Research Article

Optimization of Ultrasound Assisted Extraction Conditions on Fingerroots *(Boesenbergia rotunda)* Rhizome and its Antioxidant Activity

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ABSTRACT

Fingerroot scientifically known as *Boesenbergia rotunda*, is a widespread herb in Southeast Asia and South Asia. This experiment was conducted to investigate the effect of temperature (40 to 60 °C) and time (30 to 60 min) of the fingerroot extract yield and its antioxidant activity using Ultrasound-Assisted Extraction (UAE). The study compared the performance of UAE with the traditional maceration method in terms of extract yield and antioxidant activity. UAE, recognized as a more advanced and innovative extraction method, demonstrated superior efficiency in terms of being time-saving, cost-effective, and faster than maceration. The optimized UAE conditions were identified at an extraction temperature of 48 °C for 42.1 minutes, resulting in an extract yield of 8.42% and an antioxidant activity of 85.90%. In conclusion, the study affirms that UAE offers a highly effective and efficient approach for extracting valuable compounds from fingerroot, showcasing its potential for applications in various industries.

Keywords: antioxidant activity; *Boesenbergia rotunda*; fingerroot; optimization; ultrasound-assisted extraction

1. Introduction

Fingerroot, scientifically identified as *Boesenbergia rotunda* and belonging to the Zingiberaceae family, is commonly distributed throughout Southeast Asia. Its tubers serve both culinary and traditional medicinal purposes due to their advantageous qualities, notably their antioxidant, anticancer, and antimicrobial properties attributed to phenolic compounds (Lim, 2016). Phenolic compounds, prevalent in various edible plant materials like fruits and vegetables, play a crucial role in conferring health benefits. Extensive research has focused on the phenolics derived from fingerroot, elucidating their antioxidative, anti-aging, antibacterial, and antimutagenic attributes. Notably, fingerroot extract, particularly the phenolic compound

known as panduratin A, has recently exhibited remarkable anti-SARS-CoV-2 activity, surpassing other plant extracts (Kanjanassirirat *et al.*, 2020).

The conventional method of solvent extraction has been extensively utilized for the retrieval of bioactive compounds from natural sources (Rodsamran and Sothornvit, 2019). Nevertheless, this traditional approach presents various drawbacks, including prolonged extraction times leading to diminished yields, heightened energy consumption, safety concerns, environmental risks, and the production of low-quality extracts due to oxidative degradation (Sharmila *et al.*, 2016). To address these challenges and promote more sustainable and efficient extraction processes, alternative technologies such as supercritical fluid extraction, subcritical water extraction, pressurized liquid extraction, ultrasound-assisted extraction, and microwave-assisted extraction have been investigated (Abdul Aziz *et al.*, 2022; Zaini *et al.*, 2022; Rodsamran and Sothornvit 2019).

Ultrasound-assisted extraction (UAE) has proven to be highly effective in reducing extraction time and improving overall efficiency (Ma *et al.*, 2008). This positive impact is attributed to the formation of cavitation bubbles, leading to the rupture of cell membranes, thereby expediting solvent diffusion into the matrix. This phenomenon enhances mass transfer and the release of bioactive compounds (Dash *et al.*, 2021). Additionally, prior studies have demonstrated that integrating ultrasound as an extraction aid contributes to heightened antioxidant activity in the resulting extract (Savic *et al.*, 2022; Wang *et al.*, 2020).

The utilization of UAE has emerged as a contemporary, cost-effective, environmentally friendly, and sustainable alternative to traditional methods for obtaining bioactive compounds from plant sources, resulting in increased yields (Wen *et al.*, 2020; Ranjha *et al.*, 2021). Recent applications of UAE include extracting bioactive compounds from diverse natural sources such as pumpkin peel (Leichtweis *et al.*, 2023), garlic leaves (Shekhar *et al.*, 2023), edible mushrooms (Machado-Carvalho *et al.*, 2023), and raspberries (Teixeira *et al.*, 2023). Through the phenomenon of acoustic cavitation, ultrasound waves induce physical and mechanical changes in cell walls, facilitating the release of bioactive compounds from the plant-based matrix into the medium. This not only increases extraction yields but also contributes to reduced extraction time, energy consumption, and solvent usage (Shekhar *et al.*, 2023).

However, the efficiency of UAE is contingent on several extraction process factors, including the choice of solvent, extraction time, solvent-to-feed ratio, pH, and temperature. Operational variables such as frequency, amplitude, and ultrasonic power also play a crucial role (Teixeira *et al.*, 2023; Irakli *et al.*, 2018). These variables can influence the molecular structures of targeted compounds, leading to variations in their biological properties. Therefore, it is imperative to establish optimal UAE experimental conditions tailored to each specific plant-based material to maximize the recovery of bioactive compounds (Machado-Carvalho *et al.,* 2023). In this study, the effect of temperature and extraction time of UAE were investigated on the extraction of fingerroot rhizome.

2. Materials and Methods

2.1 Sample Preparation

Dried fingerroot was procured from Iman Bidara Company in Perak, Malaysia. The dried sample were ground into different average particle sizes (850 µm, 125 µm and 63 µm). The ground fingerroot powder was then placed in plastic bag and stored in a freezer at a temperature of -20 °C to preserve its freshness

2.2 Chemicals

All the chemicals were purchased by the local supplier. Gallic acid and quercetin were obtained from Sigma-Aldrich (St. Louis, USA). Analytical grade of ethanol (95 %) and methanol, Folin-Ciocalteu, Al_2NO_3, CH_3COOK, and Na_2CO_3 (Atlanta, USA) from Fisher Scientific were used.

2.3 Preliminary Study

Before proceeding with optimization, an initial study was conducted to determine constant parameters. The flow for the preliminary study of the constant parameters were average particle sizes (63 µm, 125 µm, and 850 µm), solvent types (ethanol, methanol, and water), and solid-to-solvent ratios (1:3, 1:6, and 1:9). The experiment employed a consistent 5 g of fingerroot powder. For the preliminary investigation, extraction temperature and time were set at 50 °C for 30 minutes. Subsequently, these selected temperature and time conditions would undergo further refinement in the optimization process.

2.4 Ultrasound-Assisted Extraction (UAE)

UAE was conducted using a Branson 8510 model ultrasonic bath operating at a frequency of 40 KHz. Two parameters viz. extraction temperature (40 °C, 50 °C, 60 °C) and extraction time (30 minutes, 45 minutes, 60 minutes), each with three levels, were tested to determine the optimal conditions. Throughout the extraction process, 5 g of ground fingerroot powder was consistently used. The face-centered-central composite design (FC-CCD) was employed to optimize the UAE conditions, focusing on the extracted yield and antioxidant activity of fingerroot. Table 1 illustrates the experimental design generated by Design Expert software version 13 using FC-CCD, comprising 13 number of runs. The obtained extracts underwent filtration and drying in a cabinet dryer at 40 °C. The resulting dried extract was weighted and then stored in the freezer before proceed to analysis.

Table 1 Experimental design of fingerroot at various temperature and extraction time.

Run	Temperature (°C)	Extraction Time (min)	Extract Yield (%)	Antioxidant Activity (%)
1	50	60	9.43	80.13
2	60	45	9.33	81.25
3	50	45	8.46	88.16
4	50	45	9.20	87.11
5	50	45	7.96	85.15
6	50	30	8.33	86.37
7	40	45	9.08	86.21
8	40	30	5.92	88.12
9	40	60	8.59	87.05
10	60	30	8.90	86.37
11	60	60	9.45	79.19
12	50	45	9.71	83.08
13	50	45	8.92	84.62

In this study, temperature (X_1) and extraction time (X_2) were selected to optimize the extract yield and antioxidant activity of fingerroot through UAE. Subsequently, the response surfaces of these parameters were analyzed using Analysis of Variance (ANOVA). The general second-order polynomial model was expected for the process as shown in the equation (1) below:

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{j=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j}^{n} \beta_{ij} X_i X_j \quad (1)$$

where Y is an investigated response (extract yield and antioxidant activity); β_0 is the constant; β_i, β_{ii}, and β_{ij} are the coefficients of linear, quadratic, and interaction terms, respectively; and X_i and X_j are the independent variables (i.e., temperature and extraction time, respectively).

2.5 Conventional Method of Maceration

For comparative purposes with UAE, maceration extraction was employed in this study. The same solvent used in UAE, methanol, was utilized for the maceration process. Five grams of sample powder were blended with 45 ml of methanol in a sealed glass bottle. Subsequently, the glass bottle was stored at room temperature for three days to facilitate the maceration process.

2.6 Extract Yield

Extract yields of fingerroot using UAE were determined using the equation (2) below based on dry basis:

$$Extract\ Yield\ (\%) = \frac{W_e}{W_s} \times 100\% \quad (2)$$

where W_e is a weight of the yield extracted and W_s is the weight of sample used for the extraction.

2.7 Antioxidant Activity

The antioxidant analysis was carried out using a modified method adapted from Liang *et al.* (2023). A stock solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) was prepared by dissolving 4.0 mg of DPPH in 100 ml of methanol and stored in the dark until use. For the analysis, 1 ml of the fingerroot extract (sample: methanol = 1:50, g/ml; Sample = 0.2 g) was liquefied and mixed with 1 ml of the prepared DPPH solution. The mixture underwent vigorous shaking at room temperature for 30 minutes. Subsequently, the absorbance was measured at 517 nm. This method assesses the fingerroot extract's ability to neutralize DPPH radicals, with a reduction in absorbance indicating the scavenging activity of antioxidants present in the sample. The results derived from this analysis offer insights into the antioxidant potential of the fingerroot extract, providing information on its ability to counteract oxidative stress, as expressed in Equation (3) below:

$$DPPH\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\% \quad (3)$$

where $A_{control}$ is a absorbance of the control or blank sample and A_{sample} is the absorbance of the fingerroot extract.

3. Results and Discussion

3.1 Effect of average particle size, type of solvents, solvent to feed ratio and solvent concentration on the extract yield of fingerroot

The effect of parameters, viz. average particle size (63 µm, 125 µm and 850 µm), type of solvents (ethanol, methanol and water), and solvent to feed ratio (1:3, 1:6 and 1:9) at low, mid and high levels, respectively on UAE extraction is depicted in Figure 1.

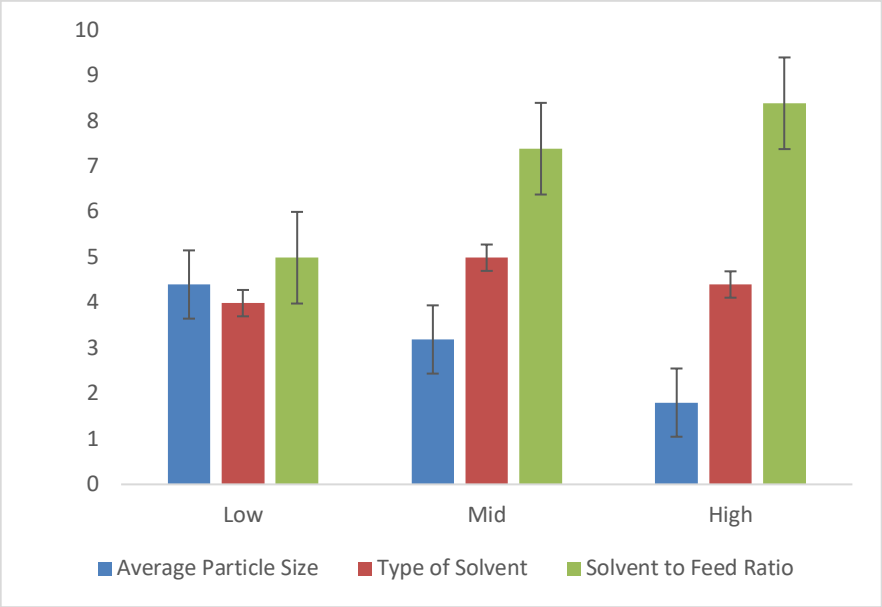

Figure 1 Preliminary studies on average particle size, type of solvent, and solvent to feed ratio.

Various average particle size (63, 125 and 850 µm) was conducted at 50 °C of temperature, 30 min extraction time with water as a solvent and 1:3 of solvent to feed ratio. Figure 1 demonstrates that particles with sizes ≤ 63 µm exhibited the highest yield (4.40%) compared to sizes ≤ 125 µm (3.19%) and ≤ 850 µm (1.80%). Reducing the size of plant substrate before extraction increases surface area, enhancing the effectiveness of extraction (Makanjuola, 2017). For example, grinding samples into powder with sizes smaller than 0.5 mm can impact plant cell walls, facilitating the release of bioactive compounds into the solvent (Wong *et al.,* 2023). As shown in a study by Prasedya *et al.* (2021), reducing the particle size of algal powder enhances the sample's surface area, leading to increased extraction efficiency with solvents and generating significantly higher extract yields. Therefore, particle sizes ≤ 63 µm was used throughout the extraction process.

The choice of solvent for UAE extraction was evaluated among ethanol, methanol and water at 50 °C of temperature, 30 min extraction time, 63 µm particle size and 1:3 of solvent to feed ratio. Figure 1 illustrates that methanol yielded the highest percentage of fingerroot extract about 4.99% followed by water about 4.40% and ethanol about 3.99%. Solvent selection is based on molecular affinity between solvent and solute, where factors viz. surface tension and vapor pressure play a crucial role in UAE (Esclapez *et al.*, 2011). Due to the diverse bioactive compounds in plants and their varying solubility in different solvents, the optimal solvent for extraction depends on the specific plant type and the compounds to be isolated. Fingerroot is particularly rich in bioactive flavonoids. Generally, organic solvents, including methanol, ethanol, acetone, and water, or combinations of these solvents, are commonly employed for extracting flavonoids from plant matrices like herbs (Chaves *et al.*, 2020). It is noteworthy that the solvents used belong to the same polarity group, indicating that compounds in fingerroot exhibit high solubility in methanol.

The influence of the solvent-to-feed ratio on the fingerroot extract yield is also depicted in Figure 1. The highest extract yield obtained at 8.39% was achieved with the highest solvent-to-feed ratio of 1:9, followed by 1:6 at 7.39% and 1:3 at 4.99%, respectively. As noted by Septiani *et al.* (2021), a larger the solid-to-solvent ratio, enhances solvent penetration into the material, thereby increasing the surface area or contact area between solvent and sample matrices. The extensive use of solvent also aids in reducing solvent saturation levels, thereby enhancing extraction efficiency. Conversely, a lower solvent volume results in diminished extract yields due to insufficient solvent support for the transfer process of extracts from the sample matrix.

3.2 Optimization of UAE conditions on the extract yield and antioxidant activity

The preceding section established the constant parameters for the UAE process. Consequently, an average particle size of 63 µm, pure methanol, and a solvent-to-feed ratio of 1:9 were employed for optimizing the temperature and extraction time in the UAE process of fingerroot. In addition, the ultrasound frequency remained constant at 40 kHz. This study presents two responses namely extract yield and antioxidant activity. The optimization was carried out using the face-centered central composite design with 13 number of runs as presented in Table 1. ANOVA was performed based on the results obtained in Table 1, thus a first-order polynomial model adequately expressed all responses for both extract yield and antioxidant activity, as follows:

$$Extact\ yield(\%) = 8.73 + 0.6817X_1 + 0.72X_2 \quad (4)$$

$$Antioxidant\ activity\ (\%) = 84.83 - 2.43X_1 - 2.42X_2 \quad (5)$$

On the other hand, to validate the adequacy of the models, ANOVA with *F*-test was conducted, as detailed in Table 2. The R^2-value for the model regarding for extract yield was 0.5167, and *F*-calculated of 5.344, surpassing the *F*-tabulated value ($F_{2,10,0.05}$) of 4.10. For the antioxidant regression model, R^2-value was 0.6479, and the *F*-calculated was 9.201, representing an acceptable value compared to *F*-tabulated. When the *F*-calculated value exceeds the *F*-tabulated value, the null hypothesis, H_0, is rejected at the significance level, implying that the coefficient estimates are not all zero, and the variation confirms the model's significant (Rizkiyah *et al.,* 2022; Putra *et al.,* 2023).

Table 2 ANOVA table for each response of extract yield and antioxidant activity.

Extract Yield				
	df	Sum of Square Error	Mean Square Error	*F*-value
Regression	2	5.90	2.95	5.344
Residual	10	5.52	0.552	
Total	12	11.42		
Antioxidant Activity				
	df	Sum of Square Error	Mean Square Error	*F*-value
Regression	2	70.37	35.185	9.201
Residual	10	38.24	3.824	
Total	12	108.61		

RSM was employed to investigate the impact of operational parameters viz. temperature and extraction time. Figure 2 illustrates the response surface graph based on the regression models of extract yield and antioxidant activity in equation 4 and 5, respectively. In Figure 2a, the trends of fingerroot extract yield as a function of extraction temperature and extraction time are illustrated. The graph indicates a significant increase in extract yield with the rise in temperature and extraction time. Both temperature and extraction time positively influenced the extract yield of the fingerroot. This allowed the extraction solvent to penetrate the fingerroot powder through diffusion, releasing the extract yield as the sample was directly covered with the solvent. Additionally, the increase in temperature enhanced solvent mass transfer, facilitating the release of compounds from the sample. This improvement was attributed to the fact that elevated temperatures improved the solvent's efficacy, reducing viscosity and surface tension, thus allowing better solvent penetration (Anaya-Esparza *et al.*, 2023). Chan *et al.* (2023) observed similar results, indicating a significant increase in pectin yield from jackfruit rags with an increase in extraction time.

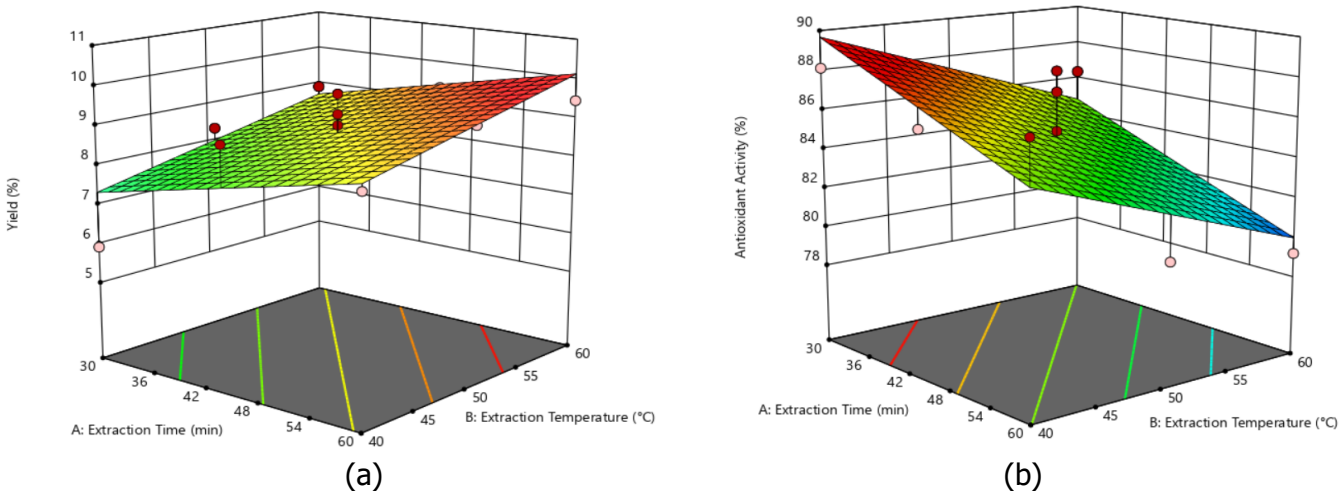

Figure 2 3D response surface graph of UAE on fingerroot at various temperature (40 - 60 °C) and extraction time (30 to 60 min) (a) Extract yield and (b) Antioxidant activity.

On the contrary, Figure 2b depicts the 3D response regarding the antioxidant activity of fingerroot extract. With an increase in temperature and extraction time from 40 to 60 °C and 30 to 60 minutes, respectively, the antioxidant activity of the fingerroot extract decreased. This decline could be attributed to the degradation of some bioactive compounds at high temperatures, and prolonged exposure to elevated temperatures might lead to further degradation. A similar finding was reported by Hashtjin and Abbasi (2015), where high temperatures and extended extraction times resulted in a reduction in the extractability of compounds.

Therefore, multiple optimizations were conducted using the Design Expert software to achieve a high-quality fingerroot extract with maximum extract yield and antioxidant activity. The optimal parameters for UAE extraction of fingerroot were determined to be 48 °C and 42 min, resulting in an extract yield of 8.42% and an antioxidant activity of 85.9%. A validation of the optimum conditions on the extract yield and the antioxidant activity have been conducted. At 48 °C and 42 min of extraction conditions the extract yield and antioxidant activity were obtained at 8.45% and 86.59%, respectively. Therefore, the regression models were significant to explain the behaviour of both responses with 0.36% and 0.80% of error for the extract yield and antioxidant activity, respectively.

3.3 Comparison UAE and Conventional Method of Maceration

A comparison of the quality of fingerroot extract between UAE and the conventional method was conducted. The maceration process, utilizing methanol as the solvent at room temperature for three days, was employed for comparison with UAE. Figure 3 presents the extract yield and antioxidant activity of both extraction processes.

As illustrated in Figure 3, the extract yield obtained by maceration (9.35%) was higher compared to UAE (8.42%). However, the antioxidant activity of fingerroot extract using UAE was higher (85.9%) than maceration (83.69%). The higher extract yield in maceration can be attributed to the longer extraction time compared to UAE. However, at a shorter extraction time of 42 minutes, the higher antioxidant activity was achieved using UAE due to increased solvent power for extracting bioactive compounds in the sample matrices. Additionally, UAE induces cavitation force, leading to the destruction of the cellular matrix and thereby improving the release of active compounds. Consequently, with the assistance of ultrasound frequency, the solvent power of methanol was improved, resulting in a high-quality extract with a high percentage of antioxidant activity. This is supported by a study conducted by Phan *et al.* (2023), where numerous micropores, fissures, and a rough surface were observed in the sample extracted by UAE, while

cracks were visible under scanning electron microscopy (SEM) in the sample extracted by the conventional process.

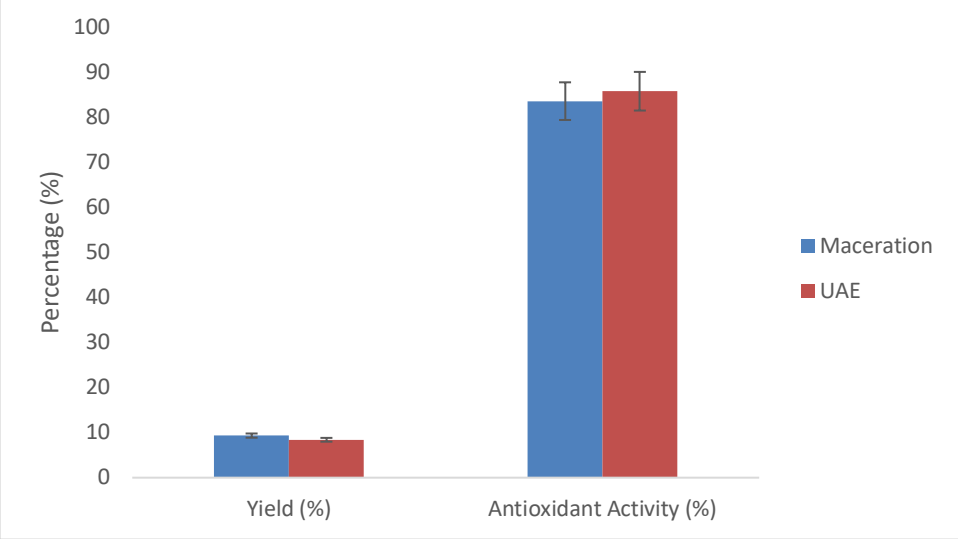

Figure 3 Comparison between conventional extraction of maceration and UAE on the extract yield and antioxidant activity.

4. Conclusion

In summary, this study explored the extraction of antioxidant compounds from fingerroot using UAE optimized through RSM. Compared to traditional maceration, UAE proved to be a more advanced and efficient method, offering advantages in terms of time, cost, and speed. The optimized UAE conditions at 48 °C for 42 minutes yielded an impressive 8.42% extract and demonstrated high antioxidant activity at 85.90%. These findings highlight UAE as a highly effective approach for extracting valuable compounds from fingerroot, with significant potential for applications across diverse industries.

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