







AN EVALUATION OF PHYSICOCHEMICAL PARAMETERS AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF DATURA METEL- A RESEARCH ARTICLE

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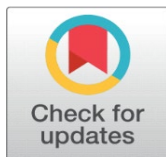
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ABSTRACT

Datura is known as a medicinal plant and plant hallucinogen all over the world. *Datura* is a rich source of alkaloids such as Hyoscyamine, hyoscyne, scopolamine, atropine, with anolides (lactones) and other tropanes. Based on the presence of alkaloids, formulations containing *Datura* exhibit antibacterial, antioxidant, herbicidal, antifungal, antiviral and antiulcer properties. Historically, *Datura* has been employed in the treatment of skin disorders, ear pain, cough, fever, and asthma, among other traditional uses. Qualitative phytochemical test indicates the identification of primary metabolites (Carbohydrate, protein, fat etc.) and secondary metabolites (alkaloids, glycosides tannin etc.). **Aim-** Physicochemical parameters and quantitative phytochemical analysis of *Datura metel*. **Material & Method-** The powdered form of *Datura* seeds was prepared, and its physicochemical or phytochemical testing & standardization were conducted in accordance with established protocols. **Results-** *Datura* was evaluated for different standardization parameters which showed Loss on drying (7.36%), Total ash (7.26%), Acid insoluble ash (3.65%), Water soluble extractive (17.65%), Alcohol soluble extractive (12.25%) and Water-soluble ash (5.19%). Molisch test was positive in aqueous extract. Benedict test was positive in alcoholic extract. Fehling test was positive in aqueous extract. Alkaloids were identified in aqueous extract due to positive of Dragondroff test, Wagner's test, and Hager's test positive in aqueous extract. Amino acid was present in aqueous extract due to show positive result in Ninhydrine test. Proteins were present in test sample due to positive in Biuret test and Xenthoprotic test in aqueous extract. Foam test was positive in aqueous extract. Borntroger's test positive in aqueous extract. Phenolic test was positive in aqueous and alcoholic extract. FeCl₃ test, Pot. Dichromate test was positive in extract of seeds that's indicate that tannin is present in sample. **Conclusion-** This paper highlights organoleptic characters and constituents of *Datura metel* and the data obtained from this study can be utilized to establish standards for *Datura metel*.

Keywords: *Datura*, Antibacterial, Antioxidant, Herbicidal, Antifungal, Antiviral, Antiulcer Activity, Standardization, Physicochemical and Phytochemical

1. INTRODUCTION

Plants possess significant potential for treating and managing various diseases. They have been utilized in different countries to treat diverse conditions. The therapeutic benefits of plants are derived from their bioactive phytochemical components, which exert specific physiological effects on living organisms. In

contemporary times, the choice of medication and method of administration heavily relies on the chemical composition or alkaloids found in the drug. *Datura* holds a prominent position in *Ayurveda*, as its leaves, flowers, seeds, and roots have been utilized for diverse medicinal purposes. It has been employed in the treatment of conditions such as leprosy, rabies, and insanity. However, it's important to note that *Datura* extract is highly toxic, and its misuse can result in delirium and severe poisoning, potentially leading to fatality. Notable active constituents found in *Datura* include scopolamine, atropine, hyoscyamine, with anolides (lactones) and other tropanes. Recent studies have demonstrated that with anolide compounds possess significant antitumor, cytotoxic, anti-inflammatory, antibacterial, hepatoprotective, sedative, cytostatic and immunosuppressive properties. Given the rising demand for medicinal remedies among the population, it is crucial to establish pharmacopeial standards. Analytical research becomes necessary to understand the phytochemical characteristics and active principles of the drug. Before administering the drug to human subjects or conducting experiments, it is essential to thoroughly comprehend and interpret its properties in the context of modern chemistry, in order to establish a solid scientific foundation. The objective of this study is to gather information about the drug, examining its benefits and ensuring its safety. Evaluating the chemical composition aids in the standardization of the drug. *Datura* is one of the most interesting plants with hallucinogenic properties, and despite having this reputation as one of the darker hallucinogens. Throughout history, *Datura* has been extensively utilized by various societies across the globe, spanning both the old world and the new. This usage continues to persist in modern times. Local studies have revealed that different parts of the plant, whether in fresh or sun-dried powdered form, are employed in Southwestern Nigeria for their psychoactive effects. Additionally, literature has indicated that *Datura metel* is among the widely abused local plants worldwide. Report of Drug Abuse in Nigeria by the United Nations Office on Drugs and Crime in 2007, have been shown 0.4% use of *Datura metel* out of the various narcotic and psychotropic substances of use in Nigeria when Cannabis took the largest proportion- 28%. [Kutama et al. \(2010\)](#), [Ganesh et al. \(2015\)](#)

2. MATERIAL AND METHOD

1) Collection of drug

Fresh fruits were collected from herbal garden, Shyampur, Nazibabad road, in the month of June. Fruits were dried in sunlight and seeds were collected for the study.



2) Drug authentication

The sample of raw drug was authenticated by expert of *Dravyaguna* department U.A.U. Rishikul campus, Haridwar.



3) Standardization

The *Datura* seeds underwent standardization following the guidelines outlined in the "Protocol for testing of *Ayurvedic Siddha* and *Unani* Medicines." During the assessment, various parameters were measured, including organoleptic characteristics, loss on drying, total ash, acid-insoluble ash, water-soluble ash, water-soluble extractive, and alcohol-soluble extractive.

4) Chemical and Consumable

Hydrochloric acid, Sodium hydroxide, Potassium ferro cyanide, Glacial acetic acid, Acetic acid, Hydrogen sulphide gas, Sodium nitropruside, Sulphuric acid, Potassium chromate, Toluene, Carbon tetrachloride, Potassium thiocyanate, Potassium iodide, Hypophosphorus, Nitric acid, Ammonia, Lead acetate, Mayer's reagent, Barium chloride, Ferric Chloride, Benzene, Perchloric acid, Sodium sulphide, Acetone, Potassium bismuth- iodide, Sodium potassium tartarate, Vanillin sulphuric acid, Ammonium chloride, Methanol, Ethanol, Chloroform, Millon reagent, Molisch's reagent, Iodine solution, Pyridine, Ninhydrin, Seliwanoff's reagent, Copper sulphate, Picric acid etc.

5) Equipments

Digital balance, Rotary Shaker, Hot air oven, Heating Mantle, Silica Crucible, Hot plate, Grinder, Water bath, Muffle furnace, TLC chamber, Common glass wear, Weighing balance etc.



2.1. METHODS THE API (AYURVEDIC PHARMACOPEIA OF INDIA) (2007)

1) Macroscopic Study/ Organoleptic Parameters:

The obtained sample was subject to organoleptic analysis using naked eyes, a magnifying lens, and a measuring tape. Pharmacognostical parameters, such as appearance, color, odor, taste, size, and shape, were observed and documented as part of the findings.

2) Pharmacognosy/ Quality Control / Microscopic study- Microscopy is a tool sample identification.

- **Powder microscopy**

Powder microscopy inspection is a crucial step in the identification of medicinal plant materials, especially for detecting broken or powdered components. To facilitate the examination, the specimen is treated with appropriate chemical reagents. It should be noted that relying solely on microscopic examination may not always lead to complete identification. However, when combined with other analytical methods, it enhances the accuracy and reliability of the identification process, it often provides valuable supporting evidence. Comparing the sample with a reference material can uncover characteristics not specified in the requirements, which could otherwise be mistaken as foreign matter rather than normal constituents.

- **Procedure**

For a thorough examination of the powder's characteristics, take a suitable quantity of the powder and place it on a slide along with different chemical reagents. Apply gentle heat over a low flame for a short duration. Next, add a small drop of glycerine to the slide, cover it with a cover slip, and observe the sample under a microscope.

The chemical reagents utilized for staining the powder samples were as follows:

- Safranin
- Dilute Ferric chloride
- Eosine
- Methylene blue

3) Physicochemical analysis: Physicochemical study is a vital step in the preparation of any formulation. Physicochemical study is the study of the relations between composition and physical properties of the elements.

- **Determination of Loss on drying at 105°C** [The Ayurvedic Pharmacopeia of India \(2007\)](#)

Moisture content refers to the water holding capacity of a sample, and a higher moisture content indicates a potential decrease in stability.

To determine the moisture content, a weighed sample of 5 grams of the drug was placed in an oven at a temperature of 105°C for a duration of 5 hours. The weight of the sample was recorded every 30 minutes until a constant weight was achieved, indicating no further variation. Afterward, the sample was allowed to cool at room temperature in a desiccator for 1 hour before being weighed.

- **Determination of Total ash** [The Ayurvedic Pharmacopeia of India \(2007\)](#)

Ash analysis is a quantitative technique used to determine the presence of inorganic substances, including silica material, in a sample. Acid Insoluble Ash specifically helps identify siliceous materials and heavy metals. Water Soluble Ash, on the other hand, indicates the quantity of water-soluble inorganic substances present in the sample.

The total ash method measures the combined residue remaining after ignition, including physiological ash derived from plant tissue and non-physiological ash originated from extraneous matter like sand and soil adhering to the plant surface.

A Silica Crucible was thoroughly cleaned, dried, labeled with glass pencils and then weighed until a constant weight was achieved. 5 gms of powdered drug sample were evenly spread in a thin layer inside the Silica crucible. Ash analysis is a quantitative technique used to determine the presence of inorganic substances, including silica material, in a sample. Acid Insoluble Ash specifically helps identify siliceous materials and heavy metals. Water Soluble Ash, on the other hand, indicates the quantity of water-soluble inorganic substances present in the sample. The ash percentage was calculated based on the air-dried drug sample.



- **Determination of Acid insoluble ash** [The Ayurvedic Pharmacopeia of India \(2007\)](#)

Acid insoluble Ash value determined as per Pharmacopoeia of India, 1996. Boiled the total ash with 25 ml of 2M hydrochloric acid for 5 minutes, collected the insoluble matter in a Gooch crucible or on an ashless filter paper. Washed with hot water, ignite, cool in a desiccator and weighed. Calculate the percentage of acid - insoluble ash with reference to the air - dried drug.

- **Determination of Water-soluble ash** [The Ayurvedic Pharmacopeia of India \(2007\)](#)

Water - soluble ash value determined as per Pharmacopoeia of India 1996. Boiled the total ash for 5 minutes with 25 ml of water; collected the insoluble matter in a Gooch's Crucible or on an ash less filter paper, Washed with hot water and ignite for 15 minutes at a temperature not exceeding 450 C. To determine the water-soluble ash, the weight of the insoluble matter was subtracted from the total weight of the ash. The resulting difference in weight represented the water-soluble ash. The percentage of water-soluble ash was then calculated by referencing it to the weight of the air-dried drug.

- **Determination of Alcohol soluble extractive** [The Ayurvedic Pharmacopeia of India \(2007\)](#)

A quantity of 5 grams of coarsely powdered, air-dried drug was macerated with 100 ml of alcohol, of the specified strength, in a closed flask for a duration of twenty-four hours. The mixture was then subjected to continuous shaking for six hours using a rotary shaker and allowed to stand undisturbed for eighteen hours. Afterward, the content was filtered using filter paper, and the resulting filtrate was transferred to a pre-weighed flat-bottomed dish. The filtrate was then evaporated to dryness on a water bath. The dish, containing the residue, was placed in an oven at 105°C until a constant weight was achieved, and it was subsequently weighed. The percentage of alcohol-soluble extractive was calculated by referencing it to the weight of the air-dried drug. The procedure was repeated three times and the mean value was calculated.



- **Determination of Water Soluble Extractive:**

Procedure was same as that of alcohol soluble extractive value and it was proceeded using distilled water instead of alcohol.



3. PHYTOCHEMICAL SCREENING **THE AYURVEDIC PHARMACOPEIA OF INDIA (2007)**

3.1. TESTS FOR CARBOHYDRATES

- 1) Molisch's test
- 2) Benedict's test
- 3) Barfoed's test
- 4) Fehling solution test

1) Molisch's Test: In a test tube, 2 ml of the test solution was added, followed by the addition of 2 ml of Molisch's reagent. The mixture was shaken carefully, and then a small amount of concentrated sulfuric acid (H_2SO_4) was added to the side of the test tube. The test tube was then allowed to stand undisturbed for one minute. If a purple colour ring appeared at the junction of the two layers, it indicated the presence of carbohydrates.

2) Benedict's test: Benedict's solution, mainly composed of copper sulphate and sodium hydroxide, is utilized for detecting reducing sugars. In the procedure, 4 ml of the aqueous solution of the drug was mixed with 1 ml of Benedict's solution and heated close to the boiling point. The appearance of a colour, ranging from green, yellow, orange, red, to brown, indicated the presence of simple sugars in increasing concentrations within the test solution. This colour change is attributed to the formation of cuprous oxide.

3) Barfoed's test: The test sample was dissolved in water and subsequently heated with a small amount of Barfoed's reagent. If a red precipitate of

cuprous oxide formed within two minutes, it indicated the presence of monosaccharides.

- 4) **Fehling solution test:** Fehling's solution is commonly employed for the detection of reducing sugars and consists of two separate solutions that are mixed in situ. Fehling solution A is composed of 0.5% copper sulfate, while Fehling solution B is made up of Sodium Potassium Tartarate.

To conduct the test, equal volumes of Fehling solution A and Fehling solution B (1 ml each) were combined. Subsequently, 2 ml of the aqueous solution of the drug was added to the mixture, followed by boiling on a water bath for a period of 5-10 minutes.

3.2. TESTS FOR ALKALOIDS

- 1) Mayer's reagent test
- 2) Dragon Droff's reagent test
- 3) Wagner's Test
- 4) Hager's Test

- 1) **Mayer's reagent test:** In a test tube, 2 ml of the test solution was combined with 2 ml of Mayer's reagent (Potassium Mercury Iodide solution). The formation of a white or pale-yellow precipitate indicated the presence of alkaloids, except for alkaloids belonging to the Purine groups and a few others.

- 2) **Dragondroff's reagent test:** In a test tube, 2 ml of the test solution was mixed with 2 ml of Dragendorff's reagent (a mixture of Potassium Iodide and Bismuth subnitrate solution). The appearance of an orange precipitate indicated the presence of alkaloids.

- 3) **Wagner's Test:** A few drops of Wagner's reagent (dilute iodine solution) were added to the drug solution. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

- 4) **Hager's Test:** This test involved using a saturated aqueous solution of picric acid. When the test filtrate was treated with Hager's reagent, an orange-yellow precipitate was obtained, indicating the presence of alkaloids.

3.3. TEST FOR AMINO ACIDS

- 1) **Ninhydrin test:** The Ninhydrin test is employed to detect the presence of alpha-amino acids and proteins containing free amino groups. When a protein solution is heated with ninhydrin, it results in the formation of a characteristic deep blue or pale-yellow colour, indicating the complex formation between ninhydrin molecules and the nitrogen of free amino acids.

3.4. TESTS FOR PROTEINS

- 1) Biuret test
- 2) Xanthoprotic test
- 3) Millon's test

- 1) **Biuret test:** A small amount of the residue was dissolved in water, and 1 ml of 4% sodium hydroxide solution was added, followed by a drop of 1%

copper sulphate solution. The development of a violet or pink colour indicated the presence of proteins.

- 2) **Xanthoprotic test:** A small quantity of the test sample was mixed with 2 ml of water, and 0.5 ml of concentrated nitric acid was added. The appearance of a yellow colour indicated the presence of proteins.
- 3) **Millons test:** A small quantity of the test sample was taken and 2 to 3 ml of Millon's reagent was added. The formation of a white precipitate slowly turning to pink indicated the presence of proteins.

3.5. TEST FOR SAPONIN

- 1) **Foam test:** In a test tube, a small quantity of the test sample was vigorously shaken with a small amount of sodium bicarbonate and water. The presence of a stable, characteristic honeycomb-like froth indicated the presence of saponins.

3.6. TEST FOR GLYCOSIDES

- 1) **Borntragar's Test:** To the ethanolic extract, 1 ml of benzene and 0.5 ml of dilute ammonia solution were added. The mixture was observed for the formation of a reddish-pink colour, indicating the presence of glycosides.

3.7. TEST FOR PHENOLIC COMPOUND

The extract was mixed with water and heated, then 2 ml of ferric chloride solution was added, and the formation of green and blue colour was observed, indicating the presence of phenolic compounds.

3.8. TEST FOR FLAVONOIDS

- 1) **Shinods test:** A small quantity of the test sample was dissolved in 5 ml of ethanol (95% v/v) and reacted with a few drops of concentrated hydrochloric acid and 0.5 gm of magnesium metal. The presence of flavonoids was indicated by the appearance of a pink, crimson, or magenta colour within a minute or two.

3.9. TEST FOR STEROIDS

- 1) **Salkoweski reaction:** A few milligrams of the extract were placed in a test tube, and 2 ml of chloroform was added. Then, 2 ml of concentrated sulfuric acid was carefully added to the test tube from the side. The test tube was shaken for a few minutes. The appearance of a red colour indicated the presence of steroids.

3.10. TEST FOR TANNINS

- 1) Ferric chloride solution
 - 2) Lead acetate
 - 3) Pot. Dichromate
- 1) **Ferric chloride solution:** A 5 percent solution of ferric chloride in 90% alcohol was prepared. A few drops of this solution were added to a small

amount of the above filtrate. The appearance of a dark green or deep blue colour indicated the presence of tannins.

- 2) **Lead acetate:** A 10 percent w/v solution of basic lead acetate in distilled water was added to the test filtrate. Development of precipitate indicated the presence of tannins.
- 3) **Pot. Dichromate:** A solution of potassium dichromate was added to the filtrate. The development of a dark colour indicated the presence of tannins.

4. CHROMATOGRAPHY [10]

- **Chromatography plates:** Thin layer chromatography (T.L.C.) plates coated with a 0.25 mm layer of silica gel 60 F₂₅₄ with fluorescent indicator were used. (Each plate had dimensions of 10 cm in length and 2 cm in width)



- **Activation of pre-coated Silica gel 60 F₂₅₄ -**

The Plates were dried in hot oven at 105° C for one and half hours.



- **Test solution: Alcoholic Extract**
- **Preparation of mobile solution: Toluene: Ethyl Acetate: Formic acid (6:3:1)**



- **Visualization:** Iodine Vapours
- **R_f Value:** The distance of each spot from the point of application was measured and recorded. The R_f value was calculated by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

• **Calculation of R_f Value-**

$$R_f = \frac{\text{Distance travelled by solute from origin line}}{\text{Distance travelled by solvent from origin line}}$$

5. OBSERVATIONS AND RESULTS

Organoleptic characters, Physicochemical analysis, Phytochemical screening and Chromatography was done, and results were summarised in Table (1), Table (2), Table (3).

Table 1

Table 1 Showing Result of Organoleptic Characters of Datura Seed Churna		
S. No	Organoleptic	Observation
1	Colour	light brown to yellowish-brown
2	Odour	Odourless
3	Size	0.6 cm long, 0.4 cm wide
4	Shape	Reniform, compressed, flattened, surface finely pitted
5	Taste	Bitter
6	Appearance	thicker towards the curved edge, which is rugose; large, pale strophiole near micropyle

Table 2

Table 2 Showing Results of Physicochemical Characters of Datura Seed Churna			
S. No	Tests	Value	Test method
1.	Loss on drying (%)	7.36	A.P.I, Part I, Vol-III, Appendices- 2.2.10
2.	Aqueous Extractive Value (%)	17.65	A.P.I, Part I, Vol-III, Appendices- 2.2.7.
3.	Alcoholic Extractive Value (%)	12.25	A.P.I, Part I, Vol-III, Appendices- 2.2.6.
4.	Total Ash (%)	7.26	A.P.I, Part I, Vol-III, Appendices- 2.2.3
5.	Acid Insoluble Ash (%)	3.65	A.P.I, Part I, Vol-III, Appendices- 2.2.4
6.	Water Soluble Ash (%)	5.19	A.P.I, Part I, Vol-III, Appendices- 2.2.5
7.	T.L.C.	R _f Value: 0.27, 0.35, 0.41, 0.69, 0.74, 0.89, 0.94	A.P.I

Table 3

Table 3 Showing Results of Phytochemical Screening of Datura Seed Churna		
Name of Test	Aqueous Extract	Alcoholic Extract
Carbohydrate		
Molish test	+ ve	+ ve
Benedict test	-ve	+ ve
Fehling test	+ ve	-ve
Alkaloids		
Dragendorff test	+ ve	-ve

Wagner's test	-ve	+ ve
Hager's test	+ ve	-ve
Amino acids		
Ninhydrine	+ ve	-ve
Protein		
Biuret test	+ ve	-ve
Xanthoprotic test	+ ve	-ve
Saponin		
Foam test	+ ve	-ve
Glycosides		
Borntrager's test	+ ve	-ve
Phenolic compound		
Phenolic test	+ ve	+ ve
Steroids		
Salkowaski	-ve	-ve
Tannins		
FeCl ₃	+ ve	-ve
Lead acetate	-ve	+ ve
Pot. Dichromate	+ ve	-ve


Table 4

Table 4 Showing Results of Microbial Contamination				
S. No.	Microbial contamination	Value	Reference Value	Test method
1.	Total bacterial count	10 ² /g	10 ⁵ /g	A.P.I, Part I, Vol-III, Appendices- 2.4
2.	Total fungal count	10 ¹ /g	10 ³ /g	

Table 5

Table 5 Showing Results of Aflatoxin				
S. No.	Aflatoxin	Value	Reference Value	Test method
1	Aflatoxin B1	Not Detected	0.5 PPB	A.P.I, Part I, Vol-III, Appendices- 2.7
2	Aflatoxin B2	Not Detected	0.1 PPB	
3	Aflatoxin G1	Not Detected	0.5 PPB	
4	Aflatoxin G2	Not Detected	0.1 PPB	

Table 6

Table 6 Showing Results of Thin Layer Chromatography		
TLC image	R _f Value	Test method
	0.27, 0.35, 0.41, 0.69, 0.74, 0.89, 0.94	Stationary Phase: Silica gelG60F254 Mobile solution Toluene: Ethyl Acetate (6:4) Visualization: Iodine Vapours

6. DISCUSSION

The physicochemical analysis of *Datura* seeds was conducted following the standard protocol mentioned in the *Ayurvedic Pharmacopeia of India*. The organoleptic study involved the use of sense organs. The appearance of *Datura* seeds was observed as coarse powder, brown in colour, and odourless. The determination of ash value is a widely used technique for detecting adulteration of inorganic substances, making it crucial for quality control and standardization purposes. The results indicated that *Datura* seeds have a low total ash value, suggesting a minimal amount of inorganic material. The acid-insoluble ash content indicated the presence of silica and silicate impurities, with *Datura* seeds containing 3.65% siliceous content. The extractive values demonstrate the presence of various constituents, and it is possible to develop a TLC fingerprint for identification and semi-quantitative analysis of these extracts. The water-soluble extractive value is a significant parameter in assessing crude drugs. A lower extractive value suggests the presence of depleted material, potential adulteration, incorrect processing during drying or storage, or formulation issues. The water-soluble extractive value of *Datura* seeds was determined to be 17.65%. Similarly, the alcohol-soluble extractive value serves the same purpose as the water-soluble extractive value and indicated that *Datura* seeds have a 12.25% extractive value. Phytochemical screening involved the extraction, screening, and identification of bioactive compounds present in *Datura* seeds, using alcoholic and aqueous extracts. Qualitative analysis revealed the presence of various functional groups, including carbohydrates, alkaloids, amino acids, proteins, saponins, glycosides, phenolic compounds, steroids, and tannins. It is important to note that *Datura* seeds were found to be free from aflatoxins, which can cause illness and liver damage when present in high levels.

7. CONCLUSION

In *Ayurveda*, when plants are utilized with strategic wisdom, it broadens the horizons for humanity, unlocking new possibilities and insights. Every plant bestowed upon us by nature holds the potential to contribute to the well-being of humanity. However, harnessing this potential requires a deep understanding of the physicochemical and phytochemical properties of each plant. The present study provides valuable information regarding the Physicochemical and phytochemical analysis of *Datura* seeds. This data contributes to the standardization of *Datura* seeds and paves the way for further research in the field of *Ayurveda*. It offers opportunities for researchers to explore new avenues and discover cost-effective treatments for various diseases.

CONFLICT OF INTERESTS

None.

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None.

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