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Research Article

Antioxidant and Anti-glycation Properties of a Combination of Seaweed and Mushroom and its Isolated Flavonoid over the Individual Extracts

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ABSTRACT

Onset and progression of type 2 diabetes occur due to oxidative stress and the formation of advanced glycation end products (AGEs). Dietary sources that contain natural antioxidants are being explored for their ability to combat these processes. In the current study, *Sargassum wightii, a* brown seaweed which is edible in nature and *Pleurotus djamor,* the pink oyster mushroom, were investigated individually and in combination for their antioxidant and anti-glycation activities. Water and ethanol extracts of the seaweed (SW) and mushroom (MS), as well as their mixtures (SW+MS), were prepared and evaluated using phosphomolybdate assay, reducing power assay, hydroxyl radical scavenging assay, and BSA-glucose antiglycation assay. The SW+MS water extract showed the highest total antioxidant activity (4.389 mg AAE/mg extract/mL) and a very high reducing power and hydroxyl radical scavenging capacity in comparison to individual extracts. Further, the flavonoid fraction isolated from the SW+MS combination showed the highest hydroxyl radical scavenging activity (IC_{50} = 0.8 µg/mL), which was much higher than that of the standard antioxidant. Moreover, the SW+MS ethanol extract showed the strongest anti-glycation potential among all tested samples. Our results show a synergistic interaction between *S. wightii* and *P. djamor*, thus providing a source of natural, antioxidant-rich food supplement with therapeutic potential against diabetes.

Keywords: Antioxidant activity, Anti-glycation, *Pleurotus djamor*, *Sargassum wightii*, Seaweed-mushroom combination, Free radical scavenging, Flavonoids.

INTRODUCTION

Free radicals cause the formation of advanced glycation end products, resulting in the formation of sugar protein adducts, which in turn lead to type 2 diabetes. These advanced glycation end products can, in turn, enhance free radical formation, which in turn starts a vicious cycle of advanced glycation end product formation (Ishrat et al. 2021). To combat free radical formation, oxidative stress and its associated health risks, natural sources of antioxidants are being increasingly explored.

The edible brown algae *Sargassum wightii* have been known to have high antioxidant and antidiabetic activity (Vijayan et al. 2023; Emilin et al. 2020). *S. wightii* methanolic extracts have been shown to have antidiabetic potential in mice models of diabetes (Emilin et al. 2020). Seaweeds, which are edible in nature, show the presence of a large number of bioactive components like antioxidants, proteins, soluble dietary fibers, minerals, vitamins and polyunsaturated fatty acids (Emilin et al. 2020). Seaweeds have been used in traditional medicine in Asia against many diseases (Emilin et al. 2020). Studies have shown that seaweeds, when consumed daily, can prevent the occurrence of diseases like cardiovascular disease, cancers, hyperlipidemia, etc. *Sargassum wightii,* a seaweed, is present in Tamil Nadu, a state in India, abundantly. These are macroscopic, linear to ovate, with a height of 20 to 30 cm and length of 5–8 cm respectively (Emilin et al. 2020).

Mushrooms have been known to provide protection against cancer and show antioxidant, anti-inflammatory, antitumor, and antimicrobial activities. Mushrooms have been used as a source of natural food across the entire worldbeing rich in protein, carbohydrates, crude fiber, vitamins, and minerals, as well as crude fat (Boobalan et al. 2020). However, mushrooms have not been explored fully with respect to their therapeutic values. Mushrooms have also shown antibiotic and antioxidant properties (Ramanaiah et al. 2022).

Pleurotus djamor, pink oyster mushroom, possesses excellent flavor and is rich in nutrients (Dharmaraj et al. 2014; Cheung et al. 2020; Hasan et al. 2015). Pink Oyster Mushroom (*Pleurotus djamor*) is known to have antioxidant activity (Medeiros et al. 2024). However, not much is known about its antidiabetic potential.

An imbalance between antioxidants and oxidants leads to the accumulation of free radicals (oxidative stress), which damage macromolecules like proteins, lipids, and nucleic acids. This damage can result in aging, abnormal gene expression, disruption of receptor activity, cell proliferation, immune perturbation, mutagenesis, tissue damage, and various disease conditions (Martemucciet al. 2022)

like Alzheimer's disease, Parkinson's disease, muscular dystrophy, cataract, Rheumatoid Arthritis, diabetes, progeria, atherosclerosis, respiratory distress syndrome, Werner's syndrome, and ageing (Vendemialeet al. 1999; Martemucciet al. 2022; Jaeschke et al. 2002).

Seaweeds are known to contain excessive heavy metals, which can adversely affect the body if consumed in huge amounts (Lori et al. 2025). Mushrooms have been shown to detoxify heavy metals from the body (Zhang et al. 2023).

Hence, in this study, a combination of the mushroom *Pleurotus djamor* and seaweeds *Sargassum wightii* has been explored for their antioxidant and anti-glycation potential to shed light on their future use as a combinatorial food product which has antioxidant and antidiabetic potential, but without the danger of experiencing heavy metal toxicity.

MATERIALS AND METHODS

Materials

- The pink oyster mushroom (*Pleutorous djamor*) was procured from, Green Aperon Ltd. Bangalore.
- The Brown seaweed algae (*Sargassum wightii*) was procured from Mandapam, Tamil Nadu.
- All the required chemicals and reagents were SRL labs, Bangalore.

Methods

Water and ethanol extracts were prepared from *Sargassum wightii* (brown seaweeds)to make SW water and SW ethanol extracts. Water and ethanol extracts were prepared from *P. djamor* (pink oyster mushroom) to make MS water and MS ethanol extracts. Further, a 1:1 combination of these extracts where prepared to make SW+MS water and SW+MS ethanol extracts

Preparation of extracts

Sargassum wightii seaweed, *P. djamor* oyster mushroom alone or in ratio of 1:1 were dried overnight in a hot air oven overnight. The dried samples (*Sargassum wightii* seaweed, *P. djamor* oyster alone or in ratio of 1:1) were powdered. For preparing ethanol extracts, 50g of powdered samples were mixed with 50ml of 100% ethanol and homogenized and filtered using filter paper. The filtrates (of each sample) were collected in petriplates and left overnight for solvent evaporation. This results in preparation of *Sargassum wightii* seaweed (SW) ethanol extract, *P. djamor* oyster mushroom (MS) ethanol extract and *Sargassum wightii* seaweed, *P. djamor* oyster mushroom combination (SW+MS) ethanol extract.

For preparation of water extracts, 50g of powdered samples were mixed with 50ml of distilled water and homogenized and filtered using filter paper. The filtrates (of each sample) were lyophilized to obtain *Sargassum wightii* seaweed (SW) water extract, *P. djamor* oyster mushroom (MS) water extract and *Sargassum wightii* seaweed, *P. djamor* oyster mushroom combination (SW+MS) water extract.

Isolation of flavonoids

Flavonoids were isolated from *Sargassum wightii* seaweed, *P. djamor* oyster mushroom using maceration method where the plants were groundin mortar and pestle containing10g of sample to which 10-15ml of methanol is added. This isfollowed by filtrationand drying of extracts (Tzanovaet al. 2020).

Total antioxidant assays (Phosphomolybdate assay)

Different concentrations of Ascorbic acid and 1mg/ml extracts were prepared. To each tube,ascorbic acid /extracts were taken and phosphomolybdate reagent ((70ml of reagent contained 0.23g of sodium phosphate monobasic and 0.33g of ammonium molybdate and 1.5 ml of concentrated Sulphuric acid) was added making the total reaction volume in each tube to be 2ml. This was incubated in hot water bath (95°C) for 90 minutes. After 90min, the tubes are cooled to room temperature and their absorbances are read at 695nm. Results are expressed as mg ascorbic acid equivalent/ mg extract/ml of solution (Umamaheswariand Chatterjee, 2007).

Reducing power assay

Different aliquots of ascorbic acid and extracts of 0.002g/ml to 0.080g / ml were taken, to which phosphate buffer 0.2mM, pH 6.6, 0.1% of Ferric chloride and 1% of potassium ferricyanide were added and centrifuged at 6000rpm for 19 minutes. To the supernatant obtained after centrifugation, 10% TCA (trichloroacetic acid) wasadded and the solutions were incubated at room temperature for 30 minutes until – green colour appears. The absorbance is read at 700nm (El Jemliet al. 2016).

Hydroxyl radical scavenging assay

The total reaction mixture (3.0ml) contained different aliquots of standard gallic acid/ extracts (2-10mg/ml), to which 1ml of ferrous -EDTA (0.13% of ferrous ammonium sulphate and 0.26% of EDTA) 0.5ml of EDTA, 1.0ml of chilled DMSO in phosphate buffer were added. To this reaction mixture, 0.22% of ascorbic acid solution was added and incubated for 20 minutes.After cooling the solutions (in different tubes), ice cold 17.5% of TCA was added to all tubes. To this 1ml Nash reagent (0.075g of ammonium acetate, 0.03ml of glacial acetic acid,0.02 ml of acetyl acetone in 50 ml water) was added and incubated at room temperature until the development of a pale yellow colour which was read at 412nm. Results were expressed as IC_{50} (concentration of extract/standard causing 50% inhibition of hydroxyl radical production (Suseela et al. 2021).

Antiglycation assay

Different aliquots of extracts/standard were prepared and then 250 microlitre of 0.5mol/L glucose and 0.5mol/L Bovine serum albumin solutions are added into the reaction mixture, along with 0.2M pH 7.2 phosphate buffer with sodium azide. The solutions were incubated at 37°C for 3 days. After this, each reaction mixture wasdialyzed against phosphate buffer,pH 7.2 for 24 h. After 24h, each of the solutions were mixed with 5% TCA &TBA and centrifuged at 3500rpm for 20 minutes. After this, the supernatants were collected in different tubes and absorbances were read at 412nm. Results were expressed as IC_{50} (concentration of extract/standard causing 50% inhibition of glycation) (Kennedy et al. 1993; Halliwell, et al.1987).

Total phenolic content estimation.

Different concentrationsare taken from stock gallic acid whose concentration was 1mg/ml. 50 microlitre of different concentrations

of standard and 1mg/ml extractwere added in different tubes and to these tubes, 65 microlitre of 8% sodium carbonatewere added and incubated for 6minutes.Later FC reagent (1:1 ratio) was added to all tubes and incubated in dark for 1 hour. The blue colour developed was read at 765nm. Results were expressed in µg Gallic acid equivalent/ mg extract/ml of solution. (Samidha et al. 2014).

Estimation of Total flavonoid content

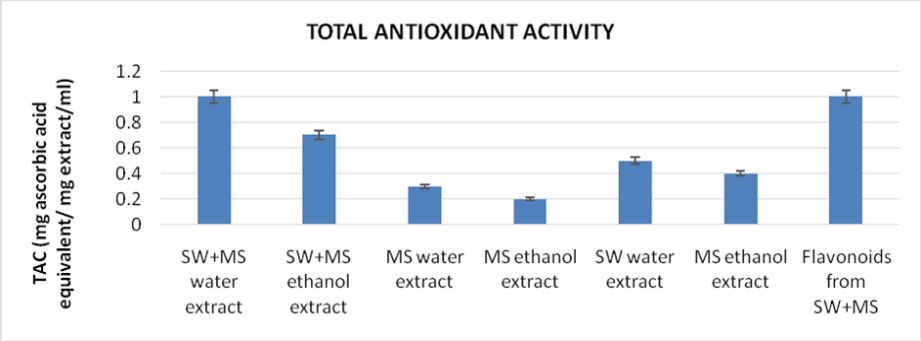
Total flavonoid content was measured by preparing the following solutions: 0.5g of sodium nitrite in 10ml of distilled water, 10% Aluminium chloride solution and 0.1N sodium hydroxide. 10 microlitres of different concentrations of standard flavonoid myricetin (stock 1mg/ml) and 1mg/ml extracts were taken in different tubes.5microlitres of sodium nitrite and then Aluminium chloride solution were added to the tubes and incubated for 10 minutes. To this 50microlitres of sodium hydroxide solution was added and incubated for 5 minutes and absorbance was read at 510nm. Results were expressed in µg Myricetin equivalent/mg extract/ml of solution (Samidha et al. 2014).

Statistical analysis

All the above experiments were performed in duplicatesand statistically analyzedusing ANOVA and Tukey test.

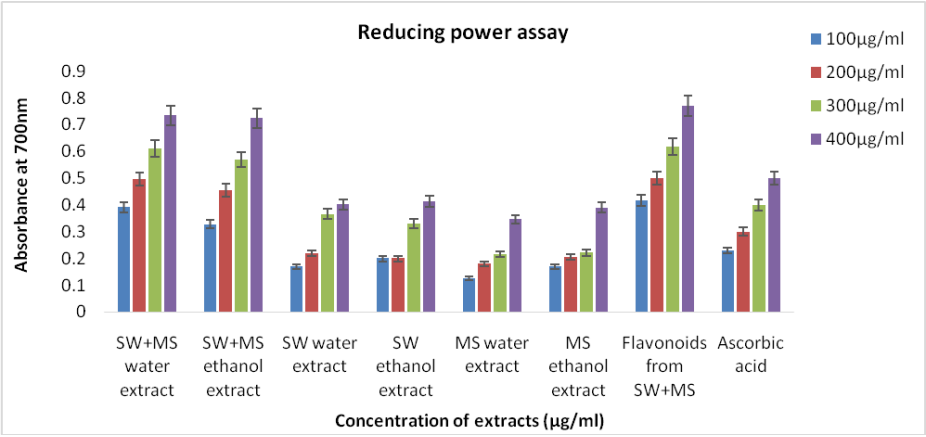
RESULTS AND DISCUSSION

Seaweeds are rich in iodine, phenolic compounds, flavonoids, tannins, carotenoids and polysaccharides which enhance immunity and confer protection against free radicals (Michalak et al. 2022). *P. djamor* mushrooms were also shown to be rich in phenolic and flavonoid compounds which possess high antioxidant activities. However, the particular phytochemical responsible for its antioxidant activity has not been pinpointed and also the antioxidant activities of the combination of seaweeds and mushrooms remain unexplored till date.The present study focused on the phytochemical composition,



SW- Seaweed, MS-Mushroom, SW+MS- mixture of seaweed and mushroom

Fig. 1: Total antioxidant activity (as assessed by phosphomolybdate assay) of seaweed-mushroom mixture water and seaweed-mushroom mixture ethanol extracts are compared with those of seaweed ethanol extract alone, seaweed water extract alone, mushroom ethanol extract alone, mushroom water extract alone and flavonoids isolated from seaweed-mushroom mixture. The results are expressed as mg ascorbic acid equivalent/ mg extract/ml. Each value represents mean ± S.D. (n = 2).



SW- Seaweed, MS-Mushroom, SW+MS- mixture of seaweed and mushroom

Fig. 2: Reducing power assay. The reductive abilities of seaweed-mushroom mixture water and seaweed-mushroom mixture ethanol extracts at different doses. The reductive abilities are compared with those of seaweed ethanol extract alone, seaweed water extract alone, mushroom ethanol extract alone, mushroom water extract alone, flavonoids isolated from seaweed-mushroom mixture and standard Ascorbic acid (at different concentrations). The absorbance (A700) was plotted against the concentration of sample. Each value represents mean ± S.D. (n = 2).

antioxidant, anti-glycation, and free radical scavenging activities of different solvent extracts of seaweed (SW), mushroom (MS), and their mixture (SW+MS). The findings show that combining seaweed and mushroom increases bioactive compound content and associated biological activities.

Antioxidant activity

Antioxidant activity was assessed using total antioxidant capacity (phosphomolybdate assay) and reducing power assays. The SW+MS water extract showed the highest total antioxidant activity (4.389 mg ascorbic acid equivalent/mg extract/ml), which was significantly higher than that of the SW+MS ethanol extract and extracts of SW or MS alone. Interestingly, the flavonoid fraction isolated from SW+MS showed equivalent antioxidant activity to the SW+MS water extract (Fig. 1), which further shows that flavonoids are major contributors to the antioxidant potential of the SW+MS mixture.

The reducing power assay revealed that SW+MS water and ethanol extracts, as well as the flavonoid fraction extracted from the mixture, had higher reductive abilities than the individual extracts. The activity was found to be significantly higher than the standard antioxidant ascorbic acid (Fig. 2). The combinatorial interaction between SW and MS could potentially increase electron-donating capacity, which is important in neutralizing free radicals and preventing oxidative damage.

Previous studies have reported similar findings in seaweed and mushroom extracts, where phenolic compounds and flavonoids were identified as the primary contributors to antioxidant activity (Cotas et al. 2020; *Azieana et al. 2017)*. The results from this study stress the role of flavonoids in participating in the antioxidant potential of the SW+MS mixture, making it a promising natural source of antioxidants.

Free radical scavenging activity

Hydroxyl free radical starts off the free radical chain reaction, which destroys lipids, proteins and nucleic acids (Ronald et al. 2015). Plants that scavenge hydroxyl free radicals can be protective against damage to biomolecules.

Hydroxyl radical scavenging assay results showed the highly significant scavenging activities of SW+MS water and ethanol extracts (3.85 and 3.9 µg/mL of $IC_{50,}$ respectively) in comparison to individual SW and MS extracts, whose activities were much lower than SW+MS extracts. The activities of SW+MS extracts were much higher than standard antioxidant mannitol, with IC_{50} = 200 µg/mL. Similar to the total antioxidant activity, the flavonoid fraction isolated from SW+MS showed the highest hydroxyl radical scavenging activity with IC_{50} = 0.8 µg/mL, which was much higher than even the crude SW+MS extracts as well (Table 1).

This shows that the flavonoid fraction of the SW+MS mixture can scavenge hydroxyl radicals at extremely low concentrations and thus confer therapeutic activity to the SW+MS mixture. Previously, it was seen that the aqueous extract of *S. wightii* possesses hydroxyl radical scavenging activity. However, the bioactive compound responsible for its activity was not reported in the work (Sradhasini et al. 2022). Similarly, previously, mushroom extracts have been shown to have high hydroxyl radical scavenging activity (Muna et al. 2015). However, the mixture of seaweeds and mushrooms has never been explored for their combined antioxidant activities and compared with those of their individual extracts.

Table 1: Hydroxyl radical scavenging assay (in the form of IC_{50}) for seaweed-mushroom mixture water and seaweed-mushroom mixture ethanol extracts, seaweed ethanol extract alone, seaweed water extract alone, mushroom ethanol extract alone, mushroom water extract alone, Flavonoids isolated from seaweed-mushroom mixture compared with the standard antioxidant Mannitol.

Extracts	*IC_{50} (µg/mL)*
SW+MS water extract	3.85 ± 0.005^{a}
SW+MS ethanol extract	3.9 ± 0.000^{a}
MS water extract	80.5 ± 0.500^{b}
MS ethanol extract	72.5 ± 0.500^{b}
SW water extract	76.5 ± 0.500^{b}
SW ethanol extract	78.5 ± 0.500^{b}
Flavonoids from SW+MS	0.8 ± 0.000^{c}
Mannitol	200 ± 0.700^{d}

SW- Seaweed, MS-Mushroom, SW+MS- mixture of seaweed and mushroom
The values shown here have been expressed as mean ± SD, $p < 0.05$. The experiments have been performed in duplicates. The letters given in superscript indicate the significance differences between the values after ANOVA and Tukey's test.
Note: Lower IC_{50} value indicates higher activity.

Anti-glycation activity

Glycation of globin proteins in hemoglobin is a major feature of diabetes. Plants that have anti-glycation activities confer protection against diabetes (Mahfuza et al. 2025). Advanced glycation end products (AGEs) are known to play a significant role in the pathogenesis of diabetes and associated complications. Among the ethanol extracts tested, SW+MS mixture had the highest anti-glycation activity (IC_{50} = 101 µg/mL) compared with that of the positive anti-glycation standard rutin drug (IC_{50} = 300 µg/mL). The SW+MS water extract also showed significant anti-glycation activity, with an IC_{50} value of 125 µg/mL. The flavonoid fraction isolated from the SW+MS mixture showed an IC_{50} value of 140 µg/mL, indicating that flavonoids could be responsible for the significant antioxidant activity of the mixture. In comparison to individual SW and MS extracts, which possessed relatively weaker anti-glycation activity (IC_{50} range of 208–278 µg/mL), the SW+MS mixture showed a significant synergistic effect (Table 2). Thus, we can conclude that SW+MS extracts possess higher anti-glycation activity than individual extracts because their combined flavonoids can possibly scavenge more free radicals. The observed anti-glycation activity was consistent with previous studies where flavonoid compounds were found to inhibit protein glycation (Patil et al. 2019).

Phytochemical analysis

The phytochemical screening of the extracts has shown a higher concentration of total flavonoid content (TFC) and total phenolic content (TPC) in SW+MS ethanol and water extracts than when each is extracted separately into SW or MS. Further, TFC and TPC of the water extracts ofSW+MS mixture were found to be extremely high-about 2732.5 µg myricetin equivalent/mg extract/mL and 2128.571 µg gallic acid equivalent/mg extract/ml respectively (Table 3). These

Table 2: Antiglycation activity (in the form of IC_{50}) for seaweed-mushroom mixture water and seaweed-mushroom mixture ethanol extracts, seaweed ethanol extract alone, seaweed water extract alone, mushroom ethanol extract alone, mushroom water extract alone, Flavonoids isolated from seaweed-mushroom mixture compared with the standard anti-glycation drug Rutin.

Extracts	*IC_{50} (µg/mL)*
SW+MS water extract	125[a]
SW+MS ethanol extract	101[b]
MS water extract	218[c]
MS ethanol extract	210[c]
SW water extract	208[c]
SW ethanol extract	278[d]
Flavonoids from SW+MS extract	140
Rutin	300[d]

SW- Seaweed, MS-Mushroom, SW+MS- mixture of seaweed and mushroom
The values shown here have been expressed as mean ± SD, $p < 0.05$. The experiments have been performed in duplicates. The letters given in superscript indicate the significance differences between the values after ANOVA and Tukey's test.
Note: Lower IC_{50} value indicates higher activity

Table 3: Total flavonoid and total phenolic content of seaweed-mushroom mixture water and seaweed-mushroom mixture ethanol extracts, seaweed ethanol extract alone, seaweed water extract alone, mushroom ethanol extract alone, mushroom water extract alone and Flavonoids isolated from seaweed-mushroom mixture. Results have been expressed as microgram Myricetin equivalent/mg extract/ml for total flavonoid content (TFC) and as microgram Gallic acid equivalent/mg extract/ml for total phenolic content (TPC).

Extracts	*TFC (µg myricetin equivalent/mg extract/ml)*	*TPC(µg gallic acid equivalent/mg extract/ml)*
SW+MS water extract	2732.5[a]	2128.571[b]
SW+MS ethanol extract	2700[a]	2142.857[b]
MS water extract	2060[c]	2007.143[d]
MS ethanol extract	2095[c]	2071.429[c]
SW water extract	1060[e]	1071.429[e]
SW ethanol extract	1407.5[f]	1378.571[f]
Isolated flavonoid fraction from SW+MS	3571[g]	

SW- Seaweed, MS-Mushroom, SW+MS- mixture of seaweed and mushroom
The values shown here have been expressed as mean ± SD, $p<0.05$. The experiments have been performed in duplicates. The letters given in superscript indicate the significance differences between the values after ANOVA and Tukey's test.

values were much greater ($p < 0.05$) than those of individual extracts, highlighting a synergistic effect that occurred upon combining SW and MS. The TFC isolated with the flavonoid-rich fraction of SW+MS was also the greatest observed (3571 µg Myricetin equivalent/mg extract/ml), and serves as another confirmation that SW and MS are rich in flavonoids.

Flavonoids have been found to act as antioxidants by preventing free radical formation and also by scavenging free radicals (Pietta 2000). Their increased concentration in the SW+MS mixture could be potentially responsible for the superior biological activities observed in this study.

CONCLUSION

The present study shows the synergistic effects of combining seaweed and mushroom, which highly enhance the phytochemical content and antioxidant activities. The SW+MS water and ethanol extracts showed superior antioxidant, anti-glycation, and hydroxyl radical scavenging activities compared to the individual extracts and the standard antioxidants. The isolated flavonoid fraction further showed excellent biological activity, thus indicating the importance of flavonoids as key bioactive compounds.The results indicate that mixtures of SW+MS, especially their aqueous and ethanolextracts, could prove to be powerful natural antioxidantandanti-glycationagents. The research presented here creates a very good foundationfor further exploration of therapeutic effects of seaweed and mushroom combinations in the treatment of oxidative stress, diabetes, and related disorders. This should be followed by a focus on the isolation and structural characterization of active components and their in vivo evaluations to validate their efficacy.

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