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Original Article

Biochemical and Physicochemical Characterization of Phytochemical from *Hygrophila schulli* (Buch. Ham.) M.R. Almeida and S.M. Almeida

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ABSTRACT

Background: Plants are an important source of medicine. Most of the plants are studied for their therapeutic values and to treat various diseases. *Hygrophila schulli* is one of the important medicinal plants belonging to the family Acanthaceae. The medicinal uses of this plant are mentioned in traditional systems of medicine such as Ayurvedha, Unani, and Sidha. **Materials and Methods:** An attempt is made to decipher the phytocomponents present in *H. schulli* using standard techniques. **Results:** Different parts of *H. schulli* were extracted using methanol as a solvent and were subjected to several Instrumental analyses for identification of various phytochemical analyses such as UV-VIS to observe the major peaks, FT-IR to identify the functional groups and GC-MS to identify and quantify the phytocompounds present in the plant. **Conclusion:** The various phytochemicals present in different parts of *H. schulli* is identified and tabulated in this research article.

Key words: *Hygrophila schulli*, UV-VIS, FT-IR, GC-MS, Phytochemical, Phytocompound.

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INTRODUCTION

Plant and plant products have been used as food and medicine right from the origin of human life. Various system of medicinal practice has been documented in India. The folk care medicine and traditional system of medicine is the backbone for Ayurvedha, Siddha, and Unani. Plants have been used to treat various diseases due to the phytoconstituents present in them. Plants produce primary and secondary metabolites.[1] Primary metabolites are used for their physiological function, whereas secondary metabolites are produced in plants to overcome the stress conditions, such as physical, environmental, or chemical stresses. Secondary metabolites are the source of chemicals like alkaloids, steroids, terpenoids, flavonoids, phenolic compounds, etc., which possess medicinal property.[2]

More than 95% of the population still rely on herbal medicine, where herbal medicine have less or no side effect when compared to allopath system, moreover these medicines increase their immunity and cure the disease.[3,4] Herbal and traditional medicines not only cure but also protect from diseases, in this context there is an urgent need for scientific research for identifying and documenting the phytoconstituents present in plants and document the pharmaceutical properties for the effective uses of these plants.

One of such important medicinal plant is *Hygrophila schulli* belonging to the family Acanthaceae has been selected for our present study. This plant is well known for its medicinal property since ancient time. *H. schulli* is mentioned in Sushruta Samhita and Charak Samhita for treating male fertility, urinary infections, and stomach related diseases.[5] *H. schulli* has been used as a crude extract in treating the disease in traditional system of medicine, hence we have made an attempt to identify the bioactive phytocompounds found in this medicinal plant *H. schullii* and to study the Biochemical and Physicochemical Characterization of phytocomponents using various instruments UV-VIS, FT-IR, GC-MS, and the findings and results are presented in this paper.

MATERIALS AND METHODS

Collection of Plant

The plant *H. schulli* was collected from Alappatti, Krishnagiri district, Tamil Nadu, India and identified by the botanical experts at Research Department of Botany, Government Arts College for Men, Krishnagiri. Further, this plant was authenticated with the help of a Botanical Survey of India, Coimbatore, Tamil Nadu, and India (BSI/SRC/5/23/2017TECH/3207).

Preparation of Plant Extract

The fresh leaves, stem, and root of *H. schulli* were cleaned by washing several times with running water for removing dust particles. The plant parts were processed through the drying process by exposing them at room temperature until all moisture was lost 25-30 days. Dried plant parts were finely crushed then grind to yield fine powder. The extract was prepared using 100g of the dried powder plant materials in 250 ml of methanol using a soxhlet extractor continuously for 10 hr. The extracts were concentrated using a vacuum evaporator and the concentrated samples were stored in labeled sterile bottles and kept at -20°C for further experimental uses.[6,7]

Ultraviolet and Visible Spectroscopy

The methanol extract of leaf, stem, and root of *H. schulli* was subjected to Ultraviolet and visible Spectroscopy (UV-VIS) studies using standard methodology.[8] The plant sample was centrifuged at 3000 rpm for 10 min and filtered through Whatman No.1 filter paper. The samples were diluted to a 1:10 ratio with the same solvent. The extract was scanned at a wavelength ranging from 200-700 nm using a Perkin Elmer spectrophotometer, Shimadzu UV-1800, Japan and the characteristic peaks were detected. The peak values of the UV-VIS were recorded.

Fourier Transform Infrared Spectrophotometer

Fourier Transform Infrared Spectrophotometer (FT-IR) is perhaps the most powerful tool for identifying the types of chemical bonds present (functional groups) in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined.[9] Dried powder of leaf, stem, and root of *H. schulli* in methanol solvent extracts was used for FT-IR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant parts was loaded in FT-IR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

Gas Chromatography and Mass Spectrophotometer

To identify the presence of active constituents and the chemical composition of *H. schulli* plants parts leaf, stem, and root in methanol extract was characterized by the use of gas chromatography and mass spectrometry (GC-MS) Agilent Technologies GC systems with GC-7890A/MS-5975C model Agilent Technologies, Santa Clara, CA, USA GC-MS. The chemical composition of the methanol fraction of the *Hygrophila schulli* was analyzed by Gas Chromatography-Mass Spectrometry using the standard procedure.[10]

RESULTS

Ultraviolet and Visible Spectroscopy

The Methanol extracts of different parts of *H. schulli* plant samples were scanned at the range 200- 700nm in UV-VIS Perkin Elmer Spectrophotometer showed different distinct peaks and absorption values as follows (Table 1; Figure 1 A, B, C).

The leaf extract of *H. schulli* showed five peaks at a different wavelength at 229.3, 233.3, 239.8, 248.2, and 338.2 nm at absorbance 10.00, 9.99, 10.01, 10.02, and 3.30 (Figure 1 A). In the stem extract, the peaks were noticed at 229.9, 236.2, 243.8, 247.5, 291.6, and 335.0 nm with absorbance 10.0, 10.02, 9.99, 10.0, 1.12, and 1.20 (Figure 1 B). The root extract of *H. schulli* showed six peaks at 228.9, 234.0, 259.5, 293.7, 330.9, and 337.1 nm with absorbance 9.99, 1.42, 1.85, 2.71, 3.33 and 3.28 (Figure 1 C).

Fourier Transform Infrared Spectrophotometer

The FT-IR spectrum was used for *H. schulli* plant leaf, stem, and root extract, and the results are tabulated in Table 2 (Figure 2). The leaf extract gave a broad peak at 3543 cm^{-1} which indicated the presence of O-H stretching and the functional group Alcohol. It gave a strong peak at 2176 cm^{-1} that indicated the presence of C≡C stretching and

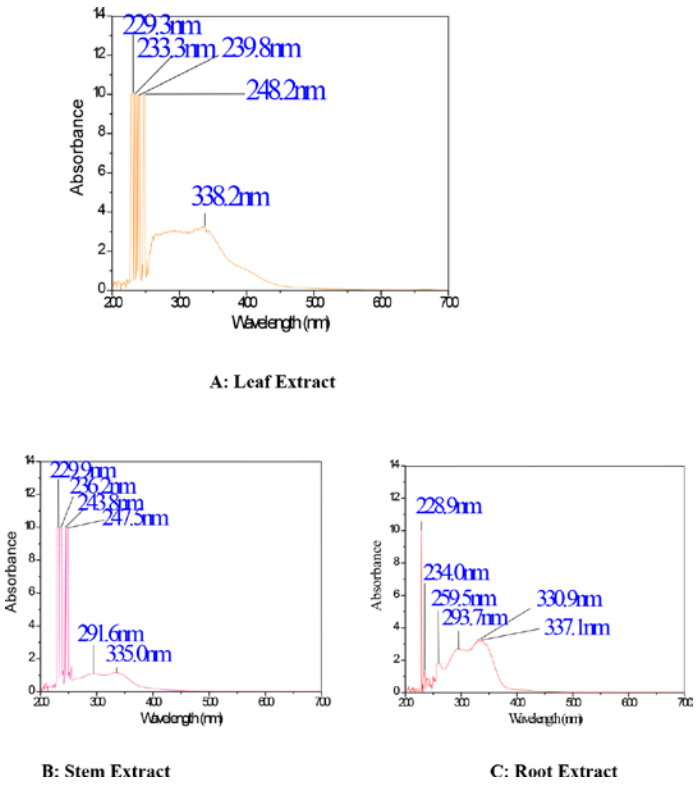

Figure 1: UV-VIS Spectra of *Hygrophila schulli*.

the functional group of Alkyne. The peak obtained at 1979 cm^{-1} shows the presence of C-H bending for the functional group of the Aromatic compound. The peak at 1652 cm^{-1} is noted with C=N stretching and the functional group Imine /oxime. The low peak obtained at 1079 cm^{-1} which indicated the presence of C-N stretching and the functional group of Amine (Table 2; Figure 2).

The stem extracts gave a strong peak at 3141 cm^{-1} which mentioned the presence of O-H stretching and the functional group of Alcohol. The peak obtained at 2930cm^{-1} which indicated the presence of C-H stretching and the functional group Alkane. The peak at 2098cm^{-1} indicated the presence of N=C=S stretching and the functional group of Isothiocyanate. The peak obtained at 1608cm^{-1} mentions the presence of C=C stretching and the presence of conjugated Alkene. The peak received at 1398cm^{-1} which indicated the presence of O-H bending and the functional group of Alcohol. The peak obtained at 1265cm^{-1} which indicated the prescience of C-O stretching and the functional group of Aromatic ester. The low peak obtained at 1040cm^{-1} which referred to the presence of C-N stretching and functional group Amine (Table 2; Figure 2).

The plant root extract gave a strong peak at 3297cm^{-1} which induced the presence of O-H stretching and the functional group of Alcohol. The low peak obtained at 1028 cm^{-1} induced the presence of C-N stretching and the functional group of Amine (Table 2; Figure 2).

Table 1: UV-Vis Spectrum analysis of Methanol extract of *Hygrophila schulli*.

Methanol extract of *H. schulli*						
	Leaf		Stem		Root	
S.N	λ (nm)	Abs	λ (nm)	Abs	λ (nm)	Abs
1.	229.3	10.00	229.9	10.0	228.9	9.99
2.	233.3	9.99	236.2	10.02	234.0	1.42
3.	239.8	10.01	243.8	9.99	259.5	1.85
4.	248.2	10.02	247.5	10.0	293.7	2.71
5.	338.2	3.30	291.6	1.12	330.9	3.33
6.	-	-	335.0	1.20	337.1	3.28

Gas Chromatography and Mass Spectrometry Analysis

Gas Chromatography (GC) and Mass Spectrometry (MS), is used to analyze complex organic and biochemical mixtures. GC can separate volatile and semi-volatile compounds with great resolution. MS can provide detailed structural information (Molecular weight, Molecular formula) based on the retention time and peak area percentage on most compounds such that they can be exactly identified and quantified (Table 3; Figures 3 A, B, C).

Table 2: FT-IR analysis of Methanol extract of *Hygrophila schulli*.

S.N	*H. schulli*	Peak Value	Functional group	Functional group name	Vibrations
		3543	O-H	Alcohol	stretching
		2176	C≡C	Alkyne	stretching
1.	Leaf extract.	1979	C-H	Aromatic compound	bending
		1652	C=N	Imine/oxime	stretching
		1079	C-N	Amine	stretching
		3141	O-H	Alcohol	stretching
		2930	C-H	Alkane	stretching
		2098	N=C=S	Isothiocyanate	stretching
2.	Stem extract.	1608	C=C	Conjugated Alkene	stretching
		1393	O-H	Alcochol	bending
		1265	C-O	Aromatic ester	stretching
		1040	C-N	Amine	stretching
		3297	O-H	Alcohol	stretching
		2920	C-H	Alkane	stretching
		2122	C≡C	Alkyne	stretching
3.	Root extracts.	1601	C=C	Conjugated alkene	stretching
		1258	C-O	Alkyl aryl ether	stretching
		1028	C-N	Amine	stretching

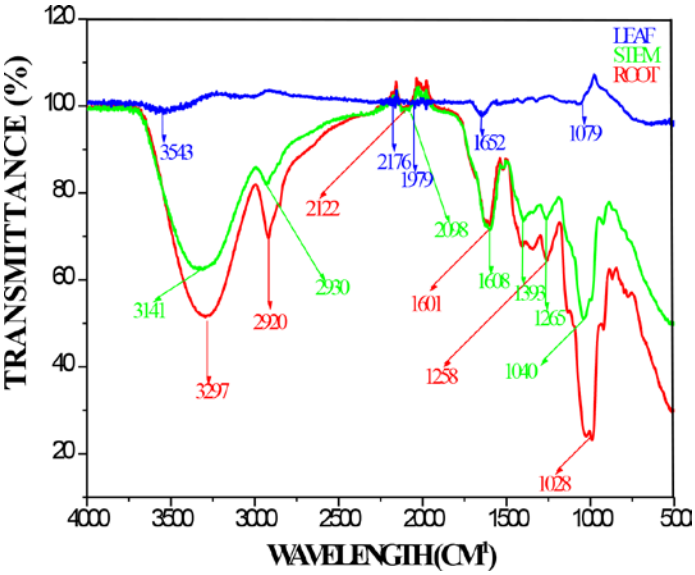

Figure 2: FT-IR Spectra of *Hygrophila schulli*.

The different parts of the experimental plant *H.schulli* were extracted using methanol as a solvent and subjected to GC-MS analysis, where five different compounds were observed from leaf sample such as 2-Methylthiolane, S, S-Dioxide (11.24%), (S)-(+)-6-Methyl-1-Octanol (26.72%), (S)-(+)-5-Methyl-1-Heptanol (32.8%), 2-Fluoro-6-Trifluoromethylbenzoic acid, 4-Nitrophenyl ester (13.70%), and Thiophen-2-Methylamine, N-(2-Fluorophenyl)- (16.20%) (Table 3; Figure 3 A). Two from stem samples such as Benzene, 1,1-Oxybis,3-phenoxy (5.75%), and Lupeol (94.24%) (Table 3; Figure 3 B) and eleven compounds from root sample such as 2-Propenoic Acid, 2-Phenylethyl Ester (48.58%), (S)-(+)-5-Methyl-1-Heptanol (1.28%), (S)-(+)-6-Methyl-1-Octanol (1.41%), 3-Tosyl Sedoheptulose (0.79%), Ethylene Diacrylate (1.18%), Cyclopentaneethanol,.Beta.,2,3-Trimethyl- (0.92%), Trans-2,4-Dimethylthiane, S,S-Dioxide (1.37%), 1,4-Bis (Trimethylsilyl) Benzene (0.80%), Di-N-Decylsulfone (4.09%), Cyclotrisiloxane, Hexamethyl (0.80%), and Arsenous acid, tris (trimethylsilyl) ester (38.72%) (Table 3; Figure 3 C).

DISCUSSION

Spectroscopic techniques are currently being used to identify compounds in plant extracts and to determine their quality and quantity.[11-15] The GC-MS analysis supports the same and shows the presence of important bioactive compounds. The height of the peak corresponds to the relative concentration of the compound. The compounds which are eluted at different timings through gas chromatogram are picked up by the mass analyzer and produce a particular fragmentation pattern. This fragmentation pattern is compared to the compounds present in the reference library (NIST) on which the structure of compounds is determined.[16-18]

Among several phytocompounds identified from leaf, stem and root of *H. schulli* in this study the most significant compound found is Lupeol, whereas rest other phytocompounds identified has meager biochemical activity. The active biocompound identified in this study is Lupeol which has a chemical formula $C_{30}H_{50}O$ and molecular weight is 426.386 with a melting point 215-216°C. Lupeol is a very active pentacyclic triterpenoid used in various treatment. A similar compound has been extracted from different plants and plant parts as per the literature and studies show that lupeol has high biological activity. Lupeol extracted from leaf extract of *Visnea mocanera,*[19] *Sapium baccatum,*[20] from the stem of *Buchholzia coriacea,*[21] roots of *Aloe hijazensis*[22] and whole plant *Curtisia dentate*[23] shows that lupeol has significant antimicrobial activity. Studies have been reported that lupeol is a potential drug against cancer cells[24-27] and has anti-mutagenic activity.[28] This phytocompound also has anti-diabetic activity[29-32] and anti-inflammatory activity.[33-35] It is very clear from the earlier literature that Lupeol is a very important phytochemical having high biological activity and is significantly present in our experimental plant.

CONCLUSION

The present study qualitative UV-Vis spectrum of *H. schulli* plant extract showed various peaks in different absorption. The highest peak of leaf, stem, and root at λ 229.3, 229.9, and 228.9, the lowest peak at 338.2, 335.0, and 337.1 nm with the absorption of 10.00, 10.0, 1.20, 9.99, 3.28 and 3.30 respectively. The FT-IR spectrum of *H. schulli* plant leaf, stem, and root extract indicates the presence of O-H, C-H, and C-N stretching and the functional group of Alcohol, Alkane, and Amine respectively. Eighteen compounds were identified from the leaf, stem, and root of *H. schulli* by GC-MS. The most significantly noted phytocompounds are (S)-(+)-5-Methyl-1-Heptanol (32.8%), Lupeol (94.24%), Arsenous acid, tris(trimethylsilyl) ester (38.72%) etc. The molecular formula, molecular weight, structure, and peak area were identified for all these compounds.

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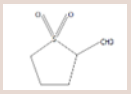
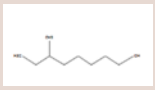
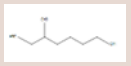
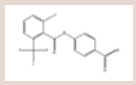
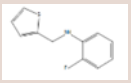
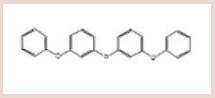
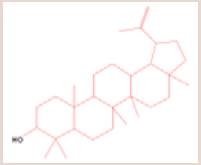
Table 3: GC-MS analyses of *Hygrophila schulli*.

A: Methanol extract of *H. schulli* leaf.

S.N	RT	Name of the Compounds	Molecular Formula	Molecular Weight	Peak Area %	Compound structure
1	3.549	2-Methylthiolane, S,S-Dioxide	$C_5H_{10}O_2S$	118.194	11.24	
2	4.877	(S)-(+)-6-Methyl-1-Octanol	$C_9H_{20}O$	144.258	26.76	
3	6.029	(S)-(+)-5-Methyl-1-Heptanol	$C_8H_{18}O$	130.231	32.8	
4	6.626	2-Fluoro-6-Trifluoromethylbenzoic Acid, 4-Nitrophenyl Ester	$C_{14}H_7F_4NO_4$	329.203	13.70	
5	7.965	Thiophen-2-Methylamine, N-(2-Fluorophenyl)-	$C_{11}H_{10}FNS$	207.266	16.20	

B: Methanol extract of *H. schulli* stem.

S.N	RT	Name of the Compounds	Molecular Formula	Molecular Weight	Peak Area %	Compound structure
1	3.264	Benzene, 1,1'-Oxybis[3-Phenoxy-	$C_{24}H_{18}O_3$	354.403	5.75	
2	10.642	Lupeol	$C_{30}H_{50}O$	426.729	94.24	

C: Methanol extract of *H. schulli* root.

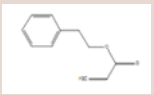
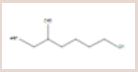
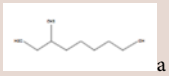
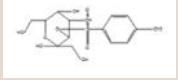
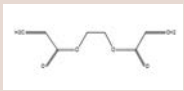
S.N	RT	Name of the Compounds	Molecular Formula	Molecular Weight	Peak Area %	Compound structure
1	3.533	2-Propenoic Acid, 2-Phenylethyl Ester	$C_{11}H_{12}O_2$	176.215	48.58	
2	4.872	(S)-(+)-5-Methyl-1-Heptanol	$C_8H_{18}O$	130.231	1.28	
3	6.024	(S)-(+)-6-Methyl-1-Octanol	$C_9H_{20}O$	144.258	1.41	 a
4	6.62	3-Tosyl Sedoheptulose	$C_{14}H_{20}O_9S$	364.368	0.79	
5	7.046	Ethylene Diacrylate	$C_8H_{10}O_4$	170.164	1.18	

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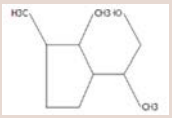
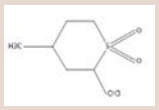
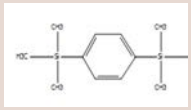
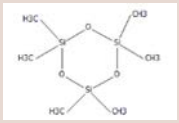
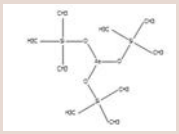
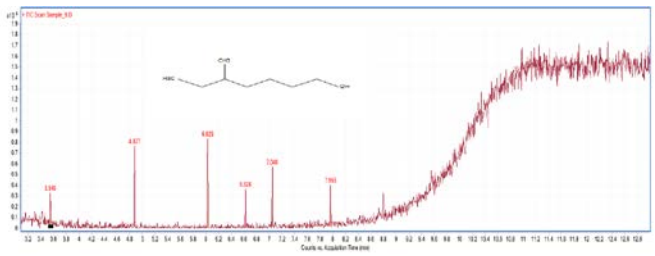
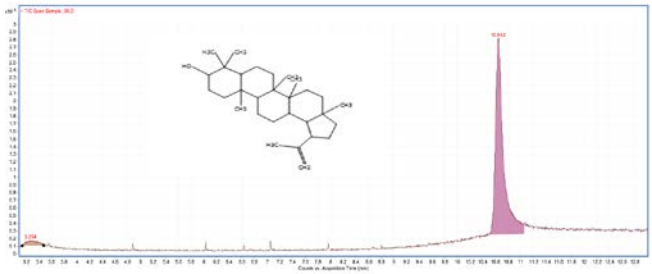
Table 3: **Cont'd.**

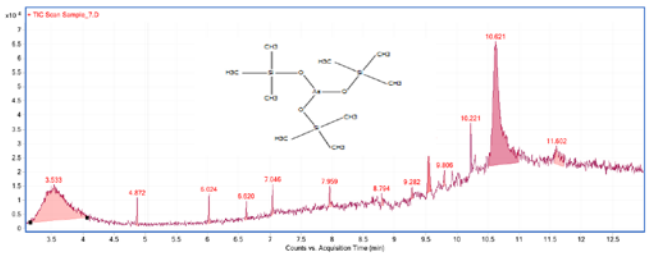
S.N	RT	Name of the Compounds	Molecular Formula	Molecular Weight	Peak Area %	Compound structure
6	7.959	Cyclopentaneethanol,. beta.,2,3-trimethyl-	$C_{10}H_{20}O$	156.269	0.92	
7	8.794	Trans-2,4-Dimethylthiane, S,S-Dioxide	$C_7H_{14}O_2S$	162.247	1.37	
8	9.282	1,4-Bis(Trimethylsilyl)Benzene	$C_{12}H_{22}Si_2$	222.478	0.80	
9	9.547	Di-N-Decylsulfone	$C_{20}H_{42}O_2S$	346.614	4.09	
10	9.806	Cyclotrisiloxane, Hexamethyl	$C_6H_{18}O_3Si_3$	222.462	0.80	
11	10.621	Arsenous Acid, Tris(Trimethylsilyl) Esters	$C_9H_{27}ASO_3Si_3$	342.489	38.72	



A: Leaf extract and the significant compound - (S)-(+)-5-Methyl-1-heptanol.



B: Stem extract and the significant compound - Lupeol.



C: Root extract and the significant compound - Arsenous acid, tris(trimethylsilyl) ester.

Figure 3: GC-MS Chromatogram of methanol extract of *Hygrophila schulli*.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

***H. schulli*:** *Hygrophila schulli*; **UV-VIS:** Ultraviolet and visible Spectroscopy; **FT-IR:** Fourier Transform Infrared Spectrophotometer; **GC–MS:** Gas chromatography–mass spectrometry; **RT:** Retention time.

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