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# Characterisation of *Maclura cochinchinensis* (Lour.) Corner Trunk Heartwood Extract and its Toxicity Evaluation

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## ABSTRACT

**Introduction:** *Maclura cochinchinensis* is widely used as a natural dye for clothing in Indonesia. Besides, there are some researches about its activities as an antioxidant, antimicrobial, and antidiabetic. However, there is a lack of comprehensive information regarding the standard characteristics and safety of use of its heartwood extract. Therefore, this study aimed to characterize *M. cochinchinensis* heartwood extract and to evaluate its toxicity.

**Method:** To obtain the extract, coarse powder of *M. cochinchinensis* heartwood was macerated using 70% ethanol and evaporated by vacuum rotavapor. Subsequently, phytochemical screening and thin-layer chromatography profiling were carried out, while the toxicity evaluation was conducted using brine shrimp lethality test.

**Results:** The phytochemical screening showed that this extract contained flavonoids, saponins, tannins, steroids, triterpenoids, coumarins, and essential oils. The extract exhibited a dark brown colour, distinct odour, flavourlessness, the value of water-soluble content, ethanol-soluble extract content, loss on drying, moisture content, moisture content, total ash content, acid-insoluble ash content, and water-soluble ash content of 31.44%±1.31, 50.44%±8.48, 9.51%±0.32, 1.62%±0.48, 4.93%±0.27, 1.10%±0.11, 3.81%±0.19, respectively. The residual solvent and heavy metal contamination were undetectable. Microbial contamination was minimal and dominated by a semipolar compound, and brine shrimp assay indicated low toxicity with an $LC_{50}$ value of 174.40 mg/L.

**Conclusion:** *M. cochinchinensis* heartwood extract has good quality and tends to be safe for the environment. This research obtained data that could be used as a supporting evidence for more specific utilization of *M. cochinchinensis* heartwood and its isolated compounds.

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## Introduction

Indonesia has the second-largest biodiversity after Brazil, and among its diverse array of species is *Maclura cochinchinensis*, also referred to as tegeran (synonym, *Cudrania javanensis* Trécul). This plant is commonly used as a natural dye for clothing due to the presence of a flavonoid compound called morin, which is also the major compound of *M. cochinchinensis*. Natural dye from plants, like *M. cochinchinensis*, is used as an alternative to reduce environmental pollution caused by synthetic dye. However, there has been limited attention to the activities of this plant (Darsih et al., 2020). To date, some studies indicated *M. cochinchinensis* heartwood exhibits some biological activities such as antioxidant, antidiabetic, antiinflammation, and anti-hyperuricemia (Chewchinda et al., 2021; Darsih et al., 2020; Sato et al., 2020).

To determine the safety of using *M. cochinchinensis* and the effectiveness of remediation methods for hazardous substances, comprehensive testing is required. Various methods, including the use of brine shrimp (*Artemia salina*), can be used for testing. Brine shrimp (*A. salina*) are widely used in analysis of pesticide residues, anaesthesia, and toxins in the marine environment. The principle behind this testing is to compare the number of shrimp larvae that die when exposed to a solution of the sample compound until the $LC_{50}$ value is obtained (Marzuki et al., 2019; Meyer et al., 1982). Therefore, this study aims to analyse toxicity of *M. cochinchinensis* heartwood extract using Brine Shrimp Lethality Test (BSLT) method in order to provide a comprehensive understanding of the safety of *M. cochinchinensis* wood extract in terms of its potential impact on human health and the environment.

## Methodology

### *Plant Collecting and Determination*

*M. cochinchinensis* plant and the heartwood of the trunk pieces were obtained from Cirebon, Indonesia. The plant was taken near the neighbourhood, since it was not cultivated. Then, it subsequently identified in the Biota Collection Room at Universitas Indonesia on 20 June 2023 with specimen code JI23-P-077 by the Botanical and Zoological Specimen Curator, Alexander Tianara. Then, the *M. cochinchinensis* heartwood was dried in room temperature for 48 hours.

### *Extraction*

The heartwood of the trunk from *M. cochinchinensis* were sorted and ground into a coarse powder. A total of 500 grams of coarse powder were macerated with 5 L of 70% ethanol. The mixture was stirred continuously at 290 rpm during a soaking period for 6 hours, followed by 18 hours of undisturbed soaking at room temperature. The resulting macerate was filtered using a Whatman No. 1 filter paper. The obtained filtrate was then evaporated into a thick extract using a vacuum rotavapor (Heidolph, Germany) at 40°C (Directorate General of Pharmaceutical and Medical Devices, 2017).

### *Phytochemical Screening*

Phytochemical screening of the coarse powder and thick extract of *M. cochinchinensis* heartwood was performed using the Farnsworth method. This screening included testing for the presence of secondary metabolites such as alkaloids, flavonoids, saponins, quinones, tannins, steroids, triterpenoids, coumarins, and essential oils (Farnsworth, 1966).

### *Extract Quality Characterisation*

Characterisation of extracts quality included evaluating various parameters, such as organoleptic properties, water-soluble content, ethanol-soluble content, loss on drying, moisture content, total ash content, acid-insoluble ash content, water-soluble ash content, residual solvent, total plate count (TPC), yeast and mould number (YMN), Pb and Cd metal contamination. The procedures were conducted based on general standard parameters for medicinal plant extract (Directorate General of Pharmaceutical and Medical Devices, 2000).

### *Thin Layer Chromatography (TLC) Profiling*

Silica gel 60 GF254 (Merck, Germany) was used as the stationary phase, cut into 10 cm lengths and 2 cm in width, and then was activated by heating in an oven at 120 °C for 30 minutes. Each chromatographic chamber was saturated with the respective mobile phases of toluene/ethyl formate/formic acid (50/40/10), methanol/chloroform/n-hexane (7/2/1), ethyl acetate/formic acid/water (8/1/1), and chloroform/n-hexane/methanol (5/4/2) using filter paper. The sample solution was prepared by diluting 1 mg of *M. cochinchinensis* extract into 10 mL of methanol, and 5 µl of the sample solution was spotted using a capillary pipette on the TLC plate. After undergoing the elution process in each saturated chamber, the TLC plates were subsequently allowed to aerate at room temperature, and their spots were observed under UV light (CAMAG, Swiss) at a wavelength of 254 and 366 nm (Gwatidzo et al., 2018; Maleš et al., 2004; Poole et al., 2000).

### *BSLT*

#### Hatching Process of *A. salina*

The container used for brine shrimp hatching consisted of two sections, the dark side (hatching compartment) and the light side (illuminated compartment), both filled with synthetic seawater. The eggs of *A. salina* were placed within the hatching compartment, while the opposite area was illuminated using an 18-watt TL lamp. After 24 hours, the newly hatched Artemia nauplii, which had migrated to the illuminated compartment, were isolated into a separate container and left undisturbed for the next 24 hours. Therefore, the tested nauplii were 48 hours old (Meyer et al., 1982).

*Preparation of Tested Sample Solution*

The stock solution of extract was prepared by dissolving 20 mg of *M. cochinchinensis* thick extract in 2 mL of 96% ethanol, resulting in a concentration of 10000 mg/L. This stock solution was used to prepare vials containing concentrations of 1000, 100, and 10 mg/L, each with three replications. In cases of insolubility, 1% DMSO was added to the vials. Control vials were prepared by adding 96% ethanol without extract. Then all of the vials were put in a water bath to dry the solvent (Marzuki et al., 2019; Meyer et al., 1982).

*Data Analysis*

Each vial, free of solvent was filled with 10 nauplii and 5 mL of synthetic seawater. The surviving nauplii were counted after 24 hours. The lethal concentration for 50% mortality after 24 hours of exposure ($LC_{50}$) was established through probit analysis, serving as an indicator of extract toxicity. Then toxicity of extract was classified based on the $LC_{50}$ value obtained (Marzuki et al., 2019; Meyer et al., 1982).

## Results

***Extraction***

Extraction of 500 g coarse powder with a solvent ratio of 1/10 produced 122.1 grams of thick extract, with 24.42% yield and 4.0950 for the DER-native.

***Phytochemical Screening***

The coarse powder and the thick extract of *M. cochinchinensis* heartwood qualitatively contained flavonoids, saponins, tannins, steroids, triterpenoids, coumarins, and essential oils, as shown in Table 1.

***Extract Quality Characterisation***

*M. cochinchinensis* heartwood extract obtained a thick, dark brown colour, distinct odour, and no taste. Table 2 shows the results for *M. cochinchinensis* extract quality characterisation, which had good quality by fulfilling the requirements value of The Indonesian Food and Drug Authority Regulation No. 32 of 2019.

Table 1: Phytochemical screening result.

| Phytochemical Screening | Results | |
|---|---|---|
| | Coarse Powder | Thick Extract |
| Alkaloids | - | - |
| Flavonoids | + | + |
| Saponins | + | + |
| Quinones | - | - |
| Tannins | + | + |
| Steroids | + | + |
| Triterpenoids | + | + |
| Coumarins | + | + |
| Essential oils | + | + |

***TLC Profiling***

The obtained spots indicated the presence of compounds as shown in Figure 1.

***BSLT Analysis***

The result from the 24-hour observation of *A. salina* Leach larvae towards *M. cohinchinensis* heartwood extract obtained the $LC_{50}$ value of 174.40 mg/L, which was in the range of 100–1000 mg/L based on Meyer index showed low toxicity (Marzuki et al., 2019). The $LC_{50}$ value were obtained by an antiLog of the x value from the linear regression equation below as shown in Figure 2.

## Discussion

Using natural sources in the form of extract must meet criteria related to quality, safety, and effectiveness. Maintaining quality of extract is crucial throughout the entire process, starting from the collection of plant materials. This includes accurate plant identification and classification to prevent any inadvertent mixing with other plant species, and this stringent method should continue until the final extract is obtained. The proper identification for medicine plant is also critical for maximizing its efficacy and minimizing potential toxicity adulteration (Klau & Hesturini, 2021; Upton et al., 2020). In this study, extract yield was lower than in the previous investigation, where a yield of 30.2% was achieved using the same method and solvent (Sato et al., 2020). This may be caused by the amount of remaceration carried out, which the more remaceration was performed, the more extract yielded (Siddiq et al., 2022). However, this maceration with a solvent of 70% ethanol was preferable to the other conventional method to extract *M. cochinchinensis*, because it yields more extract than other solvent and 70%

Table 2: Extract quality characterisation results.

| Formulation | Smell | Taste |
|---|---|---|
| **Specific characteristics** | | |
| Water-soluble extract content | 31.44 ± 1.31 | - |
| Ethanol-soluble extract content | 50.44 ± 8.48 | - |
| **Non-specific characteristics** | | |
| Loss on drying (%) | 9.51 ± 0.32[a] | ≤10 |
| Moisture content (%) | 1.62 ± 0.48[a] | ≤10 |
| Total ash content (%) | 4.93 ± 0.27[b] | - |
| Acid-insoluble ash content (%) | 1.10 ± 0.11[a] | - |
| Water-soluble ash content (%) | 3.81 ± 0.19[a] | - |
| Total plate count (CFU/g) | Too few to count | $\leq 1\times10^4$ |
| Yeast and mould number (CFU/g) | Too few to count | $\leq 1\times10^3$ |
| Pb metal contamination (mg/L) | Undetectable | 10 |
| Cd metal contamination (mg/L) | Undetectable | 0.3 |
| Residual solvent (%) | Undetectable | ≤1 |

[a] The data were presented in mean ± SD, n = 3
[b] The data were presented in mean ± SD, n = 6

ethanol extract produces the lowest colour degradation on fabric, which is beneficial for dye as the common use of *M. cochinchinensis* extract in Indonesia (Atika, 2017; Sato et al., 2020).

*M. cochinchinensis* produces colour as a natural dye for clothing due to its secondary metabolites (Darsih et al., 2020). To determine the phytochemical compound contained in the plant, phytochemical screening was conducted. The result of the phytochemical screening of *M. cochinchinensis* showed both the coarse powder and extract did not contain alkaloids, while previous investigation indicated alkaloids in the methanol extract of *M. cochinchinensis* (Swargiary & Ronghang, 2013). The difference in phytochemical screening results can occur according to several factors, such as geographic location and climate, various types of soil, or extraction methods (Farida et al., 2023).

Extraction method is also possibly affecting the safety and stability during storage for a long period of extract obtained. Therefore, extract's quality should be characterized to ensure it is safe and feasible to use. Quality characteristics of extract can be divided into specific and nonspecific categories. The specific characterisation provides information about the plant itself, whereas the non-specific characterisation mostly shows the safety and stability parameters that should be fulfilled (Directorate General of Pharmaceutical and Medical Devices, 2000).

The specific characterisation parameters consist of organoleptic properties to identify extract using human senses and dissolved compounds in certain solvents that provide an initial description of the number of compounds contained. The value of ethanol-soluble content in *M. cochinchinensis* heartwood extract was higher, indicating the plant contained more secondary metabolites dissolved in ethanol solvent. This can happen because ethanol is a solvent that has universal properties, which can attract compounds that are polar, semipolar, and nonpolar. Subsequently, compounds that tend to be extracted in ethanol are saponins, flavonoids, steroids, and triterpenoids, while in water, the presence of saponins and flavonoids (Farida et al., 2023).

The nonspecific characteristics consist of loss on drying, moisture content, total ash content, acid-insoluble ash content, water-soluble ash content, residual solvent, TPC, YMN, Pb, and Cd metal contamination. All the results fulfilled the requirements from Indonesian Food and Drug Authority Regulation No. 32 of 2019 (Indonesian Food and Drug Authority, 2019).

The loss on drying analysis was conducted to establish an upper threshold for the loss of compounds during the drying process. This consists of volatile compounds such as essential oils, thermolabile compounds, and water content within extract. This parameter is measured based on the remaining substance after drying at 105°C until a constant weight is expressed as a percent value. An ideal loss on drying value should be below 10%, as this figure also accounts for the evaporated water content within extract. The moisture content shows the amount of water in extract, which should be controlled since it affects the stability of extract

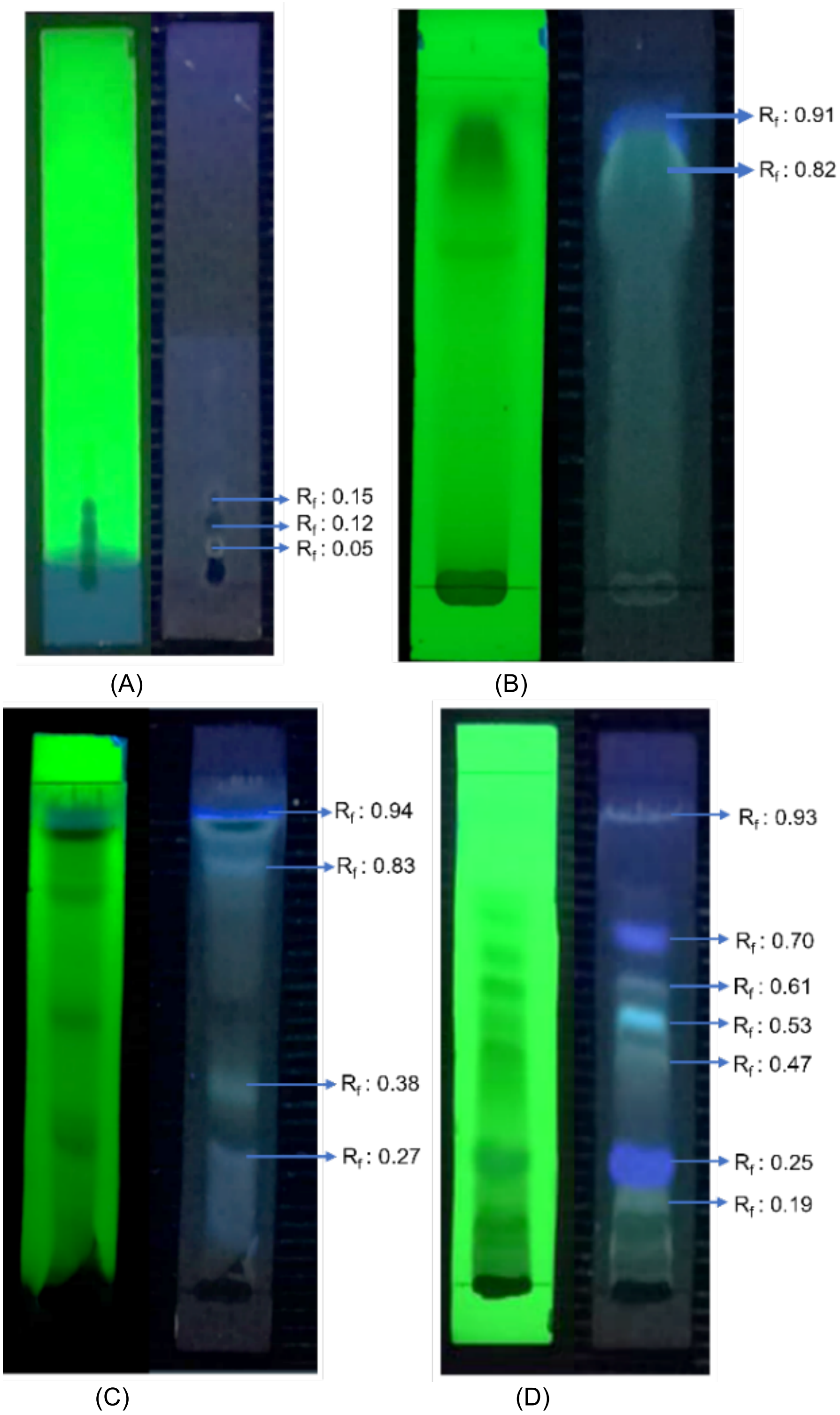


Figure 1: TLC result of *M. cochinchinensis* heartwood extract with mobile phases of (A) toluene/ethyl formate/formic acid (50/40/10) (B) methanol/chloroform/n-hexane (7/2/1) (C) ethyl acetate/formic acid/water (8/1/1) (D) chloroform/n-hexane/methanol (5/4/2)

against microbe contamination. The ash content represents an overview of the internal and external mineral composition from the initial process to extract yield. Additionally, the ash content is determined by the

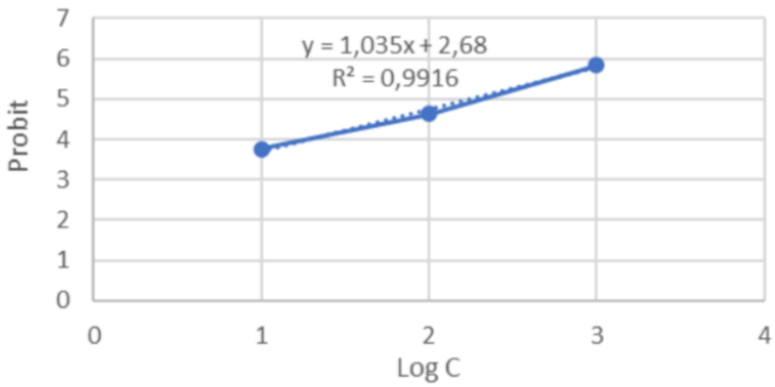


Figure 2: Correlation between log concentration and probit to determine $LC_{50}$

gravimetric method, which has the principle of heating the material to a temperature where the organic compounds and their derivatives are destroyed and evaporated leaving behind only mineral and inorganic elements. The total ash content indicates the amount of physiological and non-physiological ash, while the acid-insoluble ash content indicates the presence of silica and heavy metals such as Pb, Hg, and Cd. No regulation specifies the requirement value for *M. cochinchinensis* heartwood extract, however, it can be assessed by comparing it to similar plants with wood or trunk components. The result of *M. cochinchinensis* heartwood total ash content was a little higher than the average total ash content value of wood extract that are listed in the Indonesian Herbal Medicine Pharmacopoeia Second Edition, namely bidara laut, sanrego, secang, and kuning wood. The microbial contamination test, including YMN and TPC, was carried out to confirm that extract did not contain pathogenic or non-pathogenic microbes that exceeded the established limit because they could affect the stability of extract and be toxic to health. The result showed that *M. cochinchinensis* heartwood extract is safe to be used in pharmaceutical preparations and can be stored for a long time. Subsequently, heavy metal contamination testing was conducted to ensure that extract did not exceed the permissible levels of heavy metals, as these metals pose health risks. Extract that fulfils the requirement for heavy metal contamination can be used as a preparation material as it is considered safe for use. The determination of residual solvent was conducted to ensure that no solvent residues were left during the process, as they should ideally be absent. Extract should be less than 1.0% to be declared safe from residual solvent remaining from the maceration process (Directorate General of Pharmaceutical and Medical Devices, 2000, 2017; Farida et al., 2023).

Chromatogram profiling, an integral part of extract quality characterisation, offers a detailed view of the potential chemical composition (Directorate General of Pharmaceutical and Medical Devices, 2000). Among the four chromatograms produced, only chromatogram (D) has the best quality. Subsequently, the chromatogram is considered of high quality when spots are within the Rf value range of 0.2-0.8, exhibit no tailing, and have symmetrical shapes. This phenomenon can occur because eluents with lower polarity often enhance compound visibility, resulting in favourable Rf (retention factor) values (Wulandari, 2011). The solubility of compounds from each flavonoid group in specific solvents is a crucial factor in selecting eluents to achieve well-separated chromatogram profiles. A diverse group of compounds is indicated in *M. cochinchinensis*, with flavonoids being the most prominent class. Flavonoids are subdivided into various groups based on their polarity, where compound solubility is influenced by hydroxyl and sugar groups binding together. For example, isoflavones, flavanones, and flavonols are relatively less polar, making them more soluble in ether or chloroform. These unknown compound spots can be further identified to determine the specific compounds present in the chromatogram (Medic-Saric et al., 2008). Subsequently, prior to these results, *M. cochinchinensis* extract characterisation lacked scientific investigation, making this study a potential starting point for establishing uncharted parameter requirements.

Toxicology tests were performed to ascertain the degree of toxicity of a chemical. In this study, the BSLT method was used because it is mostly used, cheap, fast, easy, and reliable (Hamidi et al., 2014). The result showed low toxicity, suggesting the safe use of *M. cochinchinensis* heartwood extract in the environment. When used as a dye, its waste is less likely to result in water contamination, thereby reducing potential risks to aquatic ecosystems. This assertion is supported by the study's results, which indicate that *A. salina*, a sensitive environmental stress indicator, exhibited minimal mortality rates. Therefore, the presence of *M. cochinchinensis* heartwood extract is unlikely to have a significant adverse effect on aquatic ecosystems and humans (Hamidi et al., 2014; Meyer et al., 1982). This result is in line with previous investigations that showed low toxicity of chloroform, ethyl acetate, and methanol extract of *M. cochinchinensis* against *A. salina* with the same range of $LC_{50}$ (Sato et al., 2020).

## Conclusion

In conclusion, 70% ethanol extract of *M. cochinchinensis* heartwood showed high quality, primarily containing a dominant compound. Its TLC (Thin-Layer Chromatography) profile indicated semi-polar characteristics. Furthermore, it exhibited low toxicity, as evidenced by an $LC_{50}$ value of 174.40 mg/L, suggesting its relative safety for the environment. As the research on the characteristics and toxicity of 70% ethanol extract of *M. cochinchinensis* heartwood for human utilization with its environmental impact is limited, this study provides valuable data that can serve as supporting evidence for more targeted utilization of *M. cochinchinensis* heartwood and its isolated compounds. Moreover, it is recommended that further research be conducted to explore the secondary metabolite for the targeted use of *M. cochinchinensis.*

## Author Contribution

Conceptualization, E.M. and D.Y.K.; methodology, E.M., D.Y.K., R.D. and D.K.P.; formal analysis, D.Y.K.; resources, E.M., D.Y.K., R.D. and D.K.P.; writing—review and editing, E.M., D.Y.K., R.D. and D.K.P.; supervision, E.M., R.D. and D.K.P; project administration, D.K.P.; funding acquisition, R.D.

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## Conflict of Interest

The authors declare that we do not have any conflicts of interest related to this work.

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