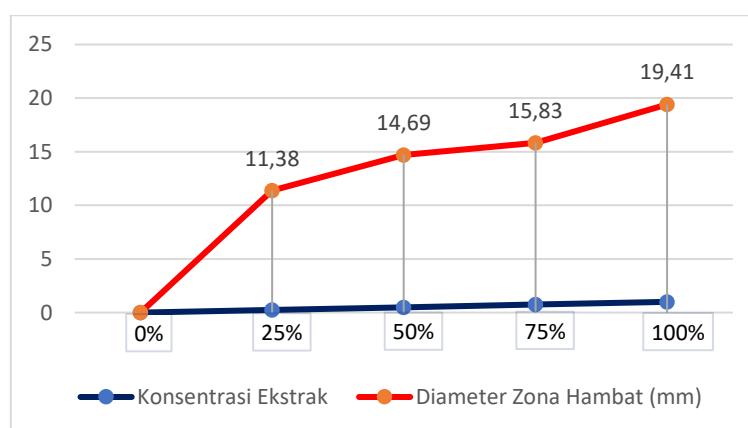


**Fig 2.** Fungal growth inhibition zone *Candida albicans*

#### Information:

1. Concentration of local onion root extract in Palu 100%
2. Concentration of local shallot root extract in Palu 75%
3. Concentration of local shallot root extract in Palu 50%
4. Concentration of local shallot root extract in Palu 25%
5. Concentration of 0% sterile aquades (without shallot root extract)

The diameter of the inhibition zone formed from each concentration is different from the average size of the inhibition zone that is formed as shown by the monotonous graph of the rise as presented in Figure 3.



**Fig 3.** Average diameter of inhibition zone

Graph in Figure 3. showed an increase in the average diameter of the inhibition zone following the high concentration of shallot root extract. This is because the higher the concentration of the extract used, the wider the antifungal activity so that the inhibition zone formed is wider. This shows that the root extract of local shallots in Palu is able to inhibit the growth of *Candida albicans* fungus.

### Analysis of Variants of Red Baang Root Extract in Inhibiting the Growth of *Candida albicans* Fungus

The results of the analysis of the variation in the diameter of the growth inhibition zone of *Candida albicans* fungus due to anti-fungal onion root extract, the results were obtained as shown in Table 1.

**Table 1.** Results of analysis of various inhibition zones of *Candida albicans* fungus

Diversity Sources	Free Degree	Total Diversity	Middle Square	F Count	F Table
Treatment	4	883,4075	220,85	138,9*	3,06
Error	15	23,93	1,59		
Tota	19	907,34			

Based on the data in Table 1. The results of the analysis of the variant of the inhibition zone of local shallot root extract in Palu against the fungus *Candida albicans* through the well technique showed that the calculated value of  $\geq F$  table ( $\alpha = 0.05$ ) with dB Error 15 obtained the value of F Count which was 138.9 greater than F Table 5% which was 3.06. The results of the analysis can be interpreted as  $H_0$  rejected and  $H_1$  accepted, which means rejecting  $H_0$ . shallot root extract is not effective in inhibiting the growth of *Candida albicans* fungus and receiving  $H_1$  namely there is an inhibition of local shallot root extract in Palu against the growth of *Candida albicans* fungus. This shows that there is a real effect of the treatment of local shallot root extract in Palu on the growth of *Candida albicans* fungus with the formation of an inhibition zone.

Based on the results of the analysis, namely  $\geq$  then continued with the Smallest Real Difference test to see the most effective concentration of shallot root extract in inhibiting the growth of *Candida albicans* fungus.

Based on the data from the BNT test results, the difference between treatments at concentrations of 100%, 75%, 50%, 25% was obtained. All of these concentrations have a value greater than the BNT value at the level of 5%, which is 2.131. These results show that there is a real difference from the significant and very significant concentration treatment. The 100% concentration was the most effective in inhibiting the growth of *Candida albicans* fungi because the average difference between treatments was greater than the difference between the concentrations of 25% to 75%, which had a difference value of 19.41\*\*. The 0% concentration (control) is not given shallot root extract, so the 0% concentration is not calculated by the calculation starting from the 25% concentration.

### The Feasibility Assessment of Digital Flipbook Learning Media

The overall average assessment of the feasibility of learning media in the form of a digital *flipbook* can be seen in the Table 2.

**Table 2.** Average Data Analysis of Digital *Flipbook* Teaching Materials Assessment

No	Valuation	Assesment Scale	Percentage (%)
1	Material Expert	4	82,5
2	Design Expert	4	80
3	Media Expert	4,875	97,5
4	Small Group Trial (15 Students)	4,36	87,2
5	Large Group Trial (25 Students)	4,41	88,2
	Sum	21,645	361,5
	Average	4,329	87,08

The results of the assessment of teaching media in the form of digital *flipbooks* show positive assessments from various experts. The assessment was carried out by content experts, media experts, and design experts, and tested in a small group of 15 students and a large group of 25 students. The assessment from the design expert showed a score of 80% which was included in the feasible category, while the content expert showed a score of 82.5%, and the media expert of 97.5%, both of which were

included in the very feasible category. In addition, the results of the trial in a small group obtained a score of 87.2%, while the large group received a score of 88.2%, which also shows that this digital *flipbook* is in the very feasible category. Based on the results of this assessment, it can be concluded that the digital *flipbook* is very suitable to be used as a learning medium (Elza et al., 2023).

The results of the microscope observation obtained an oval-shaped blastopore and an image of the budding cell of the fungus *Candida albicans*. In accordance with research (Juariah & Maritza, 2019) which states that the colony of *Candida albicans* is round or oval in shape and on microscopic observations blastospores are found.

Shallot root extract tested on the fungus *Candida albicans* through the well technique showed the presence of an inhibition zone formed. According to (Putri et al., 2016) the existence of an inhibition zone is marked by a clear area around the well from the test mushroom growth medium that is not overgrown with mold. The formation of the inhibition zone is due to the presence of secondary metabolite compounds that prevent or inhibit the growth of microorganisms around the well.

In each test with 5 treatments, the surrounding clear zone was shown with varying sizes. The size of the clear zone in each treatment of 0% extract did not indicate the presence of inhibition, then the treatment of 25% extract treatment of the diameter of the inhibition zone was increasingly enlarged consecutively until the treatment of 100% extract marked 1 showed the largest diameter of the inhibition zone because it had a higher extract. The consistency of the inhibition zone in all four replicates showed that shallot root extract had an antifungal effect in inhibiting the growth of *Candida albicans* fungus.

The secondary metabolite compounds found in the root of the hammer shallot are flavonoids, saponins, alkaloids and tannins (Faidah et al., 2020). According to (Susila Ningsih et al., 2023) flavonoid compounds inhibit fungal growth by inhibiting fungal wall synthesis. Flavonoids as anti-fungicide work by inhibiting the growth of pathogenic fungal conidia because these flavonoids are lipophilic which can damage microbial membranes. Genesteine in flavonoids functions as an inhibitor of the division or proliferation of fungal cells by penetrating the fungal cell wall towards the cell membrane. Phenolics in flavonoids damage cytoplasmic cells and cause leakage of fungal cell nuclei.

The saponins found in the root of the shallot also inhibit the growth of the fungus *Candida albicans*. According to Yulia et al., (2023) Saponins work as antifungics by lowering surface tension, increasing cell permeability, and causing cell leakage and the release of intracellular compounds. The surfactant properties of polar saponins break down fat on cell membranes, disrupting their permeability, so that fungal cells break down and swell due to disruption of the diffusion process and absorption of important substances

Alkaloid compounds as skunde metabolites are antifungal because these compounds work by interfering with the constituent components of peptidoglycan in fungal cells, causing the failure of the cell wall formation process as a whole and will cause the cell to die. Alkaloids are compounds that have antifungal activity by inhibiting DNA esterase and RNA polymerase, so that they can inhibit the growth of the fungus *Candida albicans* (Maisarah et al, 2023).

The anti-fungal mechanism possessed by tannins is its ability to inhibit the synthesis of chitin which is used for the formation of cell walls in fungi and damage cell membranes so that fungal growth is inhibited. Tannins are lipophilic compounds so they are easily related (Komala, 2020).

The results of observations in the well technique showed that the diameter of the inhibition zone formed around the well varied according to the difference in the concentration of shallot root extract given. The concentrations given consecutively are 100% as the highest concentration, and the concentrations of 75%, 50%, 25% and 0% as negative concentrations using the solvent aquades which are neutral compounds. Aquades does not contain toxins or substances that can inhibit the growth of *Candida albicans* fungi.

The diameter of the inhibition zone around the well was measured using a caliper with an accuracy of 0.05 mm. As for the 5 treatment concentrations, the results showed that the inhibition zone was only found at concentrations of 25%, 50%, 75% and 100% concentration. Meanwhile, at a concentration of 0%, no inhibition zone was formed. This means that the negative control of treatment, namely the antimicrobial test using aquades solvents, does not show the presence of an inhibition zone. This reason was put forward by (Toria Sangadji et al., (2022) who stated that aquades (water) are neutral compounds that do not have an effect on bacterial growth or do not have antimicrobial power.

The diameter of the inhibition zone formed varies due to the difference in the concentration of shallot root extract given. The average inhibition zone formed at each concentration was 11.38 mm at 25% concentration, 14.69 mm at 50% concentration, 15.83 mm at 75% concentration, 18.41 mm at 100% concentration. The results obtained can be interpreted that the higher the concentration of the extract given to feed, the wider the inhibitory zone formed. This is in accordance with the results of the study (Alouw, 2022) which argues that the increase in the concentration of the extract indicates the larger the diameter of the inhibition zone formed, because the more active compounds in the extract, the more the ability of the active substance increases, which can inhibit the growth of the fungus.

The average diameter of the inhibition zone formed at concentrations of 25%, 50%, 75% and 100% based on the Clinical & Laboratory Standards Institute, (2012) determines the category of fungal growth inhibition response of antifungal activity, namely susceptible (the state of microbes having sensitivity to antimicrobials) has an inhibition zone diameter of  $\geq 20$  mm, Intermediate (Shift from sensitive to resistant but not yet fully resistant) 15-19 mm, and resistant (does not have sensitivity to antimicrobials) with an inhibition zone diameter of  $\leq 14$  mm.

Based on the determination of the resistance response category, the average inhibition zone diameter of the inhibition zone at the 25% concentration is 11.38 mm inhibiting the growth of fungi in the resistance category, then the concentration of 50% has a diameter of 14.69 mm, which is inhibiting in the resistant category, then the concentration of 75% with the diameter of the inhibition zone of 15.83 mm, which is inhibiting in the intermediate category and the last concentration of 100% with an inhibition zone diameter of 19.41 mm, which is inhibiting fungal growth in the intermediate category. Based on the results of determining the response category of the inhibition zone, local shallot root extract of palu was stated to be able to inhibit the growth of *Candida albicans* fungus in the resistant and intermediate categories

This study was strengthened by the results of the analysis of the Variant Analysis Statistical Test (ANOVA) which showed a calculated F value of 108.03, which was much larger than the F value of Table 3.06 at a significant level of 5%. The F value of the calculation is greater than the F value of the table indicates that the results of this study are in line with the first hypothesis of receiving H1, namely Palu local shallot root extract is effective in inhibiting the growth of *Candida albicans* fungus. The Anova test showed that there was a real statistically significant supply, so it was continued with the BNT test.

Based on the BNT test data presented in Table 4.4, it is known that the difference between treatments for all concentrations tested is greater than the BNT value at the significance level of 0.05, which is 2.131. This means that there is indeed a significant difference in the treatment of significant concentrations and very significant differences from those concentrations.

In particular, the concentration of 100% local shallot root extract in Palu has been shown to be the most effective in inhibiting the growth of *Candida albicans* fungus. This can be seen from the average difference between treatments that is larger at a concentration of 100% compared to other concentrations (25%, 50%, 75%). The difference value of 19.41\*\* at 100% concentration shows a very significant level of effectiveness compared to lower concentrations. This is supported by the research of Agustina et al., (2021) regarding the test of anti-fungal activity of black onion extract on the fungus *Candida albicans* that the best inhibition zone value is at a concentration of 100%.

The use of the BNT test provides strong statistical evidence that a 100% concentration of Palu local shallot root extract is the most effective in inhibiting the growth of *Candida albicans* fungus. This result strengthens the potential of shallot root extract as an effective anti-fungal agent. This means accepting the second hypothesis, which is that there is a minimum concentration of shallot root extract that is most effective in inhibiting the growth of *Candida albicans* fungus.

Research on Palu local shallot root extract that can inhibit the growth of *Candida albicans* fungus, is empirical evidence against the theory that Palu local shallot root extract is effective as an antimicrobial against *Candida albicans* fungus

The learning media produced from this research is in the form of a digital flipbook on "Testing the Anti-Fungal Properties of *Candida albicans* by Utilizing the Roots of Local Shallots in Palu". Digital flipbook is an electronic book that imitates the physical format of a book, this tool makes the learning process more interesting and accessible.

The content of this digital flipbook includes an introduction to the material containing the local onion roots of Palu and the *Candida albicans* mushroom, anti-fungal work procedures, and anti-fungal test results. The digital flipbook is first validated to assess its feasibility as a learning medium by validation experts. The validation was carried out by three teams of experts, content experts, media experts, and design experts (Zainal et al., 2024). In addition to validation by experts, the book was also tested on biology education students who were divided into two groups, a small group consisting of 15 students from the class of 2020, and a large group consisting of 25 students from the classes of 2020, 2021, and 2022. The results of the assessment from the expert team and student groups will be calculated based on the category of percentage eligibility of learning media according to Arikunto & Cepi (2009).

The results of the research on the effectiveness of local shallot root extract in Palu are displayed in the form of a digital flipbook with an overall final result of 87.08% and are included in the category of very feasible based on the category of the percentage of feasibility of learning media according to Arikunto & Cepi (2009).

## CONCLUSION AND SUGGESTION

Based on the research, it can be concluded that local Palu shallot root extract effectively inhibits the growth of *Candida albicans* fungus. The most effective concentration for inhibiting fungal growth is 100%. Additionally, the results of this study are suitable as a learning resource in the form of a digital flipbook, with an average feasibility percentage of 87.08%.

As for suggestions, further tests beyond diffusion are necessary to determine the effectiveness of local Palu shallot root extract in killing *Candida albicans* fungus. Moreover, additional research is needed to identify the percentage of active ingredients contained in the extract.

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