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Evaluating the Efficiency of Soxhlet Versus Power Ultrasonic Assisted Soxhlet Extraction in Extracting Phenolic Compounds from Mexican Mint leaves (*Coleus amboinicus*)

Madhuri a/p Srethran[1], Nasrul Fikry Che Pa[1*]

[1] *Department of Chemical Engineering in Biotechnology, Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia, 84600 Pagoh, Johor, Malaysia*

*Corresponding Author: nfikry@uthm.edu.my
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Abstract

Coleus amboinicus or commonly known as Mexican mint is a perennial herb which belongs to the mint family. It is well known for its essential oils that are rich in bioactive phenolic compounds, particularly in carvacrol and thymol. Conventional techniques like Soxhlet extraction require long time extraction and high energy consumption, which can lead to the thermal degradation of heat-sensitive phenols. While ultrasound-assisted extraction is an emerging 'green' technology, there is limited research on optimizing the hybrid Power Ultrasound-Assisted Soxhlet Extraction (PUASE) specifically for maximizing the recovery of phenolic compounds from Mexican mint leaves. This study was conducted to extract phenolic compound from Mexican mint leaves using various ethanol concentrations and to compare the effectiveness of phenolic compound recovery under different parameters, specifically varying ultrasound amplitude and exposure time in PUASE. The research used PUASE with 100%, 50%, and 25% of ethanol, ultrasound amplitudes of 20 %, 30% and 60 %, together with exposure durations of 15, 30 and 45 minutes. The extracts that have been obtained were evaluated for percentage of yield, Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and antioxidant activity. The results demonstrated that pure ethanol at 20% amplitude for 15 minutes was the most efficient condition in achieving the highest yield of 15.79% , a TPC of 379.57 mg GAE/g, and a TFC of 7.69 mg QE/g, with an IC_{50} of 0.347 mg/mL, compared to Soxhlet which only demonstrated percentage of yield of 7.92%, 238.6 mg GAE/g of TPC, 6.63 QE/mg of TFC and IC_{50} of 0.494mg/mL. These findings indicate that moderate ultrasonication effectively disrupts cell walls and improves penetration while reducing thermal degradation. This study concludes that PUASE significantly enhances the quality and quantity of bioactive recovered compared to traditional methods.

1. Introduction

Essential oils are aromatic liquids which are presents in flowers, leaves, stems, seeds and they are extracted through methods like steam distillation, cold pressing, or solvent extraction [1]. They are known for their fragrance, flavor, and therapeutic properties, in which includes antioxidants, antimicrobial, antifungal, and sedative effects [2]. For an example, lavender oil improves sleep quality, orange oil has anxiolytic effects and tea tree oil treats infections [3]. Bioactive compounds such as terpenes, phenols, and flavonoids contribute to these activities and are widely applied in pharmaceutical, cosmetics and food preservations [4]. *Coleus amboinicus* is a herb based plant in mint family in where its rich in phenolic compounds. Conventional Soxhlet extraction is commonly used but it requires long time of heating, which can degrade heat-sensitive phenolics [5]. Green technologies such as ultrasound-assisted extraction (UAE) and Power Ultrasound-Assisted Soxhlet Extraction (PUASE) have emerged to improve extraction efficiency and preserve thermally sensitive compounds [6]. Ultrasound pretreatment enhances solvent penetration, disrupts plant cell walls, and accelerates mass transfer, increasing yield and quality of bioactive compounds. PUASE actually combines these advantages with Soxhlet extraction, allowing repeated solvent circulation through pretreated plant material. Although UAE has been applied for essential oil extraction, no studies have systematically optimized PUASE parameters for maximizing Total Phenolic Content, Total Flavonoid Content, and antioxidant activity. This gaps highlighted the need to investigate the effects of ethanol composition, ultrasonication amplitude, and sonication time on the yield and quality of phenolic compounds in *Coleus amboinicus* [7]. This study seeks to determine how different parameters can influence the recovery of bioactive compounds from *Coleus amboinicus* leaves. This study specifically investigates the effect of ethanol concentration which is pure, 50% and 25% on extraction of yield, total phenolic content, and antioxidant activity. In addition, it examines how varying ultrasound amplitude of 20%, 30%,60% and exposure time of 15, 30 and 45 minutes during the PUASE impact the efficiency of phenolic compound extraction. Finally, the study aims to identify the optimal PUASE conditions that maximize bioactive compound recovery while maintaining antioxidant activity, in comparison to conventional Soxhlet extraction. This study is part of SDG 3 which is about the Good Health and Well-being because it investigates natural compounds with potential antioxidant and antimicrobial activities to help develop health-promoting products, and alleviate the dependency on the synthetic chemicals.

2. Methodology

2.1 Preparation of materials

Coleus amboinicus were collected and washed with distilled water to remove dirt. The samples were then oven dried at 45°C for 24 hours [8]. After the leaves has been completely dried, they were ground into powder using mechanical grinder and stored in a dry container until further use.

2.2 Soxhlet Extraction

A total of 250 ml of 50 % ethanol was added to round-bottom flask. The flask was connected to a Soxhlet extractor and condenser. The grounded material was placed into the thimble, and the solvent was heated at 78 °C for 6 hours [9]. Following the extraction, the extract was filtered and concentrated using the rotary evaporator, and percentage of yield was calculated as in Equation 1. The extract was then stored at 4°C until analysis.

$$Percentage\ of\ Yield\ \% = \frac{weight\ of\ dried\ extract}{weight\ of\ dried\ plant\ sample} \times 100 \quad (1)$$

2.3 Power Ultrasonic Assisted Soxhlet Extraction

The PUASE was conducted at ultrasound amplitudes of 20%,30% and 60% using power ultrasonic with exposure times of 15,30,45 minutes [10].

2.4 Analysis by Total Phenolic Content (TPC)

Folin-Ciocalteu method in where gallic acid used as a standard. 0.5 mL of the sample was reacted with 2.5 mL of 10% Folin-Ciocalteu solution and allowed to stand for 5 min after vortex. Then 2 ml of 7.5% Na_2CO_3 solution added and vortex. They were then allowed to stand for 15 minutes and then absorbance estimated at 765nm [11]. The results then expressed as milligrams of Gallic Acid Equivalent per milliliter.

2.5 Analysis by Total Flavonoid Content (TFC)

The measurement of TFC was determined by the aluminum chloride colorimetric method. Quercetin was used as a standard in this. A volume off 2.5 mL of each sample was mixed with 2.5 ml of 25 aluminum chloride solution, vortexed and incubated for 60 minutes. The absorbance was then measured at 420 nm. The TFC was expressed as milligrams of Quercetin Equivalent per milliliter of extract [12].

2.6 Analysis by Antioxidant Testing using DPPH

The antioxidant activity of each sample was detected using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH). Sample of 0.5 mL was added to 3.5 mL of DPPH solution and incubated for 30 minutes in the dark. The absorbance was measured at 515 nm [12]. The percentage of DPPH inhibited was calculated using Equation 2 ;

$$\%\ DPPH = \frac{Absorbance\ of\ Control - Absorbance\ sample}{Absorbance\ sample} \times 100 \qquad (2)$$

2.7 Statistical Analysis

All the analyses were performed in triplicates and the results were expressed as mean ± standard deviation. All the figures and tables were used to illustrate trends and differences among extraction methods and conditions and Excel was used as software in creating this.

3. Results and Discussion

All the results obtained was tabulated and the graph was created using the values obtained. Discussion was done referring to the graph and literature review to give a better explanation on the results obtained.

3.1 Soxhlet Extraction

All the results obtained through Soxhlet Extraction which is Percentage of Yield, Total Phenolic Content, Total Flavonoid Content and Antioxidant Testing was analyzed and discussed in the following.

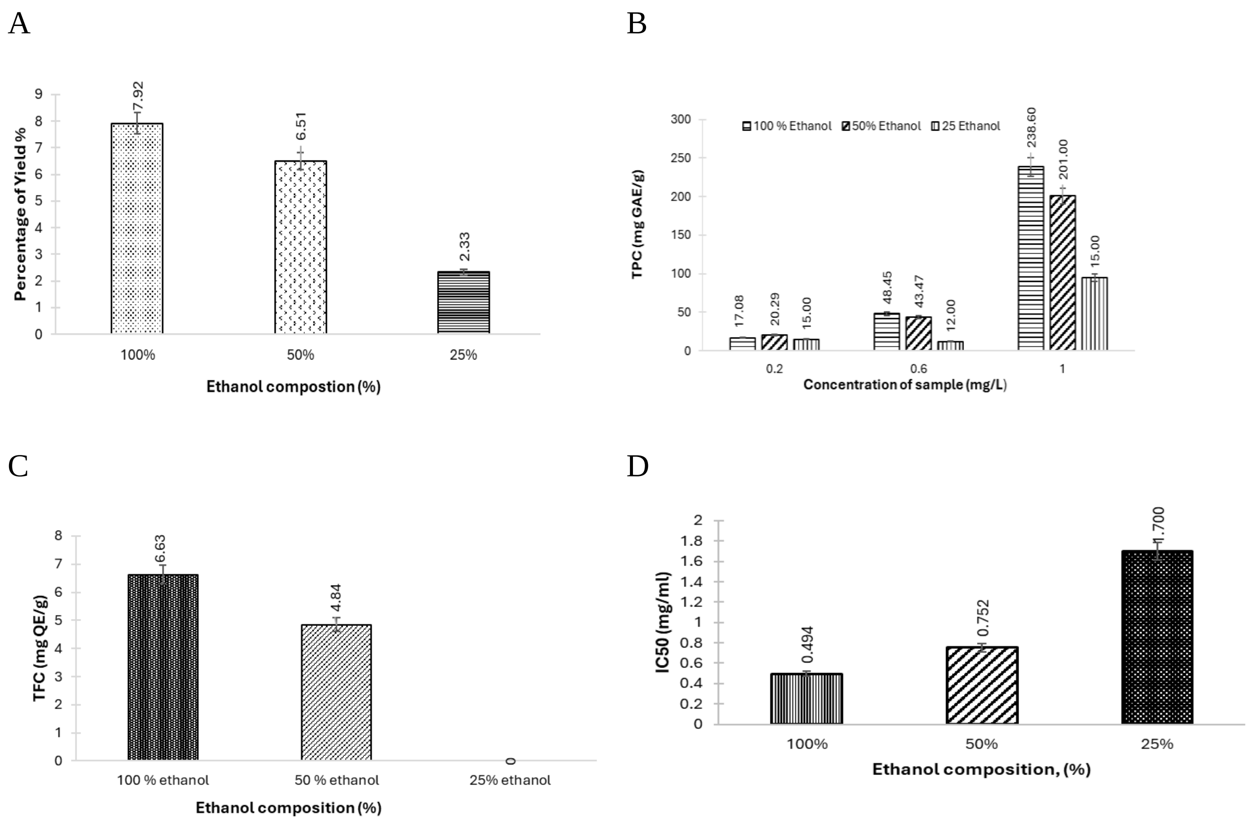

Figure 1 shows Effects of different composition of ethanol on Percentage of Yield (A), Total Phenolic Content (B), Total Flavonoid Content (C), and DPPH IC_{50} (n=3)

Extraction yield of Coleus *amboinicus* mint leaves using the traditional method which is Soxhlet method is shown in Figure 1A. Pure ethanol gave the highest yield which the value is 7.92% compared to 50% ethanol and 25% of ethanol which is 6.51% and 2.33%, in where it highlights the role of solvent polarity in extraction efficiency. Ethanol-rich solvents enhances penetration and mass transfer, on where it facilitates the release of targeted compounds (13). Moreover, the highest TPC value obtained from the pure ethanol as the concentration varies is the highest in where the values is 238.6 GAE/g which is as phenolic compounds interact favorably with ethanol for improved solubility and stability as shown in Figure 1B. Similar trends have been observed in aromatic herbs such as Mentha longifolia and oregano, where ethanol rich solvents extracted higher phenolic and flavonoid contents due to optimized solvent polarity (14). Similarly , in the TFC analysis, pure ethanol has also extracted the highest flavonoid compare to the 50% and 25% ethanol, in which the values are 6.63,4.84 and 0 mg QE/g as in Figure 1C. The value of TFC is very less in this, likely prolonged heating under Soxhlet extraction aids in the release of flavonoids when suitable solvents are used, whereas low ethanol content is insufficient to extract significant flavonoid amount. These trends were reflected in antioxidant activity as well, where higher TPC and TFC corresponded to lower antioxidant values as shown in Figure 1D. Pure ethanol extracts highest antioxidant capacity and then followed by 50% and 25% ethanol, confirming phenolics and flavonoids the main contributors (15).

3.2 Power Ultrasonic Assisted Soxhlet Extraction (PUASE)

Power Ultrasonic Assisted Soxhlet Extraction (PUASE) is a technique where it combine the efficiency of Soxhlet extraction with ultrasonic energy to enhance the extraction process. The application of ultrasonic waves facilitates the disruption of cell walls, improving the mass transfer and extraction rate of bioactive compounds from plant materials [6]. The following section explores the influence of different ultrasonic amplitudes and extraction time on the pure ethanol.

3.2.1 Percentage of Yield

This part investigates the percentage of yield that obtained through PUASE using varying ultrasonic amplitudes and sonication times.

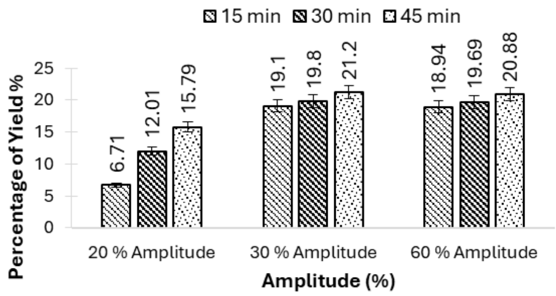

Figure 2 shows effect of different amplitude of ultrasonication on pure ethanol composition in PUASE (n=3)

The effect of ultrasonication amplitude and sonication time on the extraction yield of *Coleus amboinicus* using pure ethanol in PUASE is shown in Figure 2. At 20% amplitude, the obtained yield increased from 6.71% at 15 minutes to 15.79 at 45 minutes , suggesting that the mild ultrasonication enhances solvent penetration and mass transfer by disrupting cell walls [16].Similar observations have been reported in other studies, where prolonged sonication improved extraction yield by promoting cell wall degradation and increasing solute release into the solvent. At 30% amplitude, the highest yield ranges from 19.1-21.2% were obtained through the sonication time, where it indicating moderate ultrasonic intensity actually provides sufficient cavitation energy for effective cell wall breakage while maintaining stable conditions (17). This effect is consistent with previous reports showing that increased ultrasonic amplitude enhances extraction efficiency through stronger cavitation, which disrupts plant cell walls and improves solvent penetration. At the highest amplitude which is 60%, yields plateaued from 18.94 to 20.88% , likely due to the solvent saturation, or structural collapse of the plant matrix [18]. Such trends have been reported, where high ultrasonic power levels led to plateauing or even declining extraction yields as a results of thermal and structural limitations that restrict further solute release.

3.2.2 Total Phenolic Content and Total Flavonoid Content Analysis

This part of the section shows the analysis of both phenolic content (TPC) and total flavonoid content (TFC) in the extracts, evaluated under varying ultrasonic amplitudes and sonication time.

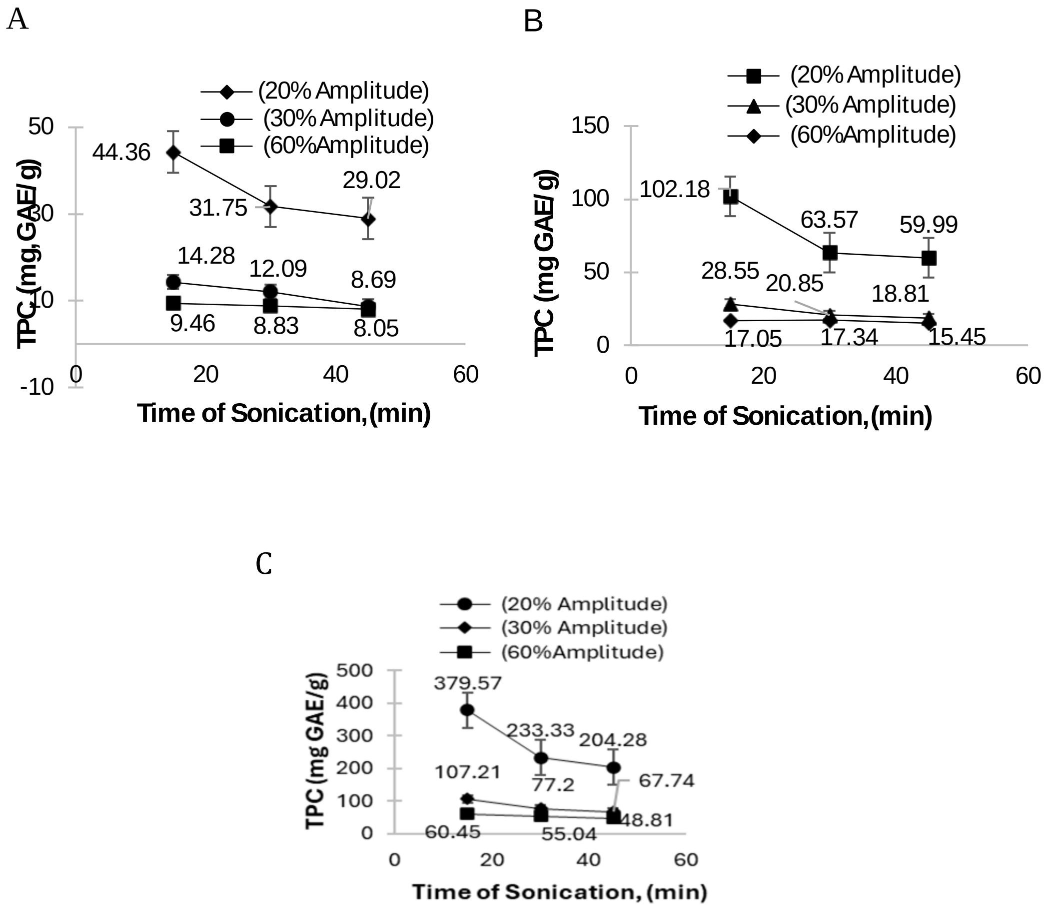

Figure 3 shows Effects of different amplitude of ultrasonication and time of ultrasonication on Total Phenolic Content of Sample Concentration of 0.2 mg/mL (A), Total Phenolic Content of Sample Concentration of 0.6 mg/mL (B), Total Phenolic Content of Sample Concentration of 1.0 mg/mL (C) (n=3)

The effect of ultrasonication amplitude and time on the TPC and TFC of *Coleus amboinicus* leaf extracts is shown in the figure 3. The sample was tested using 3 different concentration which is 0.2, 0.6 and 1 mg/ml. All the concentration sample of 20% amplitude and 15 minutes of exposure time with the reaching 44.36, 102.18 and 379.57 mg GAE/g and TFC 7.68 mg QE/g, in where it suggested that mild ultrasonication successfully disrupts cell walls, enhances solvent penetration and facilitates mass transfer [15].Similar observation have been reported in previous studies, where mild ultrasonic treatment enhanced phenolic extraction efficiency due to cavitation-induced structural disruption of plant matrices. Increasement of the amplitude and sonication time led to significant decline in both the TPC and TFC, likely due to the heat and free radicals from high level of cavitation, which degrade the phenolic and flavonoid compounds [16]. These trends were consistent and agree with the pervious reports which shows that prolonged or very high intensity ultrasonication promotes degradation rather than a improved extraction [19]. To add on, these findings emphasize that although ultrasonication enhances extraction efficiency, excessive energy input adversely affects phenolic stability. In Figure 3B, maximum TPC 102.18 mg GAE/g was achieved at 20% amplitude and 15 minutes at 0.6mg/ml concentration, followed by a significant decline with prolonged sonication. This suggesting that extended sonication promotes degradation rather than further extraction. Figure 3D illustrates the highest TFC was obtained under mild ultrasonic conditions which the value is 7.69 mg QE/g. This indicates that flavonoids better-preserved at lower ultrasonic intensities. The higher the amplitude the lower the TFC extract obtained. This reduction is likely due to the high susceptibility of flavonoids to thermal and oxidate stress generated during the cavitation bubble collapse, which produces localized heat and reactive radicals that promote flavonoid degradation.

3.2.3 Total Flavonoid Content

This section shows the impact of ultrasonic-assisted extraction on the total flavonoid content (TFC) of the plant extract. The disruption of plant cell walls via the cavitation effect enhances the release of flavonoids, which is crucial for improving extraction efficiency.

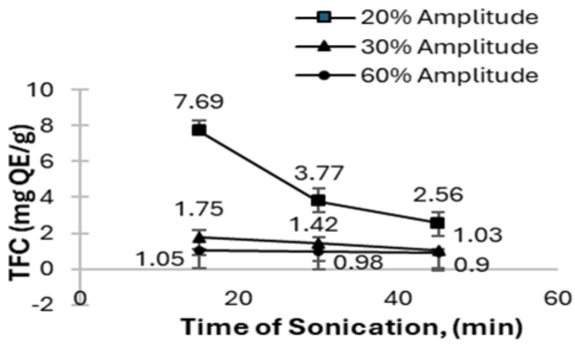

Figure 4.0 Effects of different amplitude of ultrasonication and time of ultrasonication on Total Flavonoid Content (n=3)

Ultrasonic-assisted extraction disrupts plant cell walls through the cavitation effect, which enhances flavonoid release under optimized conditions [6]. For example, *Coleus amboinicus* leaves, the maximum total flavonoid content (TFC) of 7.69 mg QE/g was achieved with 20% amplitude and 15 minutes of sonication, as shown in Figure 4.0.However, optimal sonication time or amplitude of the sonication that exceeds can reduce TFC due to heat and oxidate stress, which can lead to the degradation of flavonoids. In a study on , it was found that high ultrasound intensity or prolonged extraction times can compromise the stability of flavonoid. In contrast, milder ultrasonic conditions promote faster mass transfer, leading to higher flavonoid recovery with minimal degradation[14]. This highlights the importance of optimizing sonication time and amplitude to maximize flavonoid yield without significant losses.

3.2.4 Antioxidant Testing

This section examines the antioxidant activity of the extract, evaluated using the DPPH assay, which measure the free radical scavenging ability of the compounds. The effect of different ultrasonic amplitudes and sonication times on the DPPH IC_{50} values is analysed to determine the optimal conditions for maximizing antioxidant activity.

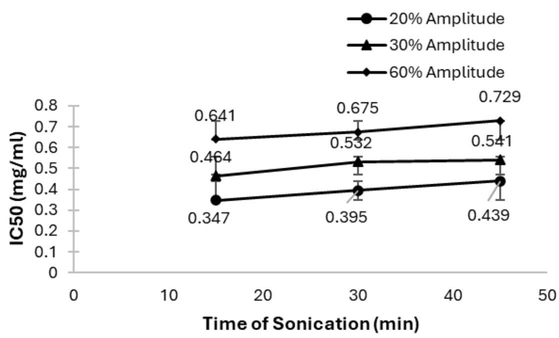

Figure 5 shows Effects of different amplitude of ultrasonication and time of ultrasonication on DPPH IC_{50}

The effect of ultrasonication and time of exposure on the DPPH scavenging activity of *Coleus amboinicus* extracts shown in Figure 5. At 20 % amplitude, IC_{50} increased from 0.345 mg/ml to 0.439 mg/ml at 45 minutes, suggesting that sonication preserves higher antioxidant activity. Moreover, at 30% amplitude, IC_{50} ranges from 0.464 to to 0.541 mg/mL, in which this suggests that moderate ultrasonic decreases the possibility of degradation of bioactive compounds in which consistent with previous studies at where high ultrasonic intensity can reduce antioxidative activity [20]. AT 60% amplitude, IC_{50} eventually increased from 0.641 to 0.729 mg/ml, suggesting that high ultrasonic intensity reduces antioxidant capacity, possibly via heat, or cavitation-induced free radicals as reported in green soybean pods [21].

4. Conclusion

The study clearly demonstrates that Power Ultrasound-Assisted Soxhlet Extraction significantly improves both the quantity and quality of phenolic compounds which has been extracted from *Coleus amboinicus* compared to the Soxhlet extraction. Moderate ultrasonication enhances cell wall disruption and solvent penetration while reducing thermal degradation, making PUASE an efficient and effective method for recovering phenolics, flavonoids and antioxidant compounds from Mexican mint leaves.

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