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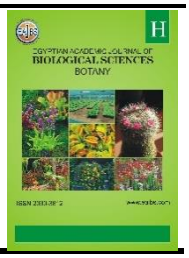

Assessment of Antimicrobial Activity and Antioxidant Properties of Three Brown Seaweeds, *Sargassum polycystum, Turbinaria triquitra* and *Cystoseria myrica*

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ABSTRACT

Within the last few decades, marine organisms have received extensive research attention from a number of scientific organizations due to their potential biological activities including antioxidant and antimicrobial properties. Among the marine species, brown seaweeds have significant role in the synthesis of a variety of bioactive chemicals. The current work was mainly focused on the screening of antimicrobial activity and antioxidant properties as well as the characterization of phenolics and flavonoids in the seaweed methanolic extract (SME) for the three brown macroalgae *Cystoseria myrica*, *Sargassum polycystum* and *Turbinaria triquitra*. The antimicrobial properties were tested against two gram-positive and two gram-negative bacteria as well as *Candida albicans* as a unicellular fungus. The obtained results revealed that all seaweed extracts showed a positive antimicrobial activity against all tested bacteria and yeast. The largest inhibition zones were recorded by the *T. triquetra* against *Staphylococcus aureus* and *Salmonella typhimurium* (2.2 cm) for both, although it had the least inhibitory effect against *Candida albicans*. Significantly, *S. polycystum* recorded the highest phenolics, flavonoids and carotenoids content (1392, 56 and 8 µg /ml) respectively which led to the highest antioxidant capacity. On the other hand, the most effective algal extract against yeast was *C. myrica.* The most dominant detected phenolic compounds in all (SME) were quercetin and apigenin which are known for their high antioxidant activity. In conclusion, all of the tested seaweed extracts exhibited considerable antimicrobial and antioxidant activities.

INTRODUCTION

Recently, marine organisms are considered the dominant among all biosources, as they can produce excellent bioactive metabolites. Except for bacteria, 20,000 compound have been identified from algae and 12,000 compounds have been recorded from other tiny marine plants, animals, and different other sources (Zheng *et al*., 2020). On the other hand, bioactive compounds from marine seaweeds have long attracted the interests of researchers studying various diseases (Hemraj *et al*., 2012). They include several structurally secondary metabolites including carotenoids, polyphenols, flavonoids and terpenoids. In addition, they contain other antioxidant pigments, vitamins, polysaccharides and polyunsaturated fatty acids. The majority of these compounds have more antibacterial and anticancer activities

(Khotimchenko *et al*., 2020). Generally, the compounds produced naturally, if compared with the antibiotics, have better potential, fewer side effects and minimal toxicity (Thanigaivel *et al*., 2015). Recently, many studies recorded the unique bioactive properties of seaweeds (Makhlof *et al*., 2023; Amala Divya *et al*., 2023).

When macroalgae are exposed to biotic and abiotic stresses, such as climate changes or microbial competition, phenolic compounds are produced as metabolites. Based on their source, structure, and function, phenolics are categorised into a variety of categories. Based on their chemical composition, phenolic compounds may be divided into two groups: flavonoids and non-flavonoids. Nearly two-thirds of all known phenolics are flavonoids, which are the most extensively distributed category of phenolics (Laura *et al*., 2019).

One of the earliest naturally occurring pigments is carotenoids. These substances give many fruits and vegetables, flowers, as well as some marine organisms their bright colours(Aryee *et al*., 2018). A broad variety of seaweeds contain carotenoids, the majority of which are brown macroalgae whose main function is to gather and transport light energy to chlorophyll and to aid in photoprotection against harmful photo-oxidation (Aryee *et al*., 2018; Pérez-Gálvez *et al*., 2020). Carotenoids have several bioactive properties, such as antiviral, anticancer, antioxidant, and anti-inflammatory effects. Additionally, these substances have a role in the management of several chronic diseases (Pérez-Gálvez *et al*., 2020; Bohn *et al*., 2021).

Seaweeds can form extremely nutritious dietary components such as vitamins, minerals, proteins, amines,peptides, lipids, fiber, steroids, and fatty acids. Apart from being used to make medications, seaweeds are stable food sources and the most popular edible macroalga in South-East Asia and a few European nations (Sangeetha *et al*., 2010). Seaweeds have traditionally been a part of Chinese, Korean, and Japanese diets. Due to its numerous health advantages, brown and red seaweeds are currently consumed by humans. Seaweed's biological attributes include antimutagenic (Osuna-Ruiz *et al*., 2016), antidiabetic (Jia *et al*., 2020), antiobesity (Sun *et al*., 2020), and anti-inflammatory prperties (Eom *et al*., 2020). Besides, marine nutraceuticals are also known to exhibit neuroprotective effect (Olasehinde *et al*., 2019).

This study aims to evaluate the antimicrobial activities of three seaweed methanolic extracts against different gram-positive and gram-negative bacteria as well as a unicellular fungus. The study extends to evaluate the seaweed's antioxidant activity and to identify the dominant phenolic compounds of the potent seaweed extracts by HPLC.

MATERIALS AND METHODS

1-Seaweeds Collection and Preparation of Methanolic Extracts:

The seaweeds, *C. myrica, S. polycystum, and T. triquitra* were collected in November 2020 from Jizan city's coastline region (about 16°49'20.8"N, 42°37'17.0"E), Red Sea, Kingdom of Saudi Arabia. The seaweeds were rinsed on-site with seawater, then with tap water three times and finally with bottled drinking water and identified morphologically according to (Guiry, 2010). The seaweeds were shade dried for 7 days at 40 °C. The dry algal biomass was cut, milled and sieved using 2 mm mesh and stored for future work.

For the preparation of SME, 3 gm of milled algal dry biomass was mixed with 30 ml 99% methanol and kept in a water bath at 35℃ for 12 hours, then the mixture was centrifuged for 5 minutes at 4000 rpm. For the antimicrobial activity test, 10 ml of the obtained extract was evaporated to 5 ml and the same volume was evaporated to 0.5 ml for HPLC analysis.

2.Antimicrobial Activity Test:

Antimicrobial activity was tested using the agar-well diffusion technique (El-Sheekh *et al*., 2014). Gram +ve bacteria; *Listeria monocytogenes, Staphylococcus aureus*, Gram -ve

bacteria; *Escherichia coli*, *Salmonella typhimurium*, and a yeast*; Candida albicans*, were used as test organisms. To allow the active compounds to diffuse through the pre-inoculated media, plates were placed in the refrigerator for six hours. After a 24-hour incubation period at 30 ± 2° C, the inhibition zones were measured in (cm).

3. Qualitative Analysis of Natural Products in (SME):

The phytochemical screening of seaweed methanolic extract (SME) by the standard method as explained by (Savithramma *et. al*., 2011). Phytochemical screening was carried out to identify the major natural chemical groups such as terpenoids, steroids, saponins, flavonoids, phenols, quinones and glycosides. General reactions in these analyses revealed the presence or absence of these compounds in the (SME) tested.

Terpenoids identification 0.5 ml of SME was mixed with 2 ml of chloroform and 3 ml of concentrated HCl to form a layer. Terpenoids are present when a reddish-brown colour develops at the interface.

Steroids identification, 2 mL of chloroform and 1 mL of sulphuric acid (H_2SO_4) were added to 0.5 mL of the SME. The occurrence of steroids is indicated by the development of a reddish-brown ring at the interface.

Saponins identification, 2 mL of distilled water was added to 2 mL SME and shaken for 15 min lengthwise. The formation of a 1 cm layer of foam demonstrates the presence of saponins.

Flavonoids identification, 1 mL of 2N sodium hydroxide (NaOH) was added to 2 mL of SME. The existence of flavonoids is indicated by the development of a yellow colour.

Phenols identification, 2 mL of distilled water followed by a few drops of 10 % ferric chloride was added to 1 mL of the SME. The formation of blue/green colour represents the existence of phenols.

Quinones identification, 1 mL of concentrated sulphuric acid (H_2SO_4) was added to 1 mL SME. The appearance of red color represents the existence of quinones.

Glycosides identification, 3 mL of chloroform and 10% ammonium solution was added to 2 mL of the SME. The appearance of pink color indicates the existence of glycosides.

4. Determination of Secondary Metabolites, and Total Antioxidant Capacity of Seaweeds Extract:

4.1. ***Estimation of Total Phenolics:***

Total phenolic content was estimated according to Singleton and Rossi (1965) using Folin-Ciocalteu reagent. The absorbance was measured at 725 nm. Using a standard curve, the total phenolics were calculated as µg gallic acid equivalent /ml of algal extract.

4.2. ***Estimation of Total Flavonoids:***

Total flavonoid content was estimated via the Aluminum Chloride Calorimetric Assay (Zhuang *et al*., 1992). The total flavonoid was calculated from the standard plot and expressed as µg catechin equivalent/ ml algal extract.

4.3. ***Estimation of Carotenoids Content:***

Photosynthetic pigments (carotenoids, chlorophylls a and b) were measured in the methanol algal extracts at wavelengths of 665, 652 and 470 nm using a spectrophotometer according to the equations of Lichtenthaler (1987).

4.4. ***Estimation of Total Antioxidant Capacity*****:**

The total antioxidant capacity of the different algal extracts was performed using the phosphomolybdenum method (Prieto *et al*. 1999). The antioxidant activity is expressed as the number of µg equivalent of ascorbic acid/ ml of algal extract.

5. Characterisation of Phenolics and Flavonoids:

The phenolic and flavonoid compounds were characterized in the algal biomass using HPLC. Analysis was performed by Waters 2690 Alliance HPLC system equipped with a Waters 996 photodiode array detector. The stock solution of 10 different standards in methanol was prepared and then filtered using a 0.22µm syringe filter then 10 µl was

injected. Separation was achieved by using Column C18 Inertsil ODS 4.6x250mm, 5µm under an ambient temperature at wavelength 280 nm and flow rate 1ml/min. 0.1 % Phosphoric acid in water: Methanol was used as the mobile phase. Three algal extracts were filtered using a 0.22 µm Nylon syringe filter then 10µl was injected.

6. Statistical Analysis:

Each experiment was conducted three times, and the results were calculated as the mean ± standard deviation (SD). SPSS software (IBM, v25) was used to run the one-way analysis of variance (ANOVA) followed by post hoc Duncan's test used to determine the difference in growth and metabolic parameters between the target treatment groups and the control group at a probability level (P) ≤ 0.05.

RESULTS AND DISCUSSION

Seaweed phenolics have developed into a desirable, sustainable source of antioxidants with a variety of bio-functional benefits during the past few decades. (80%) is either utilised to make food hydrocolloids (mostly agar, alginate, and carrageenan) or consumed by people as fresh or dried edible seaweed. Less than 20% of the remaining materials are employed in a variety of industrial processes, including the production of bioplastics, biofertilizers, cosmetics, and nutrients for animal and fish feed (ABARES 2020).

Brown macroalgae have a higher concentration of phenolic compounds, especially phlorotannins, than red and green seaweeds. Species, region, and seasons all have an influence on the phenolic content of brown macroalgae (Connan *et al*., 2004).

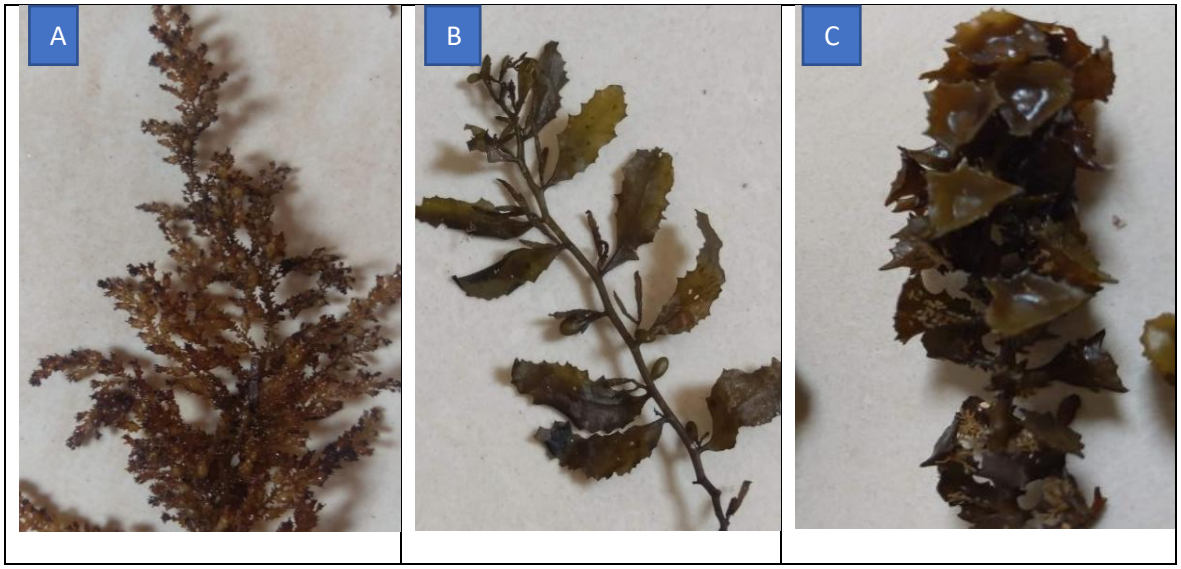

Fig. 1. Fresh parts of collected brown seaweeds: (a) *C. myrica*, (b) *S. polycystum*, and (c) *T. triquitra.*

The fresh parts of the collected seaweeds shown in Figure 1. Qualitative phycochemical analysis of an algal extract has represented the existence of several secondary metabolites which may be responsible for their edtected bioactive properties (Table 1). Methanolic extract of *S. polycystum* and *T. triquitra* showed the presence of terpenoids, steroids, phenols and flavonoids in high content, while they were detected in *C. myrica* in moderate conted. On the othed hand, both quinones and glycosides were not detected in any of the three (SME) tested.

Table 1. Qualitative analyses of phytochemical composition of algal methanolic extracts

Phytochemical parameters	***C. myrica***	***S. polycystum***	***T. triquitra***
Terpenoids	+	++	++
Steroids	+	++	++
Saponins	+	++	+
Flavonoids	+	++	++
Phenols	+	++	++
Quinones	-	-	-
Glycosides	-	-	-

++: intensely present, +: Present, - : Absent

The antimicrobial activity of different (SEM) showed in Table (2). All Seaweeds extracts exhibited antibacterial and antifungal activities. Although the most effective methanolic extract against the yeast was recorded by *C. myrica*, the same extract showed the least activity against all tested bacterial strains. On the other hand, *S. polycystum* extract had a higher effect against two bacterial isolates as well as the yeast comparing with *T. triquitra extract* as the inhibition zone was 2.2 and 1.5 cm for *E. coli* and *L. monocytogenes* respectively.

Consistent with our results, although *S. vulgare* achieved higher activity against several types of bacteria, *C. indica* was the most effective against the yeas; *Candida albicans* (Abhishek *et al*., 2021). In addition, recent studies recorded the high antimicrobial activity of *Sargassum* sp. (Alreshidi *et al*., 2023).

Table 2: Antimicrobial activity of (SME) for the tree seaweeds.

Isolate	***C. myrica***	***S. polycystum***	***T. triquitra***
E. coli	1.4	2.1	1.5
S. typhimurium	1.2	1.9	2.2
S. aureus	1.2	1.3	2.2
L. monocytogenes	1.5	1.5	1.4
C. albicans	2	1.7	0.8

*****inhibition zone in cm.

Due to their antioxidative properties, phenols and flavonoids, two frequent subgroups of polyphenolic compounds, play significant roles in stabilising lipid peroxidation. Numerous studies have shown that the number and position of hydroxyl groups in the structures of flavonoids are what gives them their antioxidant properties. These elements contribute to disease prevention. Additionally, flavonoids offer extraordinary health-enhancing properties including anti-inflammatory and antioxidant activity.The determination of total antioxidant capacity, phenolics, flavonoids, and carotenoids in *C. myrica, S. polycystum* and *T. triquitra* (SEM) was given in Table 3. Significantly, *S. polycystum* extract recorded the highest values of phenolics, flavonoids, carotenoids as well as antioxidant capacity. On contract, *C, myrice* extract showed the least values in all secondary metabolites and antioxidant activity.

Table 3: SME contents of secondary metabolites (phenolics, flavonoids and carotenoids), and total antioxidant capacity.

Seaweeds	**Total antioxidant capacity (µg/ml)**	**Total phenolics (µg/ml)**	**Total flavonoids (µg/ml)**	**Total carotenoids (µg/ml)**
C. myrica	440.05 ± 0.01^{c}	1114.00 ± 35.5^{b}	32.87 ± 2.2^{c}	3.09 ± 0.02^{c}
S. polycystum	812.12 ± 0.06^{a}	1392.14 ± 48.2^{a}	56.35 ± 2.46^{a}	7.82 ± 0.22^{a}
T. triquitra	621.09 ± 0.03^{b}	1218.51 ± 27.5^{b}	43.03 ± 1.32^{b}	4.2 ± 0.1^{b}

Flavonoids are the most most abundant and diverse forms of phenolic compounds which have a powerful antioxidant potential by chelating molecules involved in the production of free radicals or scavenging reactive oxidative species (Abubakar *et al*., 2021).

The results of HPLC phenolics and flavonoids analysis of all (SME) were shown in Table 4 and Figure 2. The most dominant compounds detected in the three extracts were apigenin and quercetin. In addition, catechin was detected in both *S. polycystum* and *T. triquitra* while it was absent in *C.myrica*. Catechin is one of flavanols with remarkable medicinal and antioxidant properties (Maestri *et al*., 2006). Catechin showed the same performances in the olive and canola oil triacilglycerols, and exerted bioctivities less than a quarter and greater than three times those of luteolin in the olive and canola oils triacylglycerols, respectively (Ahmadi *et al*., 2020), which may explain the higher antioxidant activity of both *S.polycystum* and *T. triquitra* compared with *C. myrica.*

Table 4. Detected phenolic and flavonoid compounds in (SME) using HPLC.

Seaweeds	Detected compound	RT	Area %
C. myrica	Quercetin	54.652	87.12
	Apigenin	58.078	12.88
S. polycystum	Catechin	24.021	11.34
	Quercetin	57.089	74.01
	Apigenin	58.057	14.65
T. triquitra	Catechin	24.059	10.00
	Quercetin	57.094	58.44
	Apigenin	58.061	31.56

Apigenin exhibits antidiabetic properties and promotes an increase in insulin secretion. Additionally, it inhibits sleeplessness and is neuroprotective, anti-depressant, anti-tumor, and anti-inflammatory (Salehi *et al*., 2019). Many studies have revealed that quercetin can prevent several diseases, such as some forms of cancer, tumors, and cardiovascular diseases. In addition, quercetin is currently employed in a variety of pharmaceutical preparations as an antioxidant and in the treatment of age-associated diseases (Maurya, 2022).

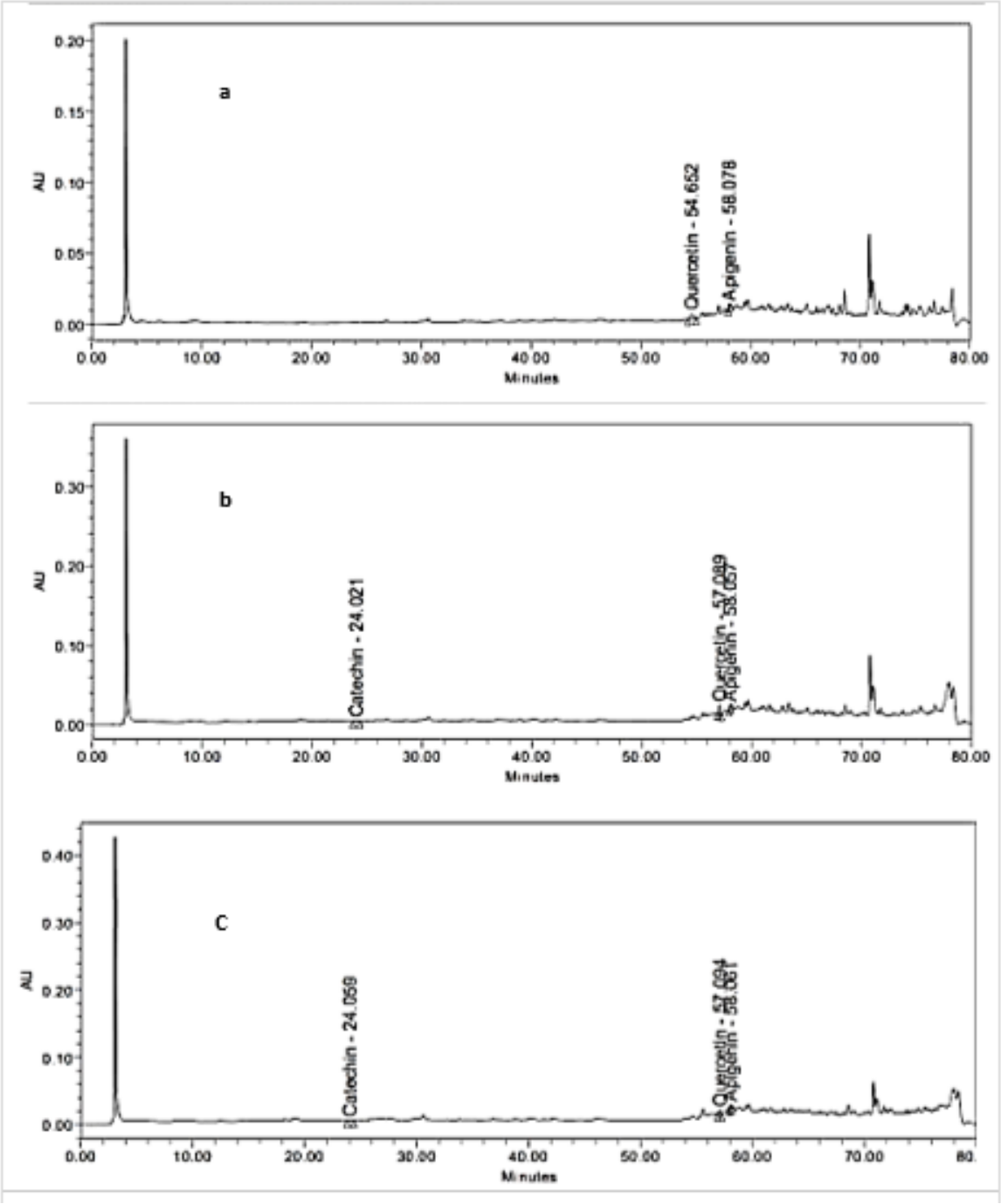

Fig.2. HPLC profile for phenolic and flavonoid of seaweed methanolic extracts : (a) *C. myrica*, (b) *S. polycystum*, and (c) *T. triquitra.*

Conclusion:

The current study showed that brown seaweed extracts of *C. myrica, S. polycystum*, and *T. triquitra* are rich in bioactive compnents such as flavonoids, steroids, terpenoids, and carotenoids. All methanolic extracts of macroalgae studied exhibited considerable antimicrobial activities against all tested bacteria and yeast. *Sargassum polycystum* showed the maximum phenolics, flavonoids, and carotenoids content as well as antioxidant capacity. Apigenin and Quercetin are the two detected flavonoids dominant in all extracts. Even though the consumption of brown seaweed is not common in several countries, owing to its antioxidant and antimicrobial efficacy, it is recommended to maximize the potential use of this seaweed in the food and pharmaceutical industry.

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