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ISOLATION & CHARACTERIZATION OF BIOACTIVE COMPOUND FROM MARINE ALGAE *SARGASSUM WIGHTII*

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Abstract:

Bioactive compound was isolated and characterized from selected marine algae Sargassum wightii and evaluated its antioxidant potential. Sargassum was dark brown algae having foul smell algae was 20-28cm in size and highly branched and leave size was 6-9 cm long and 3-10mm broad. Physiochemical screening of powdered fruit was done by the standard reported methods. The Loss on drying (0.2%), Total ash value (7%), Acid insoluble ash value (3.7%), Water soluble ash value (1.33) and Foaming index (8ml) was found. Extraction of algae Sargassum wightii was done by maceration extraction method. Obtained ethanolic extract was light brown in color, semisolid in consistency with 29.65% yield. The phytochemical analysis of the ethanolic extract of Sargassum wightii revealed the strong presence of phenolics and tannins, flavonoids and alkaloids. Total phenolic content was obtained. The phenolic content was expressed as gallic acid equivalence (GAE)/g of extract. The absorbance of all sample solutions was measured at 720 nm using a spectrophotometer. Total phenolic content was found as 0.835 GAE/g of extract.Column chromatography a brilliant technique for separating and purifying bioactive compounds from complex algal extracts of Sargassum wightii. *The bioactive compounds present in the three different solvent extracts of Sargassum wightii were identified by GC-MS analysis. The compounds present in the extracts were identified after the comparison of the Mass Spectra with NIST Library. The identification of active principles was assured by observing their retention time (RT), molecular formula, molecular weight and peak area percentage. The compound having high peak area was collected from preparative TLC run at 0.57 Rf-value by spotting concentrated fraction eluted from column chromatography. Collected compound was identified as [Bicyclo [4.3.0] nonan-2-one, 8 isopropylidene]. Screen the bioactivity of isolated compounds using in-vitro DPPH assays and found higher percentage of inhibition (66.59%) by Eluted fraction from column. The 62.50 % inhibition was achieved at 500 µg/ml concentration of Ethanolic extract of Sargassum wightii.*

KEYWORDS: *Sargassum wightii, Marine, Bioactives, Isolation, Algae, Antioxidant*

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INTRODUCTION:

A large body of research has focused on the bioactive compounds derived from marine algae, particularly those from Gracilaria, Sargassum, Ulva, Spirulina, and Codium, among others[1]. Bioactive compounds such as polysaccharides (e.g., fucoidan and alginate), polyphenols, terpenoids, fatty acids (e.g., eicosapentaenoic acid), and peptides have been isolated and shown to possess significant biological activities[2]. The marine environment offers an underexplored reservoir of compounds with potential applications in drug development, which makes this field highly relevant for both scientific and commercial purposes[3].

The isolation and characterization of bioactive compounds from *Sargassum wightii* is driven by the growing interest in sustainable and natural sources of therapeutic agents. This species of brown algae is known for its rich repertoire of phytochemicals including flavonoids, alkaloids, and phenolic compounds that possess promising antioxidant and anti-inflammatory properties[4]. By isolating these bioactive compounds, researchers can explore their potential applications in pharmaceuticals, nutraceuticals, & cosmetics as eco-friendly alternatives to synthetic chemicals[5]. The process also aids in identifying specific molecules that can act as lead compounds for drug development, especially in combating microbial infections and lifestyle-related diseases[6]. Moreover, marine algae like *Sargassum wightii* are abundant and renewable, making them ideal candidates for large-scale bioactive extraction with minimal environmental impact[7]. Thus, this research holds the dual benefit of contributing to health science and supporting sustainability efforts through marine biotechnology.

MATERIAL AND METHOD:

Collection of Plant material: The Brown seaweed *Sargassum wightii* dried algae was purchased online which was collected from the coastal area near Rameshwaram, Tamil Nadu, India in the month of March 2025.

Drying and Size Reduction of Algal Material: The dried algae Sargassum wightii was already dried in packet further dried in oven at 50 ^{O}C for 3 hour. They were pulverized to make coarse powder. The coarse powder of algae was passed through sieve No. 18 to maintain uniformity and stored in cool and dry place for further study.

Screening of Powder (Physiochemical Analysis): Physiochemical screening of powdered fruit was done by the standard reported methods.

Extraction of *Sargassum wightii*: Extraction of algae *Sargassum wightii* was done by maceration extraction method.

Qualitative Phytochemical Analysis of Crude Extract: The crude extract obtained by solvent extraction was subjected to various qualitative tests with standard reported methods to detect the presence of common phytochemical constituents. All the chemicals and reagent used in phytochemical testing was of analytical grade.

Estimation of total phenolic content: A 100 µl aliquot of the crude sample was mixed with 2 mL of 2% sodium carbonate and left to stand in the dark at room temperature for 2 min. A standard solution of 30 mg/mL gallic acid was used to prepare a calibration curve with concentrations ranging from 10-20 mg/l. The phenolic content was expressed as gallic acid equivalence (GAE)/g of extract. The absorbance of all sample solutions was measured at 720 nm using a spectrophotometer.

GC-MS analysis for phytochemical constituents: A high resolution mass spectrum equipped with a data system in combination with Gas Chromatography was used for the chemical analysis of seaweeds. GC-MS analysis of the extracts were carried out by the following method of Hema et al., (2010) using a GC-MS Clarus 500 Perkin Elmer system and gas chromatography interfaced to mass spectrometer (GC MS). The detection of the compounds was employed with the NIST (National Institute of Standards and Technology). The relative % amount was calculated by comparing its peak area to the total areas. Software adopted to handle mass spectra and chromatogram was Turbomass.

Identification of phytocompounds: Interpretation on mass spectrum of GC-MS analysis was done using the database of National Institute of Standard and Technology (NIST). Mass spectrum of the unknown compounds was compared with spectrum of the known compounds stored in the NIST Library (Version, 2005).

RESULTS AND DISCUSSION:

Morphology of *Sargassum wightii*:

Table No. 1: Morphological characteristics of *Sargassum wightii*

S. No.	Character	Observation
1	Color	Dark brown
2	Odor	Foul
3	Taste	Not good
4	Size of algae	20-28 cm
5	Texture	Highly branched
6	Leaves size	6-9 cm long and 3-10mm broad

Physiochemical analysis of powder:

Table No. 2: Physiochemical analysis of powder of *Sargassum wightii*

S. No.	Parameters	Observation (%)
1	Loss on drying	0.2
2	Total ash value	7
3	Acid insoluble ash value	3.7
4	Water soluble ash value	1.33
5	Foaming index	8 (ml)

Extraction of *Sargassum wightii*

Table No. 3: Consistency and color of *Sargassum wightii* extract

Extract	Color	Consistency	Percentage Yield
Ethanol	Light Brown	Semisolid	29.65 %

Phytochemical Analysis of Crude Extract: The phytochemical analysis of the ethanolic extract of Sargassum wightii revealed the strong presence of phenolics and tannins, flavonoids and alkaloids.

Table No. 4: Phytochemical screening of ethanolic extract of *Sargassum wightii*

S. No.	Chemical Tests	Ethanolic extract
1	**Carbohydrates** i) Molisch's Test ii) Fehling's Test iii) Benedict's test	 (-) (-) (+)
2	**Phenolics and Tannins** i) with 5%ferric chloride solution ii)with 10% lead acetate solution	 (+) (+)
3	**Alkaloids** i)Dragendorff's Test ii) Mayer's Test	 (+) (+)
4	**Glycosides** i) Borntrager's Test ii) Legal Test iii) Baljet Test	 (-) (+) (-)
5	**Flavonoids** i) Shinoda's Test ii) Alkaline reagent test iii) Lead test	 (+) (+) (+)
6	**Steroids and Sterols** i) Libermann-Burchard Test ii) Salkowski Test	 (-) (-)

(+) = Present, (–) = Absent

Table No: Total phenolic content of *Sargassum wightii*

S. No.	Extract	Total phenolic content
1	Ethanolic	0.835 GAE/g of extract

GC-MS Analysis of fraction obtained during isolation: Phytochemicals are secondary metabolites from plants that are essential for the plant defense against grazing animals and other predators. GC-MS technique provides the identification and quantification of chemical compounds based on their characteristic fragmentation patterns at specific retention times. Active components responsible for various biological activities could be evaluated by investigating the chemical composition of each extract using GC MS analysis.

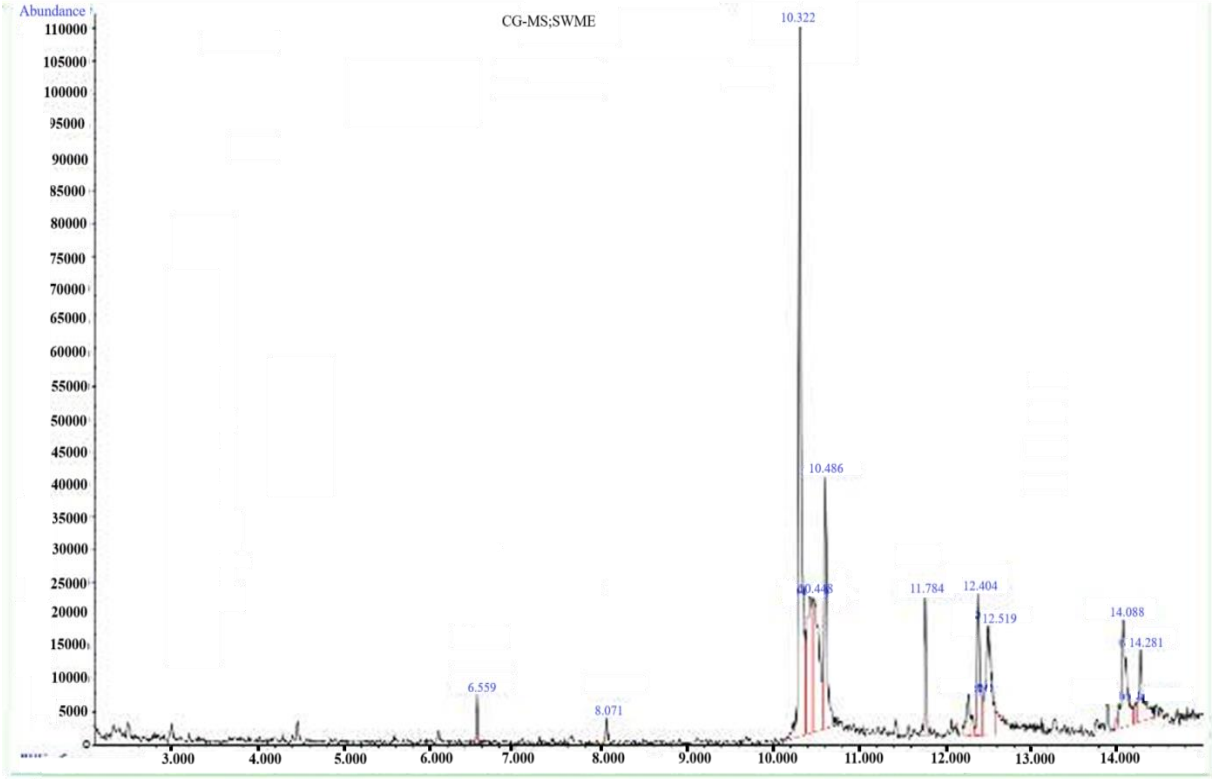


Figure 6: GC-MS Chromatogram of ethanol Extract of *Sargassum wightii*

In the present investigation, the bioactive compounds present in the three different solvent extracts of *Sargassum wightii* were identified by GC-MS analysis. The GC-MS analysis of ethanol extracts revealed the presence of different bioactive compounds. A total of 11 peaks were observed with different retention time was identified in methanol extract followed by ethyl acetate and chloroform extracts. The compounds present in the extracts were identified after the comparison of the Mass Spectra with NIST Library. The identification of active principles was assured by observing their retention time (RT), molecular formula, molecular weight and peak area percentage are presented in Table.

Figure 6: GC-MS Chromatogram of ethanol extract of *Sargassum wightii*

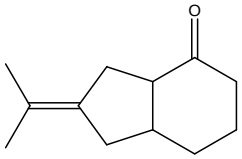
Retention Time (Min.)	Name of the Compound	Molecular Formula	Molecular Weight (m/z) (g/mol)	Peak Area (%)
6.559	Cycloheptanol, 2-methylene	$C_8H_{14}O$	126.20 g/mol	1.22
8.071	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro- 4,4,7a- trimethyl-, (R)-	$C_{11}H_{16}O_2$	180.2435 g/mol	0.90
10.322	Bicyclo [4.3.0] nonan-2-one, 8 isopropylidene	$C_{12}H_{18}O$	178.27 g/mol	29.88
10.448	9H-Fluorene, 1-methyl-	$C_{14}H_{12}$	180.24 g/mol	9.73
10.486	Diethylstilbestrol	$C_{18}H_{20}O_2$	268.3 g/mol	12.52
11.784	4′ (Trifluoromethyl)acetophenone	$C_9H_7F_3O$	188.15 g/mol	3.94
12.404	Tridecanoic acid	$C_{13}HO_2$	214.34 g/mol	5.68
12.519	Phenytoin	$C_{15}H_{12}N_2O_2$	252.27 g/mol	9.39
14.088	1-Hexyl-2-nitrocyclohexane	$C_{12}H_{23}NO_2$	213.32 g/mol	7.42
14.281	2-Hydroxyethyl vinyl sulfide	C_4H_8OS	104.17 g/mol	4.60

Characterization and structural elucidation of isolated compound

The structure of the compound was confirmed by following analytical methods.

General characters of isolated compound:

Structure:



IUPAC Name : Bicyclo [4.3.0] nonan-2-one, 8 isopropylidene

Molecular formula : $C_{12}H_{18}O$

Molecular weight : 178.27 g/mol

TLC of isolated compound: The compound having high peak area was collected from preparative TLC run at 0.57 Rf-value by spotting concentrated fraction eluted from column chromatography. Collected compound was identified as [Bicyclo [4.3.0] nonan-2-one, 8 isopropylidene].

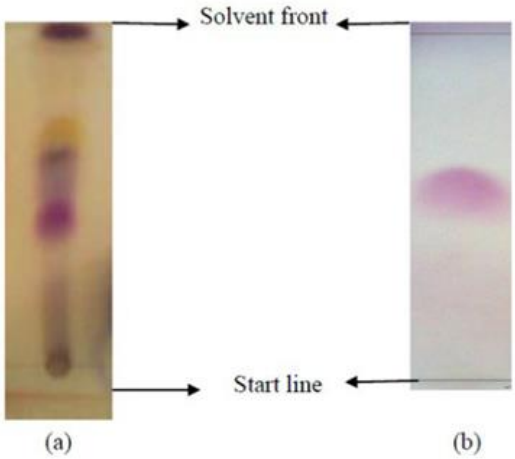


Figure 7: TLC of (a) concentrated fraction eluted from column (b) isolated compound

7.5.2 UV- Spectroscopy of isolated compound:

The isolated compound was scanned from 200-400 nm on the UV- spectroscopy and the λmax was found at 322.8nm.

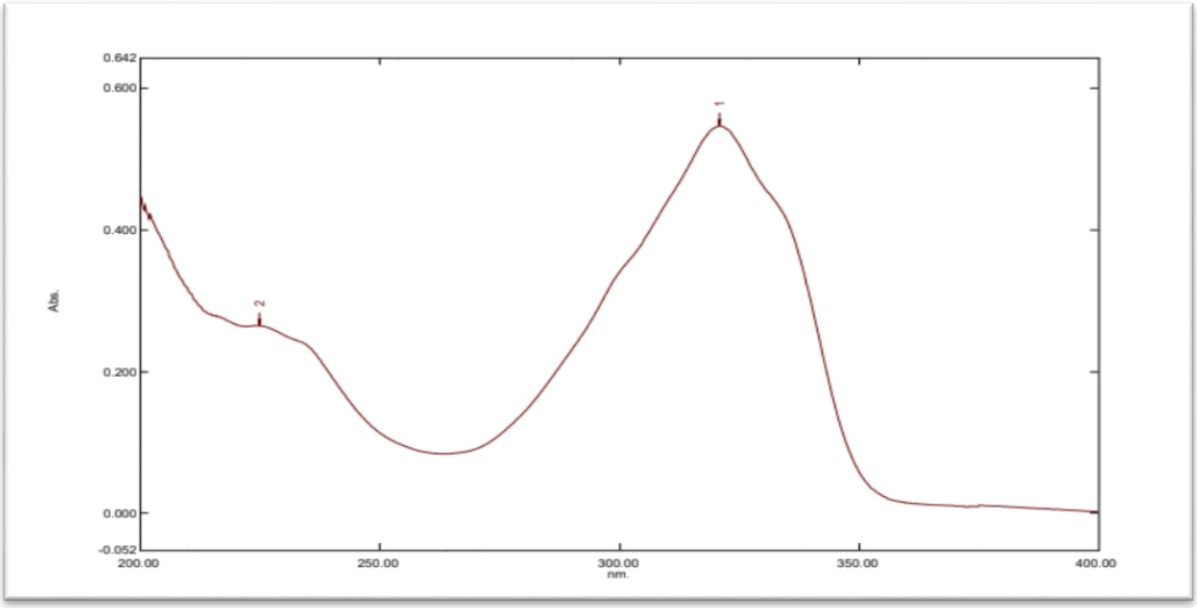


Figure 8: UV- spectrogram of isolated compound

7.5.3 FT-IR spectrograms of isolated compound:
FT-IR analysis found the C=O peak on 1787 cm^{-1}.

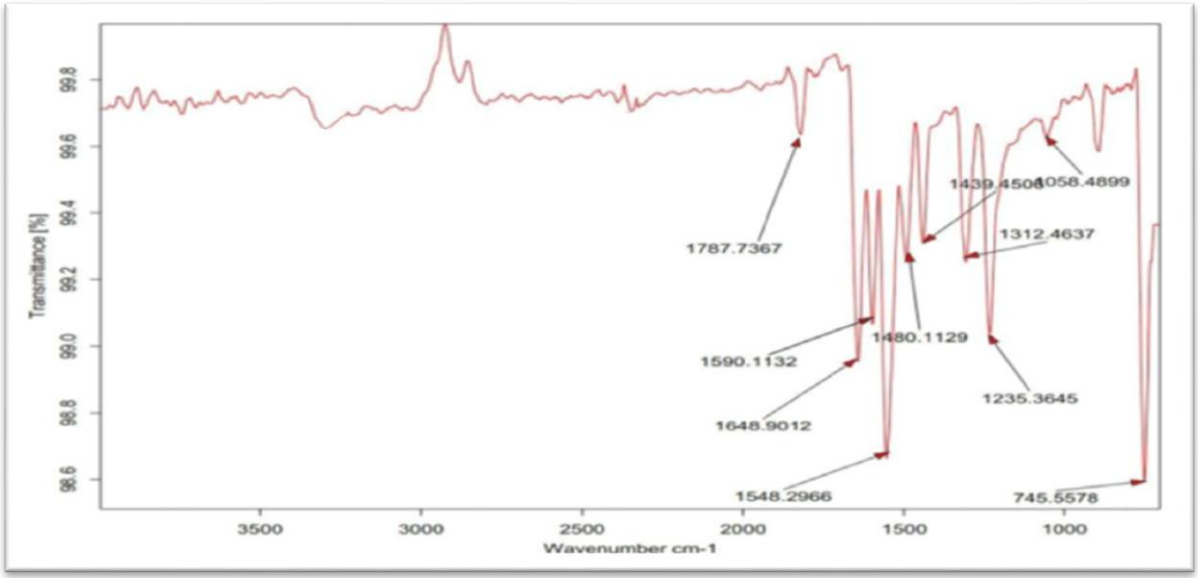


Figure 9: FT-IR spectrogram of isolated compound

7.6 Anti-oxidant activity by DPPH method:
Screen the bioactivity of isolated compounds using *in-vitro* assays:

Table No. 7: Antioxidant activity by DPPH assay

Sample	Conc.	Absorbance at 517 nm		% Inhibition
		Control	Absorbance	
Standard (Ascorbic acid) (AA)	100 µg/ml	0.488	0.266	45.49
	200 µg/ml	0.488	0.203	56.90
	300 µg/ml	0.488	0.166	58.40
	400 µg/ml	0.488	0.153	68.64
	500 µg/ml	0.488	0.112	77.04
Ethanolic extract (EE)	100 µg/ml	0.488	0.394	19.26
	200 µg/ml	0.488	0.326	33.19
	300 µg/ml	0.488	0.277	43.23
	400 µg/ml	0.488	0.233	52.25
	500 µg/ml	0.488	0.183	62.50
Eluted fraction from column (EFC)	-	0.488	**0.163**	**66.59**

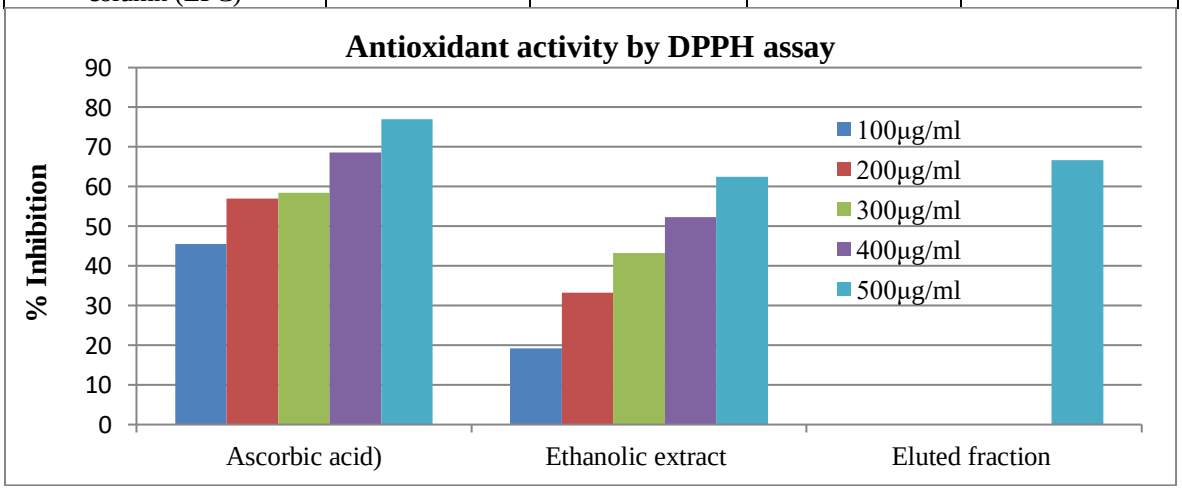


Figure 10: Antioxidant activity by DPPH assay

7.7 DISCUSSION:

Bioactive compound was isolated and characterized from selected marine algae *Sargassum wightii* and evaluated its antioxidant potential. The Brown seaweed *Sargassum wightii* dried algae was purchased online which was collected from the coastal area near Rameshwaram, Tamil Nadu, India. Morphologically, *Sargassum* was dark brown algae having foul smell algae was 20-28cm in size and highly branched and leave size was 6-9 cm long and 3-10mm broad. The dried algae *Sargassum wightii* was already dried in packet further dried in oven at 50 ^{O}C for 3 hour. They were pulverized to make coarse powder. The coarse powder of algae was passed through sieve No. 18 to maintain uniformity and stored in cool and dry place for further study.

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CONCLUSION:

The study of marine algae as a source of bioactive compounds has revealed their immense potential in pharmaceuticals, nutraceuticals, and cosmetics. Marine algae especially brown, variety is rich in polysaccharides, polyphenols, fatty acids, pigments, and terpenoids, which exhibit antioxidant, anti-inflammatory, antiviral, and anticancer properties. Advanced extraction techniques such as ultrasound-assisted, microwave-assisted, and supercritical CO_2 extraction have improved yield and purity of bioactive compounds. Characterization methods like HPLC, GC-MS, NMR, and FT-IR have enabled precise identification of chemical structures and biological activities. The isolated compounds have shown better results in free radical scavenging.Continued research and refinement of isolation protocols will enhance the scalability and application of marine algal bioactives in healthcare, food, and environmental sustainability. Also there is requirement of the screening of some more activities like enzyme inhibition and cell viability assays for supporting their therapeutic potential. In short, marine algae are emerging as a sustainable and versatile source of novel bioactive compounds, offering innovative solutions to global health and industrial challenges.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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