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RESEARCH ARTICLE

DPPH radical scavenging activity of selected Seagrasses from South East Coast of India

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Abstract

Marine ecosystem includes aquatic plants, among which seaweeds and seagrasses have more economic value. Seagrasses are marine angiosperms, completing their whole lifecycle submerged under the sea. In search of novel drugs the focus from screening terrestrial plants has recently being diverted to marine ecosysytems. The main aim of this study is to screen the selected seagrass species for DPPH radical scavenging activity. Seagrass species were extracted with polar and non polar solvents (aqueous, butanol, ethyl acetate and petroleum ether). The scavenging activity was quantified by spectrophotometric method and TLC bioautography was performed to qualitatively identify the location of the active compounds in particular fractions. This study concludes that out of 7 seagrass species out of which *Thalassia hemprichii* and *Cymodocea serrulata* showed high radical scavenging activity.

INTRODUCTION

Seagrass ecosystems are characteristic of marine as well as estuarine environments in tropical, subtropical and temperate regions (Den Hartog, 1970). Australia is the richest continent in seagrass flora having 30 species. The major seagrass meadows in India occur along the southeast coast (Gulf of Mannar and Palk Bay) and in a number of islands of Lakshadweep (Arabian Sea) and Andaman and Nicobar (Bay of Bengal) and comprises of 14 species out of 49 species throughout the world. Out of the Indian seagrass flora represented by 14 species, only 8 species have been reported from west coast (Kirkman, 1997). Among the 14 species, belonging to seven genera, which are all found to be present in Tamil Nadu coast, *Cymodocea rotundata*, *Cymodocea serrulata*, *Thalassia hemprichii*, *Halodule uninervis*, *Halodule pinifolia*, *Halophila beccarii* , *Halophila ovata* and *Halophila ovalis* are the dominant ones (Jagtap 1991,Ramamurthy *et al*., 1992).

Seagrasses are a rich source of structurally novel and biologically active metabolites which they produce inorder to sustain the extreme environmentalconditions prevailing under sea. These metabolites are not explored to their utmost potential. Many seagrass species are found to have antibacterial, antifungal, antiviral activities.Secondary metabolites extracted from *Enhalus acoroides* exhibit antibacterial, antilarval, anti feedant activity (Shu Hua Qi *et al.*, 2008). Antibacterial agents exist in *Cymodocea serrulata*, *Halophila ovalis, Zostera capensis* (Kumar *et al.*, 2008) *Halodule pinifolia* (Umamaheshwari *et al.*, 2009). *Thalassia testudinum* were shown to inhibit the growth of *thraustochytrid* (zoosporic fungus) *Schizochytrium aggregatum*. Some researchers reported increase in phenolic acid content in *Zosteramarina* and *Thalassia testudinum* during pathogen infection (Vergeer and Develi, 1997; Steele *et al*., 2005). Extracts from *Halophila decipiens, Thalasia testudinum, Ruppia maritima* and *Halodulewrightii* have inhibitory effects on fungal pathogen *Lindra thalassiae, Fusarium sp 2 and 3* (Ross *et al.*, 2007). The extracts of Caribbean seagrass *Thalassia testudinum* inhibits of HIV integrase and thus contains lead potential metabolites against HIV (Rowley *et al*., 2002).

Free radicals are highly reactive molecules produced in our body via daily metabolic processes. These are usually taken care of by our body's defense mechanism including various enzymes and metabolites (including dietary source). In case of compromised situation these free radicals react with polyunsaturated fatty acids in cellular

membranes, nucleotides in DNA, and critical sulfhydryl bonds in proteins, thereby altering their structure and hampering their function.

Various commercial synthetic antioxidants are available in markets however, due to the toxicity or side effects associated there is a need to introduce natural antioxidants. From marine plants, there are two groups that can be studied as a candidate for antioxidant sources from the class of seaweed and seagrass. Seaweeds have become part of the diet in countries like Korea and Japan where as seagrass have not yet been claimed as a dietary food for human, so that the study of antioxidants on seaweed is much done when compared to the seagrass. (Santoso, et al., 2004; Santoso, et al., 2010;Devi, et al., 2011; and O'Sullivan, et al., 2011). So the present study is undertaken to evaluate the free radical scavenging activity of selected seagrasses.

Materials and methods

Chemicals:

The stable free radical DPPH(2, 2-diphenyl-1-picrylhydrazyl),synthetic antioxidants like ascorbic acid (AA), caffeic acid (CA) and gallic acid(GA)were purchased from Himedia. All the solvents, used were of analytical grade from Sisco Research Laboratories. Silica 60 Thin layer chromatography plates were purchased from Merck.

Sampling:

Seagrasses *Halodule pinifolia* (Miki) Den Hartog (HP), *Halophila ovalis* (R.Brown) J.D.Hooker (HO), *Syringodium isoetifolium* (Ascherson) Dandy (SI), *Thalassia hemprichii* (Ehrenberg) Ascherson (TH), *Enhalus acoroides* (Linnaeus f.) Rich. ex Steud. (EA), *Cymodocea serrulata* (R. Brown) Ascherson & Magnus (CS) *and Halophila beccarii* Ascherson (HB)were collected from Mandapam coast of South India and were identified by Ganesan, Scientist from CECRI, Mandapam.

Sample preparation:

The shade dried seagrass samples were pulverized into fine powder using blender. The powdered seagrass samples (30 gms) were subjected to Soxhlet extraction using 400ml solvent mixture of petroleum ether : methanol (1:1).The crude extract thus obtained after vacuum evaporation was dissolved in 90% aqueous methanol and partitioned with petroleum ether (2x100), ethyl acetate (2x50), butanol (2x50) and water. The solvent and aqueous fractions were again evaporated under pressure and stored for antioxidant assays. The petroleum ether and ethyl acetate fractions were dissolved in chloroform and butanol and aqueous fractions were dissolved in ethanol for the assay.

Spectroscopic DPPH radical scavenging assay:

The scavenging effect of samples for DPPH radical were monitored according to the method of the previous report (Yen & Chen, 1995). 0.1 M DPPH solution was made in both chloroform and ethanol. Briefly, a 1.0 ml aliquot of extract was added to 1.0 ml of 0.16 mM DPPH solution and then left to stand at room temperature for 30 min in the dark, and its absorbancewas read at 515 nm. The ability to scavenge the DPPHradical was calculated using the follow equation:

$$Scavengingeffect(\%) = \left[1 - \left(\frac{\text{Absorbance of sample} - \text{Absorbance of sample blank}}{\text{Absorbance of control}}\right)\right] \times 100$$

Where the Absorbance of control is ofDPPH solution without sample, the Absorbance of sample is of test sample (DPPH solution plus testsample at various concentrations), and the Absorbance of sample blank is the absorbance of thesample only (sample without DPPH solution). Syntheticantioxidants like ascorbic acid, caffeic acid and gallic acid were used as positive control.

Bioautography:

The bioautography assay was performed on Silica 60 (Merck) for identifying the active DPPH radical scavenging compounds in the fractions. The samples were loaded as bands on the plates and developed using appropriate elution solvents like chloroform : petroleum (29:1) for petroleum ether fractions, chloroform : methanol (5:1) for ethyl acetate fractions, chloroform : methanol (2:1) for butanol fractions and chloroform : methanol (1:1) for aqueous fractions. The developed plate was then sprayed with 0.2% DPPH solution in methanol and dried.

Results and Discussion

DPPH is a stable free radical having a maximum absorbance at 517 nm which after donating its hydrogen to a radical scavenger, acquires its reduced form. Table 1-4 displays the percentage radical scavenging activity of the seagrass extracts where as Table 5 includes the same activity for the positive controls. Most of the samples exhibited increasing activity with increasing concentrations. It is evident that all petroleum ether extracts has very less radical

scavenging activity and are also visible as faint white bands in the bioautography assay (Figure 1 A&B). Thus proving, very few highly non polar compounds among the tested seagrass extracts have less DPPH radical scavenging activity. The ethyl acetate fractions exhibited very high DPPH radical scavenging activity which was comparable to the positive controls considered for this research highest being 94.34 % for *Thalassia hemprichii* (Figure 2 A&B). Among the butanol fractions*Thalassia hemprichii* and *Cymodocea serrulata* exhibited high radical scavenging activity with 84.9 % and 82.6 % (Figure 3 A&B). The aqueous fractions showed little or negligible DPPH scavenging activity. Mild scavenging activity was observed in *Cymodocea serrulata* (Figure 4 A&B). So the active DPPH radical scavengers seem to more polar and semi polar in nature.

Halodule pinifolia and *Thalassia hemprichii* possesses strong antioxidant activity when compared with *Enhalus acoroides* and *Syringodium isoetifolium*. This property is due to presence of phenolic compounds which act as free radical scavengers and antioxidants (Kanan *et al*., 2010). Apigenin-7-O-β-D-glucoside, chrysoeriol and luteolin were isolated from *Zosteramarina* and were found to have strong antioxidative activity. In particular, luteolin suppressed the expression of MMP-1(Matrix metalloprotease 1) because of its antioxidative effect and by inhibiting Interluekin-1α and Interleukin-6 production (Jin Hui Kim *et al*., 2004). MMP-1 degrades most components of the extracellular matrix such as collagens, laminins, fibronectins, elastins (Leung *et al*., 2000; Netzel-Arnett *et al*., 2002). Since collagen fibrils with elastin are responsible for the strength and resiliency of skin, their disarrangement causes wrinkles and skin aging. Therefore, these compounds are expected to be useful in protecting skin aging from UV irradiation (Jin Hui Kim *et al*., 2004). Santosa et al. (2012) compared the phenolic content and antioxidant activity of methoanolic extracts of seagrasses *Thalassia hemprichii Cymodocea rotundata, Enhalus acoroides* from Indonesia and their result revealed that the extracts contained more phenolic compounds and hence exhibited high DPPH scavenging activity. The bound phenolic compounds present in Posidonia oceanica from Mediterranean Sea exhibited high DPPH radical scavenging activity compared to free phenolic compounds. Athiperumalsami et al. (2010) screened 5 seagrass species and six seaweeds species from the Gulf of Mannar, for antioxidant activity. Their research concluded that methanolic and aqueous extracts *Halophila ovalis* had the highest antioxidant activity for Nitric Oxide radical scavenging. With the hydrogen peroxide scavenging method, both methanolic and aqueous extracts of many of the seagrasses and seaweeds had higher antioxidant activity.

Table 1. Percentage DPPH radical scavenging activity of petroleum ether fraction of seagrasses

Concentration	HO	TH	CS	EA	HB	HP	SI
200 ug/ml	3.5±0.62	30.87±0.91	16.24±0.74	25.16±0.61	13.05±0.58	15.6±0.88	7.32±0.89
400 ug/ml	6.68±0.45	38.24±0.65	15.28±0.98	31.84±0.36	11.14±0.94	19.1±0.57	8.14±0.22
600 ug/ml	4.77±0.39	42.67±0.52	26.75±0.52	33.75±0.85	14.33±0.44	21.02±0.71	10.2±0.94

Table 2. Percentage DPPH radical scavenging activity of ethyl acetate fraction of seagrasses

Concentration	HO	TH	CS	EA	HB	HP	SI
200 ug/ml	2.23±0.42	73.56±0.34	42.04±0.7	49.36±0.22	80.62±0.45	68.47±0.46	4.45±0.55
400 ug/ml	5.73±0.98	83.12±0.86	64.64±0.56	76.75±.0.94	82.3±0.77	75.79±0.39	9.23±0.35
600 ug/ml	6.68±0.94	94.34±0.42	89.45±0.84	80.57±0.89	84.56±0.23	80.25±0.85	6.36±0.76

Table 3. Percentage DPPH radical scavenging activity of butanol fraction of seagrasses

Table 4. Percentage DPPH radical scavenging activity of aqueous fraction of seagrasses

Concentration	HO	TH	CS	EA	HB	HP	SI
200 ug/ml	1±0.9	80.2±0.39	79.4±0.94	10.2±0.48	9.3±0.71	18.6±0.33	0.4±0.86
400 ug/ml	7±0.94	83.7±0.4	81.3±0.59	15.6±0.89	10.8±0.42	24.3±0.44	1±0.1
600 ug/ml	12.2±0.45	84.9±0.98	82.6±0.21	19.4±0.64	13.9±0.97	28.4±0.89	6.2±0.97

Concentration	HO	TH	CS	EA	HB	HP	SI
200 ug/ml	0.2±0.93	13.2±0.76	37.4±0.58	11±0.62	19±0.89	14±0.4	11.8±0.78
400 ug/ml	2±0.45	19.4±0.64	45.4±0.5	12±0.8	19.8±0.43	16.2±0.61	13±0.45
600 ug/ml	5.2±0.51	26.6±0.85	53.8±0.95	15.8±0.16	24.4±0.35	22.2±0.58	16.2±0.3

Table 5. Percentage DPPH radical scavenging activity of positive controls

Concentration	AA	CA	GA
200 ug/ml	95.1±0.22	92.6±0.24	95±0.89
400 ug/ml	96.4±0.71	92.8±0.65	95.2±0.77
600 ug/ml	97.1±0.76	93.6±0.84	95.6±0.94

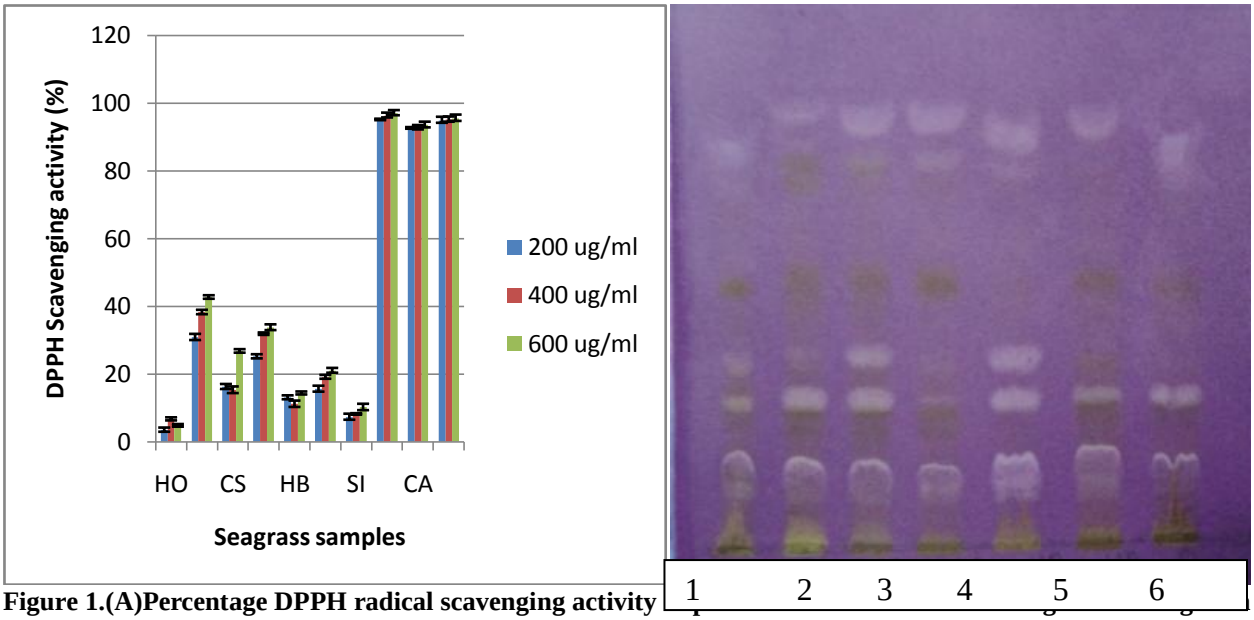

Figure 1.(A)Percentage DPPH radical scavenging activity [illegible] positive controls. Results are expressed as Mean±SD.(B) DPPH radical scavenging activity of petroleum ether fraction of seagrasses performed by TLC Bioautography method. 1-HO,2-TH,3-CS,4-EA,5-HB,6-HP,7-SI.

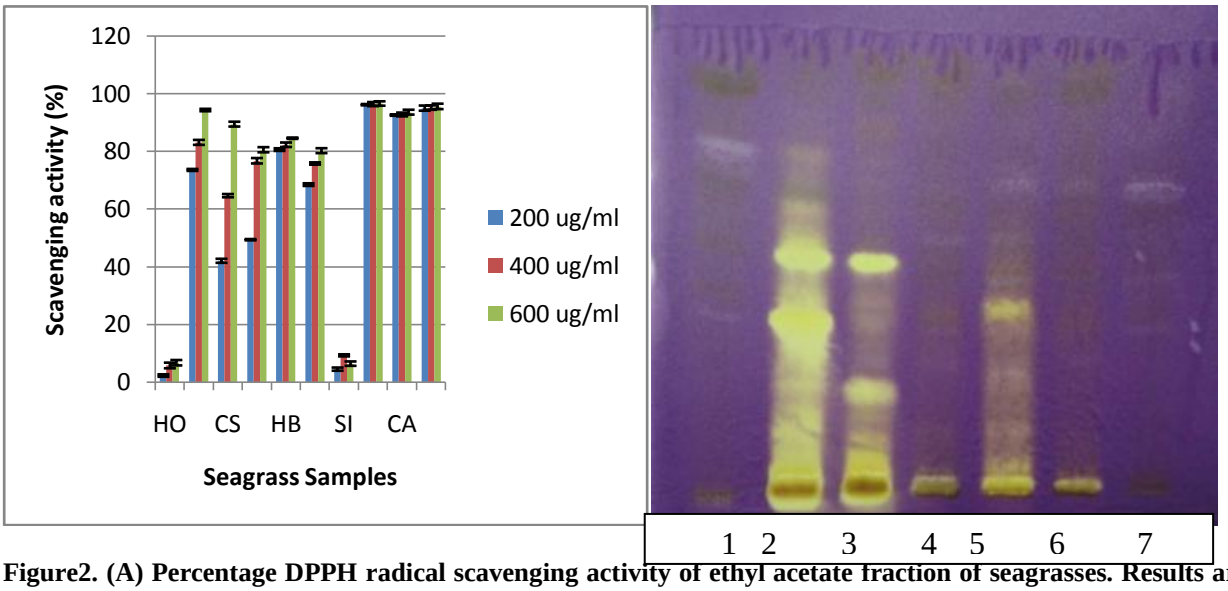

Figure2. (A) Percentage DPPH radical scavenging activity of ethyl acetate fraction of seagrasses. Results are expressed as Mean±SD. (B) DPPH radical scavenging activity of ethyl acetate fraction of seagrasses performed by TLC Bioautography method 1-HO,2-TH,3-CS,4-EA,5-HB,6-HP,7-SI.

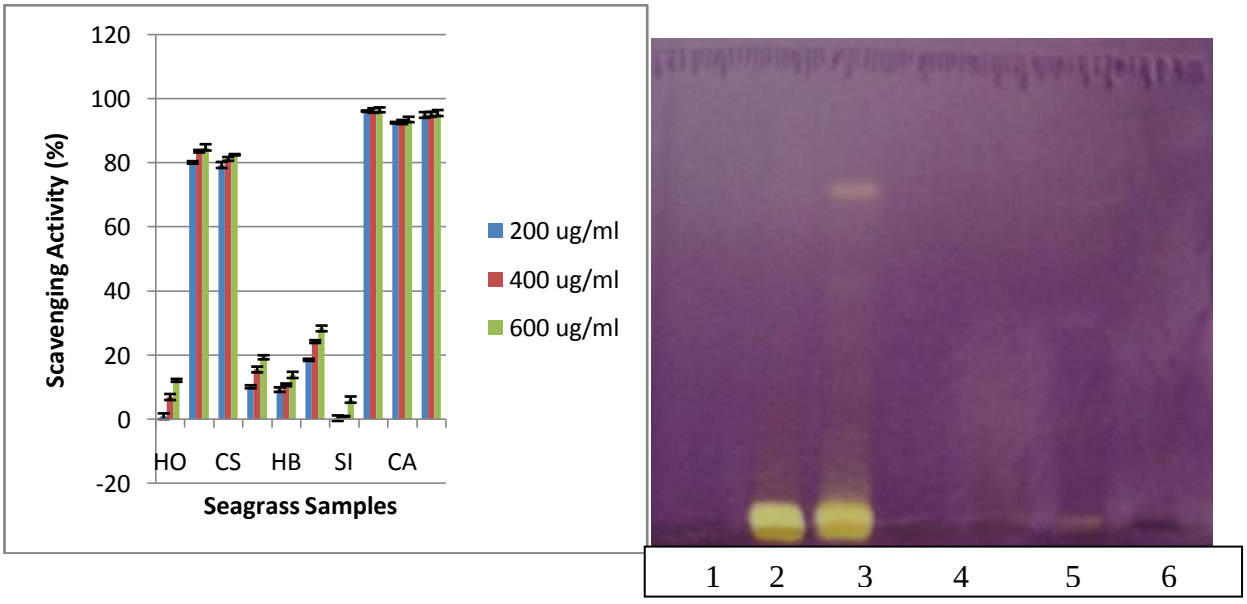

Figure 3. (A) Percentage DPPH radical scavenging activity of butanol fraction of seagrasses. Results are expressed as Mean±SD. (B) DPPH radical scavenging activity of butanol fraction of seagrasses performed by TLC Bioautography method 1-HO,2-TH,3-CS,4-EA,5-HB,6-HP,7-SI.

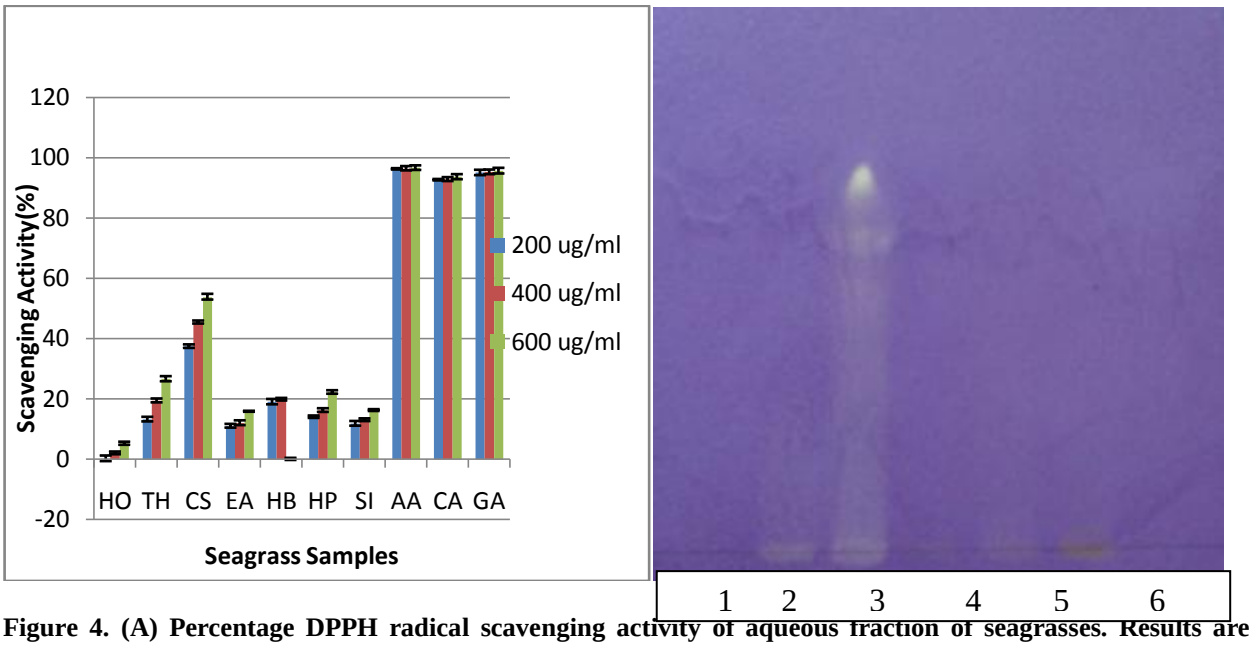

Figure 4. (A) Percentage DPPH radical scavenging activity of aqueous fraction of seagrasses. Results are expressed as Mean±SD. (B) DPPH radical scavenging activity of aqueous fraction of seagrasses performed by TLC Bioautography method 1-HO,2-TH,3-CS,4-EA,5-HB,6-HP,7-SI.

Conclusion

In search of novel and natural bioactive compounds from marine sources, seagrasses are currently under the focus and are being screened for antimicrobial, antioxidants and anti inflammatory compounds. The present study reveals that among the investigated seagrass species*Thalassia hemprichii* and *Cymodocea serrulata* possess high DPPH scavenging activity. On the basis of the platform provided by our research, the structure of the active compounds can be elucidated and identified by various other techniques.

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