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EVALUATION OF PHYTOCHEMICAL, BIOCHEMICAL AND PIGMENT CONTENT OF SEAWEEDS FROM PONDICHERRY COAST

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ABSTRACT

Edible seaweeds are widely consumed as they provide high nutritional compounds of minerals, fatty acids and free amino acids. In the present study, biochemical, phytochemical and pigment content of eight seaweeds (*Enteromorpha compressa*, *Enteromorpha intestinalis*, *Ulva fasciata*, *Ulva lactuca*, *Chaetomorpha antennina*, *Padina gymnospora*, *Grateloupia lithophila*, and *Hypnea valentiae*) collected from Pondicherry coast were analysed. Brown seaweed *P. gymnospora* showed the presence of all the phytochemicals screened. Maximum total chlorophyll and carotenoid was recorded in green seaweed *U. fasciata* (0.75±0.01 mgg^{-1}) and brown seaweed *P. gymnospora* (0.85±0.01 mgg^{-1}) respectively. Maximum protein and carbohydrate was observed in *P. gymnospora* while lipid in *U. lactuca*. The results clearly showed that brown algae *P. gymnospora* is an alternate food and feed source among the seaweeds analysed.

Keywords: Phytochemical composition, Biochemical composition, Total Chlorophyll, Carotenoid, *Enteromorpha compressa*, *Enteromorpha intestinalis*, *Ulva fasciata*, *Ulva lactuca*, *Chaetomorpha antennina*, *Padina gymnospora*, *Grateloupia lithophila*, *Hypnea valentiae*.

INTRODUCTION

Seaweeds or marine algae are among the oldest members of the plant kingdom. They have little tissue differentiation, no true vascular tissue, no roots, stems or leaves and flowers [1]. Seaweed or sea vegetables are rich in polysaccharides, vitamins, minerals, bioactive substances like polyphenols, proteins, lipids and carotenoid that possess antioxidant, antibacterial, antiviral and other beneficial functions. Marine products are currently of considerable interest in the food and pharmaceutical industries for the development of antioxidants [2].

Seaweeds greatly influence environmental conditions for other types of marine life by providing food, shade protection from waves and as substrate for attachment of other organisms. Seaweeds were one of the first groups of marine organisms whose natural product chemistry was studied extensively because of their abundance in shallow waters. During the past 30 years, marine natural product chemists have reported the discoveries of a large number of novel metabolites with useful pharmacological properties [3]. The majority of the studies on seaweeds occurred after the development of many of the useful mechanism bioassays used today.

Seaweeds have been used as food particularly in Far Eastern countries due to their high content of polysaccharides, minerals and certain vitamins. Seaweeds contain more minerals than any other food due to the surface cell wall polysaccharides that freely and selectively absorb inorganic nutrient from the sea [4]. *Porphyra umbilicalis* (purple laver) is among the most nutritious seaweeds [5]. Furthermore, seaweeds are also used as animal fodder. Algae usually contain single cell protein, which may supplement animal food.

MATERIALS AND METHODS

Sample collection and preparation

Fresh thallus of *Enteromorpha compressa*, *Enteromorpha intestinalis*, *Ulva fasciata*, *Ulva lactuca*, *Chaetomorpha antennina*, *Padina gymnospora*, *Grateloupia lithophila*, and *Hypnea valentiae* were collected from the intertidal regions of Pondicherry coast, India. The seaweeds were washed thoroughly with to remove extraneous materials. Washed samples were shade dried and ground with the help of electric mixer. The seaweed powdered samples were then stored in refrigerator for further use.

Preparation of crude extract

The seaweed powders were extracted with

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acetone in soxhlet extractor for 12 h. The extracts were then concentrated under reduced pressure using a rotary flash evaporator. The crude extracts obtained were stored in dark at 4ºC for further use.

PHYTOCHEMICAL ANALYSIS

Detection of alkaloids

To 2 g of seaweed powder, 1 g of calcium hydroxide and 5 ml of water was added and made into smooth paste and set aside for 5 min. It was then evaporated to dryness in a porcelain dish on a water bath. Alcohol (20 ml of 90%) was added and mixed well, then refluxed for half an hour on a water bath. Then it was filtered and the alcohol was evaporated in a desiccator. To this dilute sulphuric acid was added and tested with various alkaloidal reagents such as

a) **Dragendroff's reagent:** Reddish orange precipitation indicates the presence of alkaloids.

Detection of Sugar

Fehling's test: The aqueous extract of the powdered material was treated with Fehling's solution I and II and heated on a boiling water bath. Reddish brown precipitate was obtained indicating the presence of free reducing sugars.

Detection of Proteins and Amino Acids

Five ml of each extract was dissolved in 5 ml of water and were subjected to the following tests

a) **Biuret test:** One ml of each of the various extract was warmed gently with 10% NaOH solution and a drop of dilute $CuSO_4$ solution. Formation of reddish violet showed the presence of proteins and free amino acids.

b) **Ninhydrin test:** One ml of each of the various extract was treated with a few drops of ninhydrin solution. Change in colour indicated the presence of protein and free amino acids.

Detection of Sterols

The powdered seaweed sample was extracted with petroleum ether and evaporated to a residue. Then the residue was dissolved in chloroform and tested for sterols

a) **Salkowski's test:** To the chloroform solution few drops of conc. H_2SO_4 was added, shaken well and set aside. The chloroform layer turned red colour indicating the presence of sterols.

b) **Libermann-Burchard's test:** To the chloroform solution, a few drops of acetic anhydride and 1 ml of conc. H_2SO_4 were added through the sides of the test tube and set aside for a while. At the junction of two layers a brown ring was formed and the upper layer turned to green colour indicating the presence of sterols.

Detection of Saponins

About 0.5 g of each powdered material was boiled gently for 3 min with 20 ml of water. Filtered while hot and allowed to cool. Filtrate (5 ml) was diluted with water and shaken vigorously. Production of frothing indicated the presence of saponin.

Detection of Coumarins

One ml of each alcoholic extract was treated with alcoholic NaOH solution. Production of dark yellow colour indicated the presence of coumarins.

Detection of Flavanoids

Five ml of each extract was dissolved in 1 ml of alcohol (Stock solution) and then subjected to the following test.

a) **Ferric chloride test:** To one ml of stock alcoholic solution add a few drops of neutral $FeCl_3$ solution. Formation of blackish red colour indicates the presence of flavanoids.

Detection of Phenols

One ml of each extract was dissolved in 5 ml of alcohol with a few drops of neutral $FeCl_3$ solution. Change in colour indicated the presence of phenolic compounds.

Detection of Tannins

Each seaweed powdered sample (5 g) was dissolved in minimum amount of water, filtered and the filtrate was then subjected to the following tests.

a) **Ferric chloride test:** To the above filtrate, a few drops of ferric chloride solution was added. The colour change indicates the presence of tannins.

b) **Basic lead acetate test:** To the filtrate, a few drops of aqueous basic lead acetate solution was added. Formation of reddish brown precipitate indicates the presence of tannins.

Detection of Carboxylic Acids

Each seaweed extract (1 ml) was treated with a few ml of saturated solutions of sodium bicarbonate. Observation of effervescence (due to liberation of CO_2) indicated the presence of carboxylic acids.

Detection of Quinone

Each seaweed extract (1 ml) was treated with alcoholic KOH solution. Quinones give colorations ranging from red to blue.

Detection of Xanthoprotein

Seaweed extract (1 ml) was treated separately with a few drops of conc. HNO_3 and NH_3 solution. Formation of reddish orange precipitation indicated the presence of xanthoproteins.

PIGMENT COMPOSITION

Estimation of Chlorophyll

Amount of chlorophyll present was estimated by the method of Arnon [6]. 500 mg of seaweed sample was kept in a pestle and mortar with 10 ml of 80% acetone and it was ground well and the homogenate was centrifuged at 3000 rpm for 15 min and the supernatant was stored. The pellet was re-extracted with 5 ml of 80% acetone each time till the pellet became colourless. All the extracts were pooled and utilized for chlorophyll determination. Absorbance was measured at 645 nm and 663 nm in a spectrophotometer. The chlorophyll content was determined using the following formula

$$\text{Total Chlorophyll(mg/g.fr.wt.)} = \frac{22.2 \times A645 - 8.02 \times A663}{A \times 1000 \times W} \times V$$

A= Absorbance at respective wave length
V= Volume of extract (ml)
W= Fresh weight of the sample (g)

Estimation of Carotenoids [7]

The same chlorophyll extract was measured at 480 nm in spectrophotometer to estimate the carotenoids content.

Carotenoid: (µg/g.fr.wt.) = ΔA. 480 + (0.114 × ΔA. 663) – (0.638 × ΔA. 645)

ΔA = Absorbance at respective wave length

BIOCHEMICAL COMPOSITION

Estimation of Protein

The protein content was estimated by Biurette method [8]. To 5 mg of dried powdered sample, 1ml of distilled water followed by 4ml of biurette reagent were added and incubated for 30 min in room temperature. Then the mixture was centrifuged for 10 min at 4000 rpm. The supernatant was collected and the optical density was measured in a Spectrophotometer at 540 nm. The protein content was calculated using BSA as standard and expressed as mgg^{-1} protein.

Estimation of Lipid

Lipid content was estimated by chloroform-methanol mixture as described by Folch *et al.* [9]. To 10 mg of dried powder sample, 5 ml of chloroform-methanol (2:1) mixture was added. The mixture was incubated at room temperature for 24 h after closing the mouth of the test tube with aluminium foil. After incubation, the mixture was filtered using a filter paper. The filtrate was collected in a 10 ml pre-weighed beaker, which was kept on a hot plate. The chloroform-methanol mixture was evaporated leaving a residue at the bottom of the beaker. The beaker with the residue and the weight of the empty beaker was calculated to know the weight of the lipid present in the sample. The percentage of lipid present in the sample was calculated by using the following formula.

$$\% \text{ of Lipid} = \frac{\text{Amount of Lipid in the sample}}{\text{Weight of the sample taken}} \times 100$$

Estimation of Carbohydrates

The carbohydrate content was estimated by Dubois method [10]. 20 mg of dried seaweeds powder was taken and to this 1 ml of 4% phenol solution and 5 ml of concentrated sulphuric acid were added. After that, they kept in a dark room for 30 minutes. The colour intensity developed was read in a spectrophotometer at 490 nm. Sugar content was calculated by referring to a standard D-Glucose and the results have been expressed as mgg^{-1} sugar.

RESULTS

Phytochemical screening

The presence of important phytochemicals such as alkaloids, sugars, proteins and amino acids, sterols, saponins, coumarins, flavonoids, phenols, tannins, carboxylic acids, quinones, and xanthoproteins were screened and represented in the Table 1. Sugars, flavanoids and proteins and amino acids were present in all the eight seaweeds screened. Coumarins are present in all seaweeds analysed except *G.* Saponins are present only in *P. gymnospora*, and *H. valentiae*. *P. gymnospora* showed the presence of all the 12 phytochemicals screened. Ulva lactuca also showed the presence of almost all the phytochemicals screened except saponins and carboxylic acids.

Pigment composition

Photosynthetic pigments were estimated and the results were presented in Figure 1. The total chlorophyll ranged from 0.40±0.01 to 0.75±0.01 mgg^{-1} with minimum in red seaweed *H. valentiae* and maximum in green seaweed *U. fasciata*. The carotenoid content ranged from 0.37±0.01 to 0.85±0.01 mgg^{-1} with minimum in green seaweeds *E. compressa*, *U. lactuca*, *C. antennina* and maximum in brown seaweed *P. gymnospora*.

BIOCHEMICAL COMPOSITION

Protein

Quantitative analysis of protein content ranged from 10.6% to 26.3%. Maximum protein content was found in *P. gymnospora* (26.3%) followed by *G. lithophila* (23.3%), *H. valentiae* (22.3%). Minimum content was found in *C. antennina* (10.6%), *E. compressa* (11%), *E. intestinalis* (12%). The results are shown in Figure 2.

Lipid

Lipid content of seaweeds ranged from 1.5% to 2.9% as shown in Figure 3. Maximum lipid content was found in *U. lactuca* (2.9%.) followed by *U. fasciata* (2.5%), *E. intestinalis* (2.2%) and *C. antennina* (2.2%). Minimum content was found in *P. gymnospora* (1.5%), *H. valentiae* (1.6%), and *G. lithophila* (1.7%) respectively.

Carbohydrates

Carbohydrate content of seaweeds ranged from 18.6% to 50%. Maximum carbohydrate content was found in *P. gymnospora* (50%.) followed by *G. lithophila* (41.3%) and *H. valentiae* (40.6%). Minimum content was found in *E. intestinalis* (18.6%), *E. compressa* (21.3%), and *C. antennina* (23.6%) respectively as presented in Figure 4.

Table 1. Preliminary phytochemical screening of the seaweeds from Pondicherry coast

S.No	Seaweeds	Alk	Sug	Pro	Ste	Sap	Cou	Flv	Phe	Tan	Carb	Qui	Xan
1	*Enteromorpha compressa*	+	+	+	-	-	+	+	-	+	-	-	-
2	*Enteromorpha intestinalis*	+	+	+	-	-	+	+	-	+	-	+	-
3	*Ulva fasciata*	-	+	+	+	-	+	+	+	-	+	-	+
4	*Ulva lactuca*	+	+	+	+	-	+	+	+	+	-	+	+
5	*Chaetomorpha antennina*	+	+	+	+	-	+	+	+	-	+	-	+
6	*Padina gymnospora*	+	+	+	+	+	+	+	+	+	+	+	+
7	*Grateloupia lithophila*	-	+	+	+	-	-	+	+	+	-	+	-
8	*Hypnea valentiae*	+	+	+	-	+	+	+	+	-	+	-	-

Alkaloids – **Alk**, Sugar – **Sug**, Proteins and amino acids – **Pro**, Sterols – **Ste**, Saponins – **Sap**, Coumarins – **Cou**, Flavonoids – **Flv**, Phenols – **Phe**, Tannins – **Tan**, Carboxylic acids – **Carb**, Quinones – **Qui**, Xanthoproteins – **Xan**

Figure 1. Pigment content of seaweeds from Pondicherry coast

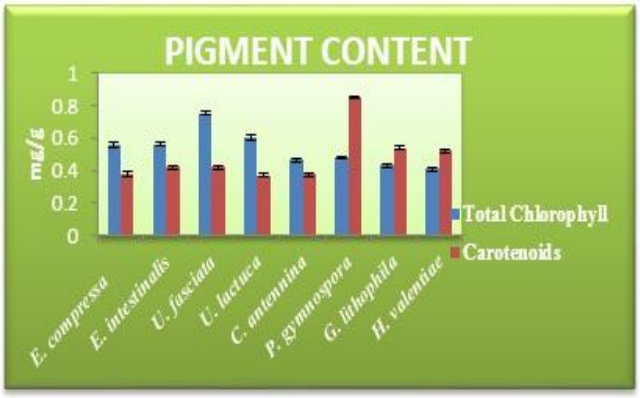


Figure 2. Protein content of seaweeds from Pondicherry coast

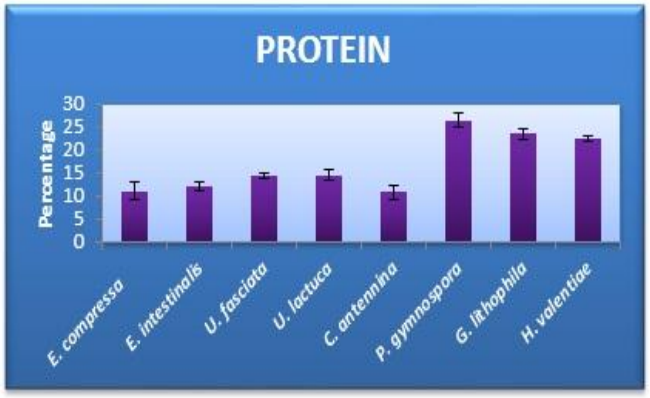


Figure 3. Lipid content of seaweeds from Pondicherry coast

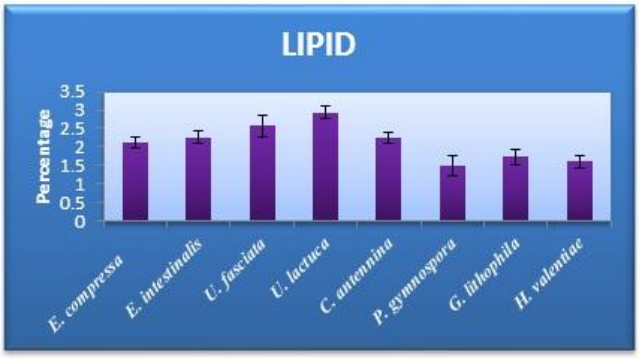
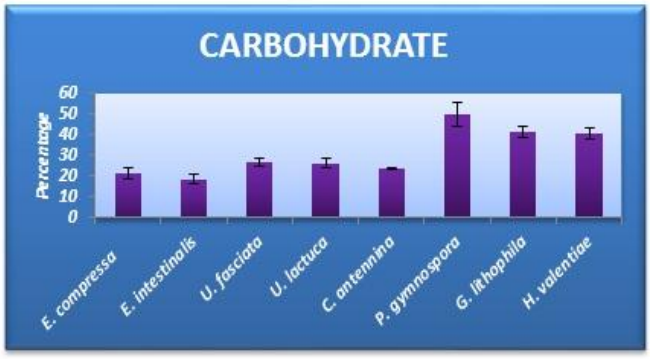


Figure 4. Carbohydrate content of seaweeds from Pondicherry coast



DISCUSSION

The results of the phytochemical analysis revealed the presence of various secondary metabolites with varied degree. Phenolic compounds are widely distributed in the plant kingdom and have been reported to have several biological activities including antioxidant properties [11]. Earlier reports revealed that marine seaweed extracts, especially polyphenols have antioxidant activity [12, 13]. Alkaloids are commonly found to have antimicrobial properties against both Gram-positive and Gram-negative bacteria [14]. Flavonoids are known as nature's tender drug which possesses numerous biological and pharmacological activities. Recent reports of antiviral, anti-fungal, antioxidant, anti-inflammatory, antiallergenic,

antithrombic, anticarcenogenic, hepatoprotective and cytotoxic activities of flavonoids have generated interest in studies of flavonoid containing plants [15, 16]. Steroids may serve as an intermediate for the biosynthesis of downstream secondary natural products and it is believed to be a biosynthetic precursor for cardenolides in plants. Marine algae have shown to be good source of unsaponofiable, non toxic sterols that have medicinal value [17, 18]. Saponins possess numerous biological properties which include antimicrobial, anti-inflammatory, anti-feedent and hemolytic effects [19]. The presence of alkaloids, sugars, proteins and amino acids, sterols, saponins, coumarins, flavonoids, phenols, tannins, carboxylic acids, quinones, and xanthoproteins in *P. gymnospora* suggest that seaweeds can be used as antimicrobial (anti-viral, anti-fungal and anti-bacterial), anti-parasitic, anti-inflammatory, anti-feedent, antioxidant, antiallergenic, anti-thrombic, anti-carcenogenic and anti-ulcer agents in the near future.

The overall observation of the pigment data revealed that maximum amount was recorded in green algae. The maximum total chlorophyll content was recorded in *U. fasciata* of 0.6 ± 0.017 mg/g and minimum in *H. valentiae* of 0.40 ± 0.01 mg/g. The highest carotenoids content was observed in *P. gymnospora* of 0.85 ± 0.01 mg/g and lowest in *E. compressa, U. lactuca* and *C. antennina* of 0.37 ± 0.01 mg/g. The maximum total chlorophyll and carotenoids content were found in green and brown algae respectively. Saranya *et al.* [20] also reported on maximum chlorophyll content observed in green algae and carotenoids in brown algae. Similarly, Sukran *et al.* [21] also reported on maximum pigment content observed in green and brown algae.

Carotenoid detected on seaweed extract could speculate on its antibacterial effect. These compounds are liposoluble tetraterpenes with series of conjugated olefinic bonds constituents [22]. The chromophoric group of carotenoids have been described to give color on food products from yellow to red [23]. Extracted carotenoid potency have been documented in the studies of El-Badrawy and El-Fadaly [24], Nannapaneni *et al.* [25] and Tao *et al*. [26] which showed variable degree of antimicrobial effect. The study of Cucco *et al.* [27], showed that β-carotene an antibacterial immune enzyme that digest bacterial cell walls which could lead to the accumulation of lysozyme, in effect it generates the antibacterial activity. In addition, neoxanthin , fucoxanthin, and violaxanthin have been detected on *P. australis* extract. These are xanthophylls, a subclass of carotenoids consisting of the oxygenated carotenes [28]. Specifically, violaxanthin is a natural constituent found in variety of plants with an orange color. Neoxanthin in plants is an intermediate in the biosynthesis of the plant hormone abscisic acid and produced from violaxanthin by the action of neoxanthin synthase [29]. While fucoxanthin is found as an accessory pigment in the chloroplasts of brown algae like *P. australis* which gave its distinct olive-green color and including other heterokonts [30]. These xanthophylls have been associated with antibacterial activity and could consider on antibacterial property of *P. gymnospora* extract. Similar results have been documented on *Toddalia asiatica* dyes where most of its pigment are xanthophylls which showed its antimicrobial activity on *Bacillus cereus, E. coli, K. pneumoniae* and *Vibrio cholera* [31]. The photosynthetic pigment fucoxanthin on *Fucus vesiculosus* a brown alga appeared to be ecologically relevant as a surface-associated antimicrobial agent, acted against the settlement of bacteria on the surface of the *F. vesiculosus* as documented by Saha *et al.* [32].

Proteins have crucial functions in all the biological processes. Their activities can be described by enzymatic catalysis, transport and storage, mechanical sustentation, growth and cellular differentiation control [33]. Similarly the brown seaweeds have higher protein content than the other two groups of seaweed and the result is very much similar to earlier observation of Wong and Cheung [34]. Marinho-Soriano *et al.* [35] have studied tropical seaweed for their chemical composition and showed that brown algae contain more protein when compared to red algae. In this present study, protein content ranged from 10.6% to 26.3% and maximum protein content was found in *P. gymnospora* as 26.3%. Parthiban *et al.* [36] studied on the biochemical composition of seaweeds from Tuticorin coast and found that maximum protein content was found in the brown seaweed *T. ornata* than red and green seaweeds analyzed. Among the different groups, brown seaweeds exhibited higher values than the other two groups. Dhamotharan [37] investigated on protein contents and found that the highest protein contents were observed in brown algae *Stoechospermum marginatum* (10.6 ± 0.162%). Protein content varied among different genera and also in different species of the same genus [38]. Protein content in the same species but collected from different localities and different seasons also showed fluctuating values [39]. Variations in the protein content of seaweeds can be due to different species and seasonal periods [40, 41].

Lipid content in the present study varied from 1.5 to 2.9%. Lipids are rich in –C=O– bonds, providing much more energy in oxidation processes than other biological compounds. They constitute a convenient storage material for living organisms. The maximum lipid content was found in *U. lactuca* of 2.9% and minimum in *P. gymnospora* of 1.5%. Murugaiyan *et al.* [42] recorded that lipid content varied from 0.9 ± 0.38 % to 3.58 ± 0.45% with maximum lipid content in green algae. Similarly, in this present study also maximum result were observed in green algae. In macroalgae, the lipids are widely distributed, especially in several resistance stages [43]. Seaweeds are relatively low in lipid (1–5% of dry weight) [44, 45].

Total sugar is one of the important components for metabolism and it supplies the energy needed for respiration and other most important processes. Present study showed that the carbohydrate content of seaweeds ranged from 18.6% to 50% with maximum in *P. gymnospora* (50%.) and minimum in *E. intestinalis* (18.6%). Ganga Devi *et al.* [46] found that carbohydrate content of seaweeds was 1.36 to 9.66% and Ganesan and Kannan [47] also reported that carbohydrate content varied from 0.02 to 1.94% for seaweeds of Gulf of Mannar. These reports showed low carbohydrate content than the present study. McDermid and Stuercke [48] reported on carbohydrate of 11.8%, 15.2%, and 16.0% in *Caulerpa*, *Gracilaria* and *Laurencia*, respectively.

Similar range was also noticed in the earlier reports [49 – 51]. As per the earlier reports, seaweeds contain large amount of carbohydrates but less amount of protein and amino acids [44].

CONCLUSION

The results of the present study conclude that seaweeds can provide a dietary alternative due to its nutritional value and its commercial value can be enhanced by improving the quality and expanding the range of seaweed-based products. Further studies focusing on bioactive secondary metabolites may lead to the development of new pharmaceutical agents.

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