Phyto-Physico chemical analysis in Standardization of *siddha* herbo mineral drug *Perungaya Chooranam* (PGC)

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Abstract

Introduction:

The Siddha system of medicine, which began in the Indian subcontinent, is one of the earliest traditions of healthcare that has been codified. Since ancient days the founders of the Siddha system manuscripted so many medicines preparation. Among that, a Herbo-mineral preparation *Perungaya Chooranam* (PGC) mentioned in classical text, which assumed to prescribe for gastritis, GERD. In this globalized world drug standardization and publication consider as a master key to propagate the genuineness. Standardization of PGC was done based on PLIM guidelines would be flashes the essential to the westernization.

Material and methods:

PGC was prepared as per GMP guidelines. Drug Standardization includes discovery of organoleptic properties, Phyto, Physico-chemical analysis, HPTLC, TLC study. According to PLIM guidelines, the study was done in laboratory of tn.mgr mu and Noble research solution.

Results:

Study data shown Loss of drying -2.51%, Total ash value - 19.69%, Acid insoluble ash -2.18%, Water soluble extraction in - 25.71%, Alcohol soluble extraction -14.10%, presence of Alkaloids, carbohydrate, saponin, tannin, flavonoid, gum and mucilage in PGC, illustration of 5 peaks in HPTLC screening.

Conclusion:

The reported results would be supportive for standardization and future clinical studies.

Key word:

Perungaya Chooranam (PGC), Gastro Esophageal Reflux Disease (GERD), pharmacopoeial laboratory for Indian medicine (PLIM), TN. Dr. MGR. Medical university (tn.mgr mu), High Performance thin layer chromatography (HPTLC),

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Introduction

The *Siddha* system of medicine, which began in the Indian subcontinent, is one of the earliest traditions of healthcare that has been codified. The fundamentals and principles largely rely upon 5 element theory, taste and three humours [(1).] It enjoys state patronage as part of the officially recognized Ayush systems and caters to considerable proportion of population through public and private health care facilities. Since ancient days the founders of the *Siddha* system manuscripted so many herbal, mineral, marine & metalic medicine preparation which are now documented and secured in paper form. Among that, a herbo mineral preparation *Perungaya chooranam* was assumed to prescribe for gastritis, GERD, abdominal bloating. In this artificial intelligence world, WHO recognized *Siddha* system of medicine has been undergone the discovery of active components and standardization of study drug based on PLIM guidelines. Standardization of *Siddha* drugs would be flashes the genuineness to the westernization. Standardization of *Perungaya Chooranam* includes many studies such as its organoleptic properties, physical characteristics, phyto-chemical properties, qualitative and quantitative analysis etc.

Material and methods

The study drug *Perungaya Chooranam* herbo mineral preparation was sort out in a long-established text "*Anuboga Vaithiya Deva Ragasiam*[(2)".] The formulation was listed in the Table-1[(4-10)]

Table 1 Ingredients of PGC

S.NO	INGREDIENTS	BOTAMNICAL NAME/CHEMICAL NAME	QUANTITY
1	*PERUNGAYAM*	*Ferula asafoetida*	1 palam(35gms)
2	*SEERAGAM*	*Cuminum cyminum*	1 palam(35gms)
3	*CHUKKU*	*Zingiber officinale*	1 palam(35gms)
4	*KARUNJEERAGAM*	*Nigella sativa*	1 palam(35gms)
5	*MILAGU*	*Piper nigrum*	1 palam(35gms)
6	*THIPPILI*	*Piper longum*	1 palam(35gms)
7	*OMAM*	*Trachysspermum ammi*	1 palam(35gms)
8	*INDHUPPU*	*Sodium chloride/Rock salt*	1 palam(35gms)

Collection, Identification and Authentication of the drug

All raw drugs materials were purchased in a raw drug shop at parry's corner, in Chennai - Tamil Nadu. Those drugs were identified and authenticated by the Botanist (GSMC/MB 572-578)[11] and Gunapadam (Pharmacology) experts in Government Siddha Medical College hospital in Arumbakkam, Chennai – 106.

Purification of the drugs [12-25]

All the drugs mentioned here were purified as per the *Siddha* literature.

- Resin of *Perungayam* (*Ferula asafoetida*) was roaster and powdered well.
- *Indhuppu* (*Sodium chloride impura*) was soaked in *Kaadi* (Vinegar) for 3 days and dried in sunlight.
- Dust particles of *Seeragam* seed *(Cuminum cyminum*) and *Karunjeeragam* seed *(Nigella sativa)* were removed and dried in sunlight.
- One part of *Chukku (Zingiber officinale)* was strewed with two parts of lime stone for 3 hours. After 3 hours, it is washed, dried and the outer skin was exfoliated.
- Seed of *Milagu (Piper nigrum)* was soaked in sour buttermilk and dried in sunlight.
- Seed of *Thippili (Piper longum), Omam (Carum copticum*) were soaked in lemon juice and dried in sunlight.[12-25]

Preparation of the Drug

Procedure

35 grams *(1 palam)* of purified raw drug in table 1-8 were grinded into fine powdered form named as *chooranam* by mortar and pestle and kept in a air tight container. It was labeled as *Perungaya Chooranam* (PGC)[2]. Then the *Chooranam* was purified by steam boiling process based on the Siddha long- established text.[26]

Organoleptic characters

The *Perungaya Chooranam* appeared to be brown in colour with a characteristic salt in taste and had a pleasant odour[27]. The results were tabulated in the Table 2

Table 2: Organoleptic Characters of PGC

S.No	PARAMETER	RESULT
1	Colour	Brown

2	Odour	Pleasant
3	Taste	Salty (alkali) taste
4	Texture	Fine powder
5	Particle size	65.04 ± 27.92 µm.[28]

PHYSICOCHEMICAL ANALYSIS OF *PERUNGAYA CHOORANAM* (PGC)

The preliminary physicochemical screening test was carried out for *Perungaya Chooranam* (PGC) as per the standard procedures mentioned hereunder.[29-32]

1.1 Loss on Drying:

An accurately weighed 1g of *Perungaya Chooranam* (PGC) formulation was taken in a tarred glass bottle. The crude drug was heated at 105°C for 6 hours in an oven till a constant weight. The Percentage moisture content of the sample was calculated with reference to the shade dried material.

1.2 Determination of total ash:

Weighed accurately 2g of *Perungaya Chooranam* (PGC) formulation was added in crucible at a temperature 600°C in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air-dried drug.

1.3 Determination of acid insoluble ash:

Ash above obtained, was boiled for 5min with 25ml of 1M Hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble was calculated with reference to the air-dried drug.

1.4 Determination of water-soluble ash:

Total ash 1g of ash was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15 min at a temperature not exceeding 450°C in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

1.5 Determination of water-soluble Extractive:

5gm of air-dried drug, coarsely powered *Perungaya Chooranam* (PGC) was macerated with 100ml of distilled water in a closed flask for twenty-four hours, shaking frequently. The Solution was filtered and 25 ml of filtrated was evaporated in a tarred flat

bottom shallow dish, further dried at 100°C and weighted. The percentage of water-soluble extractive was calculated with reference to the air-dried drugs.

1.6 Determination of alcohol soluble extractive:

1 gm of air-dried drug coarsely powdered *Perungaya Chooranam* (PGC) was macerated with 20 ml alcohol in closed flask for 24 hrs. With frequent shaking, it was filtered rapidly taking precaution against loss of alcohol 10ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100°C and weighted. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.[29-32]

Table3: Physico-Chemical Analysis of Siddha formulation *Perungaya Chooranam*

S.NO	PARAMETER	PERCENTAGE
1	Loss on drying	2.51%
2	Total ash value	19.96%
3	Acid insoluble ash	2.18%
4	Water soluble ash	15.72%
5	Water soluble extraction	25.71%
6	Alcohol soluble extraction	14.10%

2. PHYTOCHEMICAL SCREENING OF *PERUNGAYA CHOORANAM* (PGC)

The preliminary phytochemical screening test was carried out for *Perungaya Chooranam* (PGC) as per the standard procedures mentioned hereunder.[33]

2.1 Detection of alkaloids:

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

2.1.1 Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colour precipitate indicates the presence of alkaloids.

2.1.2 Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (Potassium Bismuth Iodide). The formation of a red precipitate indicates the presence of alkaloids.

2.1.3 Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

2.2 Detection of carbohydrates:

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

2.2.1 **Molisch's Test:** To 2 ml of plant sample extract, two drops of alcoholic solution of α-naphthol are added. The mixture is shaken well, and a few drops of concentrated sulphuric acid are added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

2.2.2 **Benedict's Test:** Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

2.3 Detection of saponins

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes, it indicates the presence of saponins.

2.4 Detection of phenols Ferric Chloride Test:

Extracts were treated with 3-4 drops of ferric chloride solution. The formation of bluish black color indicates the presence of phenols.

2.5 Detection of tannins Gelatin Test:

The extract is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

2.6 Detection of Flavonoids

2.6.1 Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

2.6.2 Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

2.7 Detection of diterpenes Copper Acetate Test:

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

2.8 Test for Quinones:

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones.

2.9 Gum and Mucilage:

To 1ml of extract add 2.5ml of absolute alcohol and stirring constantly. Then the precipitate was dried in air and examine for its swelling properties. Swelling was observed that will indicate presence of gum and mucilage.

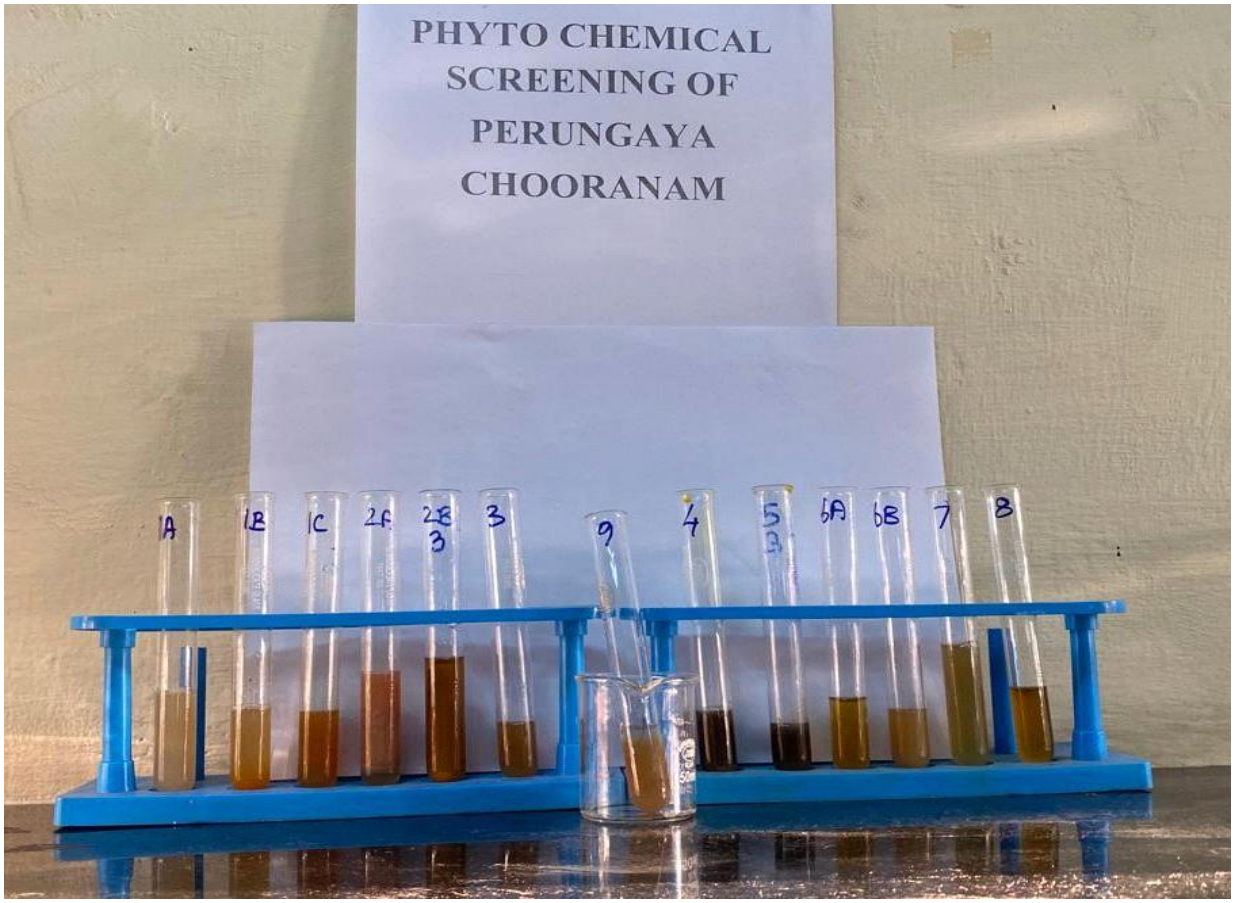
Preliminary phytochemical studies of aqueous extract of *Perungaya Chooranam* (PGC) were done using standard procedures. The results were presented in tables. The present study reveals that the bioactive compounds were present in all the extracts of *Perungaya Chooranam* (PGC)

Table 4: Phytoconstituents analysis of Siddha formulation *Perungaya Chooranam* (PGC)

S.NO	PHYTOCHEMICALS	TEST NAME	H_2O EXTRACT
1	Alkaloids	Mayer's Test Dragendroff's Test Wagner Test	+ve +ve +ve
2	Carbohydrate	Molisch's Test Benedict Test	+ve +ve
3	Saponin	Foam Test	+ve
4	Phenols	Ferric Chloride Test	-ve
5	Tannin	Gelatin Test	+ve
6	Flavonoid	Alkaline Reagent Test Lead acetate	+ve -ve
7	Diterpenes	Copper Acetate Test	-ve
8	Quinones	Test for Quinones	-ve
9	Gum & Mucilage	Test for Gum & Mucilage	+ve

+ve indicate presence and -ve indicate absence

Figure 1: Qualitative Phytochemical Investigation



3. TLC Analysis

The test sample was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipettes were used to spot the sample for TLC applied sample volume 10-micro liter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with the specified solvent system After the run plates are dried and was observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm

4. High Performance Thin Layer Chromatography Analysis

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler were used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers a

high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. Thus, this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phyto-chemicals which is suitable for confirming the identity and purity of Phyto therapeutics.

Chromatogram Development

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analyzed. After elution, plates were taken out of the chamber and dried.

Scanning

Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic fingerprint was developed for the detection of phyto constituents present in each sample and their respective Rf values were tabulated.

Figure 2: TLC Visualization of PNC at 366 nm

Figure 3: 3D – Chromatogram

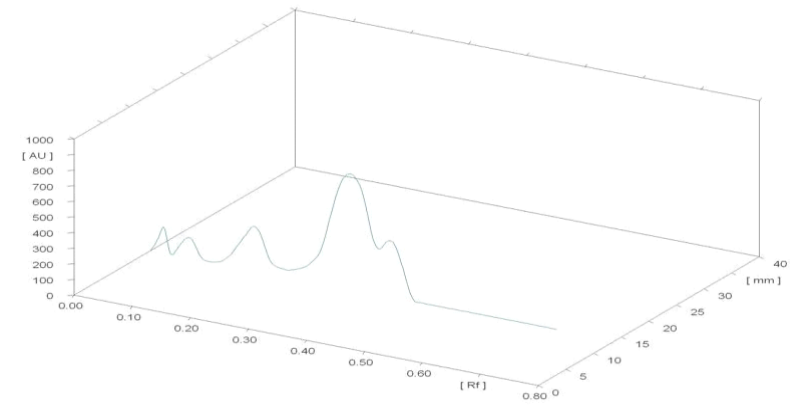
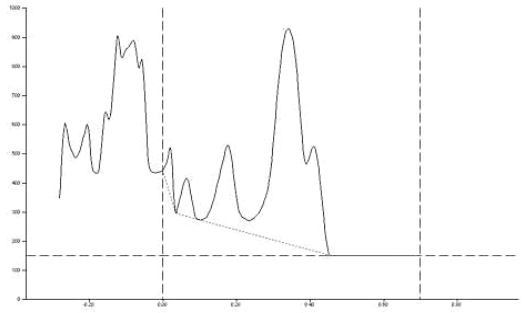
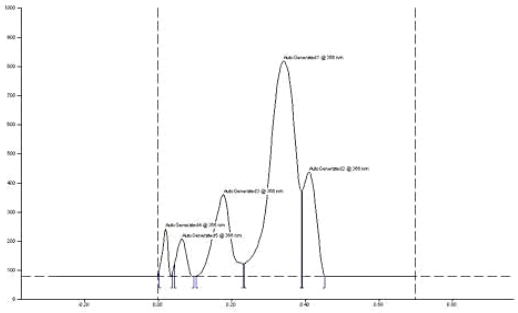


Table 5: Analysis of High Performance Thin Layer Chromatography (HPTLC) of Siddha Formulation *Perungaya Chooranam* (PGC)





Peak Table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.00	14.9	0.02	163.8	9.79	0.04	11.3	1050.2	2.93
2	0.05	44.1	0.07	129.1	7.72	0.10	0.1	1409.2	3.93
3	0.10	0.6	0.18	281.8	16.84	0.23	43.7	5712.6	15.95
4	0.24	44.0	0.34	740.0	44.23	0.39	292.1	22212.3	62.02
5	0.39	293.8	0.41	358.5	21.42	0.46	0.3	5430.0	15.16

Discussion

Siddha system of medicine was contemporary to ancient *Sangam* age in Tamil Nadu India. It's an inevitable system of practice has a multiple source of drugs. Drug standardization is a proper way to disclosure the values about the *Siddha* system to the western education of medicine. Here the study drug *Perungaya Chooranam* was treated for phyto-chemical physiochemical analysis, particle size determination, and TLC, HPTLC study. These studies were conducted in the TN. Dr. MGR Medical University laboratory and Noble Research Solution. The results are sorted out in table (3,4).

A complete combustion of sample gives out the inorganic residues which form the ash value. Ash value of the study drug was19.96%. It includes amount of minerals and other earthy material deposits. The Acid insoluble ash value of PGC was 2.18%. This test denotes the amount of siliceous matter present in the raw drug. The quality of the drug is better if the acid insoluble value is low [(34)]. Water-soluble ash is the part of the total ash content which is soluble in water. It is 15.72% for PGC.(Table 3).

The extraction test is used to measure the amounts of chemical constituents in the raw drug .The percentage of soluble matters present in the drug is determined by the values of water extractive and ethanol extractive[34]. Here water soluble extraction was 25.71% and alcohol soluble extraction was 14.10% in PGC. The total volatile content and moisture present in the drug was accessed in loss on drying. Moisture content of the drug unveils its stability and shelf-life. High moisture content can adversely affect the active ingredient of the drug. It might bring early contamination of the drug. Thus, low moisture content could get maximum stability and better shelf life[34]. The study drug's parameter of loss of drying shown 2.51% (Table 3).

In phyto-chemical screening, the availability of alkaloid in PGC was evaluated by Mayer's test (yellow colour precipitate), Dragendroff's test (Formation of a red precipitate), Wagner's test (Formation of brown/reddish precipitate) indicates the presence of alkaloids qualitatively. Availability of carbohydrates in PGC was evaluated by Molisch's test (A violet ring formation) and Benedict's Test (Orange red precipitate) indicates the presence of reducing sugars qualitatively in PGC. Saponins were detected by Foam test (foam produced & persists for ten minutes), In Ferric Chloride Test proves the absence of phenol. Gelatin Test proves the presence of tannins. Diterpenes, quinones and lead acetate were absent in the PGC which was evaluated by various tests. Alkaline reagent test proved the availability of flavonoids and gum & mucilage also identified in PGC. (Table 4).

Particle size determination was carried out by optical microscopic method. Light microscopic images (app 1/100th dilution) were drawn with scale micrometer to arrive at the average particle size. It reveals that the average particle size of the sample was found to be 65.04 ± 27.92 µm. [28]

TLC and HPTLC were performed. Thin layer chromatography (fig 2,3) was observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm. In HPTLC finger printing analysis of the sample reveals the presence of five prominent peaks corresponds to the presence of five versatile phyto-components present within it. Rf value of the peaks ranges from 0.05 to 0.39.[35,36](Table 5).

Conclusion

The data of phyto-physico chemical analysis, TLC, HPTLC, particle size determination respectively Loss of drying –2.51%, Total ash value in 600°C–19.69%, Acid

insoluble ash-2.18%, Water soluble extraction in 100°C - 25.71%, Alcohol soluble extraction in 100°C–14.10%, presence of Alkaloids, carbohydrate, saponin, tannin, flavonoid, gum and mucilage in PGC, illustration of 5 peaks in HPTLC screening, 1/100 dilution of microscopic particle size evolution. On scrutinizing above data about the *Siddha* herbo-mineral preparation *Perungaya Chooranam* is considered to satisfying the research world to accept the safety and efficacy. In this globalized world physicians depend widely on Proton Pump Inhibitors (PPI) to treat Gastritis in acute, chronic, and pan gastritis. Hence it might cause some short time side effect and cumulative effect likely chronic kidney disease (PPI induced intestinal nephritis), bacterial infection *(Clostridium difficile), H.pylori* infection, development of neuroendocrine tumors, Increased risk of fracture etc,. [(37).] In that case *Perungaya Chooranam* would be a better drug of choice. Though, this drug can be taken to the next level of preclinical and randomized clinical studies to validate the efficacy, pharmacological activities and therapeutic effect.

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