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Physicochemical and Phytochemical Assay of Rediscovered *Wissadula contracta* (Link) R. E. Fries from Karnataka, India

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ABSTRACT

Wissadula contracta (Link) R.E.Fr. is a perennial undershrub of Malvaceae, with a native range from Mexico to tropical America and is naturalized in Dharwad, Karnataka, India. The current investigation was carried out to analyse the presence of medicinally useful bioactive constituents of *Wissadula contracta* leaf extract using selected physicochemical parameters, preliminary qualitative analysis and Gas Chromatography coupled with Mass Spectroscopy. From the results, it is evident that the aqueous and ethanol extracts have high soluble extractive values, indicating the richness of secondary metabolites. A foaming index of 1000 specifies saponin content, and the swelling index of 38 was due to significant mucilage in the crude sample. Qualitative analysis revealed the presence of all secondary metabolites tested namely, phenolic compounds, saponins, flavonoids, tannins, fixed oil and fats, triterpenoids, and steroids except alkaloids. Additionally, the extensive profile of twenty-eight phytoconstituents in the sequential methanol extract was revealed by the GC-MS analysis, supporting the therapeutic potential of *Wissadula contracta*.

Keywords: Physicochemical parameters; Phytochemical screening; GCMS; Wissadula contracta; Therapeutic value 1

1 INTRODUCTION

Phytochemicals such as alkaloids, terpenes, glycosides, phenolic compounds, and flavonoids are abundant in medicinal plants. These bioactive secondary metabolites (SMs) serve as a source of remedy for various ailments amidst other functions. Numerous SMs are recognized for their potential medicinal properties in the prevention and treatment of infectious and noncommunicable ailments, as well as for their antioxidant properties. The potency, specificity, and spectrum of action against diverse diseases differ based on the structure of chemicals they possess. The unexplored plant with few phytochemical combinations of bioactive principles may do wonders as curative and preventive medicine or may act as an immune system booster. Hence there is an ever-increasing demand for new drugs to treat the cells which have developed resistance against the current drugs or to enhance the efficacy of the present treatment.

W. contracta (Link) R. E. Fr., an undershrub with a native ranging from Mexico to Tropical America and predominantly grows in the dry tropical biome. It was rediscovered in India as a garden escape and naturalized in Dharwad, Karnataka, India [1]. The genus Wissadula includes 85 scientific species, of which 23 are accepted and *W. contracta* is cultivated in the gardens of Sri Lanka, Malesia, and tropical America. Outside Malesia, it is treated as a weed, found in highly disturbed places like open roadside areas. In Indonesia, high-quality bast fiber is extracted from *W. contracta* [2–4].

The related species namely *W. periplocifolia* (L.) *C. Presl* (also found in India) has ethnomedicinal value, used in the treatment of snakebite [5] and being nontoxic finds application as diuretics, antiseptic, anti-inflammatory, and anti-rheumatic agent. The reported preliminary phytochemical work confirmed the presence of five important phenolic acids and the flavone 7,4'-di-O-methylisoscutellarein [6]. Also, *Sida contracta* (Link) and *Abutilon contractum* (Link) Sweet are the important synonyms of *W. contracta* (Link) R. *E. Fries*, which have been used for centuries in traditional medicines in many countries [7]. So, far the medicinal potentialities of *W. contracta* were not reported, though its close relatives and plants of Malvaceae have profound medic-

inal influence. Consequently, the present study was taken up to investigate the medicinal potential of *W. contracta*, using selected physicochemical and phytochemical assays namely qualitative and GC-MS analysis.

The result of the physicochemical parameters assay revealed the various aspects of SMs present in the plant. The Gas chromatography–mass spectrometry (GC-MS) data provides the qualitative profile of phytoconstituents which throws light on the biological and pharmacological applications of the test species.

2 MATERIALS AND METHODS

2.1 Collection of plant materials and authentication

Fresh leaves and twigs of *W. contracta* (Link.) R. E. Fr. were collected from in and around Dharwad, Karnataka, India. It was authenticated and a voucher specimen with Accession No. 19555 was deposited in the Department of Botany, Karnatak Science College, Dharwad. The leaves were thoroughly cleaned, shade dried, and pounded into an abrasive powder, used for the soluble and sequential extraction process to screen the SMs, and crude powder was used for physicochemical parameter study. The sequential hot extraction method was followed using Soxhlet apparatus, the chemicals of AR grade namely petroleum ether, dichloromethane (DCM), acetone, methanol and aqueous extracts whereas, rotary shaker was used in cold extraction method [8]. The extracts were dried on a rotary evaporator, collected in a sterile Eppendorf tube and refrigerated for further use.

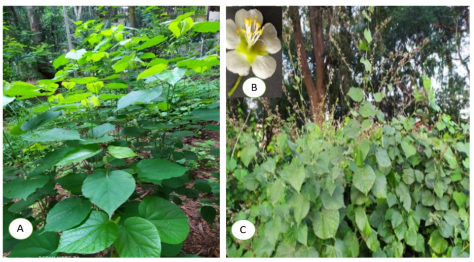

Figure 1: *Wissadula contracta*: A - Yound plant, B - Single floret, C - Habit

W. contracta is an erect, perennial plant, of height up to 3 meters, branches and petiole are cylindrical, pubescent and encircled with whitish, fasciculate, sparse stellate trichomes. Leaf lamina is cordate to ovate and surrounded with colourless, whitish, simple, glandular, fasciculate, and multiradiate hairs while the ventral surface is greyish white and velvety. The distal nodes of stem and inflorescence possess 2 or 3 condensed leaves at maturity. Small white florets in apical congested double raceme inflorescence produce subreniform seed bearing schizocarpic fruits (Figure 1).

2.2 Characterization of potential medicinal values

To characterize the potential phytoconstituents showing pharmacological activity, physicochemical and phytochemical methods were followed.

2.2.1. Physicochemical parameters

Selected parameters namely soluble extractive values (using ethanol and aqueous solvent through hot and cold methods), swelling index, and foaming index of the crude leaf sample were estimated [9–11].

2.2.2. Qualitative phytochemical analysis

The sequential leaf extracts were tested to verify the presence of various medicinally useful SMs, such as alkaloids, flavonoids, tannins, phenolic compounds, saponin, triterpenes, steroids, and gum and mucilage as per the standard procedures [12].

Statistical analysis: The experiments of physicochemical parameters were conducted in triplicates and the results were presented as mean ± standard error of the mean (SEM) using Microsoft Excel (2007).

2.2.3. GC-MS analysis of sequential methanol extract

GC-MS analysis was used for the identification of chemical constituents of *W. contracta* leaf, and was performed on Shimadzu, GC-MS, with the mass spectrometer detector QP2010S. By using an ELITE-5MS column (30 m long, 0.250 mm internal diameter, and 0.25 mm film thickness), phytochemical substances were separated. The temperature of the oven was maintained at 40°C for five minutes before being raised to 250°C for nine minutes. Then the temperature reached its maximum and stayed there for 14 minutes. The running process was continued 30 minutes altogether. For the GC-MS analysis, 2μl of methanol extract of *W. contracta* leaf was employed [13]. Results were recorded using GC-MS Software, specifically GC-MS Solutions, and the compounds were identified by comparing spectral peak values with GC-MS Library (Wiley and National Institute of Standards and Technology [NIST]).

3 RESULTS AND DISCUSSION

The therapeutic property of plants against different ailments depends on the synthesis of certain active principles, which may be effective either singly, or the synergistic activity in consortia. Nowadays, the interest among researchers in investigating and characterizing the potential phytoconstituents is increasing, to address the various health issues menacing the society. In order to identify the bioactive components, it is crucial to analyze the phytochemicals of undisclosed plants.

The soluble extractive values of *W. contracta* leaf alcoholic and aqueous extracts, were obtained using hot and cold methods (Table 1). The aqueous and cold extracts showed slightly higher values than the ethanolic and hot extracts. The high extractive values in polar solvents indicate the presence of significant quantity of medicinally valuable SMs [14],

responsible for the potential pharmacological activity of *W. contracta* leaf.

Table 1: The soluble extractive values, foaming and swelling index of *W. contracta* leaf

Soluble extractive values [%w/w]		
Solvent	**Hot extract**	**Cold extract**
Ethanol	20.60±0.071	21.30±0.046
Aqueous	22.44±0.056	23.06±0.087
Foaming index		1000
Swelling index		38

The foaming index of 1000 and swelling index of 38 gave the quantitative idea of saponins and mucilage content (Table 1). The highest amount of saponin may become the reason behind a multitude of biological properties, including antimicrobial, and as a source of dynamic plant-based anti-viral remedies could be potentially useful in treating viral diseases such as COVID-19, HIV, HSV, rotavirus, etc., in addition to profound haemolytic, anti-inflammatory, insecticidal, anticancer and cholesterol level reducing property [15–17]. Similarly, a considerable accumulation of mucilage may impart a healing effect in traditional folk medicine, to cure burns, wounds, ulcers, external and internal inflammations, irritations, diarrhoea and dysentery [18]. The mucilage-producing Persian medicinal herbs are providing specific and hopeful solutions to the complaints related to respiratory, gastrointestinal, musculoskeletal, genital and urinary systems [19]. Owing to the possession high mucilage content, the Grewia ferruginea bark was recommended as an appropriate excipient to be used in the pharmaceutical industry [20].

The preliminary phytochemical screening of sequential solvent extracts of *W. contracta* leaf, for medicinally useful SMs [Table 2] showed positive results for all the tests conducted to glycoside saponin, tannins and phenolic compounds, steroids and triterpenoids, flavonoids, fats and oils, gums, and mucilage, except alkaloid. To be precise, the petroleum ether and DCM extracts showed a positive result for only steroids and triterpenoids, fats and oils, tannins and phenolic acids, and flavonoids. The acetone and aqueous extracts contained all the tested SMs but the former is short of saponin while the latter is rich in steroids and triterpenoids, fats and oils. But the methanol extract was found to contain all the SMs tested except alkaloids.

Thus, the results were in accordance with the polarity rule of biochemicals in the sequential extraction method. Most of the screened SMs were known to impart appreciable pharmacological effects like amylase inhibitory, free radical scavenging, antimicrobial, anti-inflammatory, anticancer, and antihypertensive activities [21] and individual SMs were linked with specific pharmacological properties. Methanol extract emerged as a highly potent one owing to all the tested SMs except alkaloid. Likewise, flavonoids, tannins, phenolic acids, glycosides etc. were known to exhibit maximum free radical scavenging, anticancer, and antimicrobial activities [22, 23]. Flavonoids, prevent oxidative cell damage and act as a powerful anticancer agent and terpenoids were proved to have anticancer, antimicrobial, antiparasitic, anti-inflammatory, anti-allergenic, antihyperglycemic, antispasmodic and immunomodulatory effects [24–26]. Also, the Abutilon indicum leaves showed strong antimicrobial and antioxidant properties owing to saponin content [27]. The above results confirm the possible pharmacological properties of *W. contracta*.

The data of GC-MS chromatogram of leaf methanol extract of *W. contracta* and the names of identified phytochemical compounds along with the retention time [RT], area, molecular formula, and molecular weight and reported pharmacological effect were shown in Figure 2, Tables 3 and 4 respectively. A total of twenty-eight compounds have been listed as having RT ranging from 8.592 to 48.894 minutes. Piperazine, 2,5-dimethyl (2-Tert-butoxy-1-methylethyl) benzene showed less RT of 8.592 minutes and retinol had a high RT of 48.894 minutes. Phytol was identified with a maximum peak area percentage of 12.12 while Methyl palmitoleate was found to have a minimum peak area percentage of 0.93. N, N-Dimethylglycine, and dl-.alpha.-Tocopherol were the bioactive compounds with the lowest and highest molecular weight of 103.12 and 430.7 respectively. The major components present in the leaves were Retinol (RT:48.494), dl-.alpha.-Tocopherol (RT:48.081), 3-Oxatricyclo[20.8.0.0(7,16)]triaconta-1(22) (RT:46.726),4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4.1.0]heptane (RT:44.891), Lupeol (RT:43.408) etc.

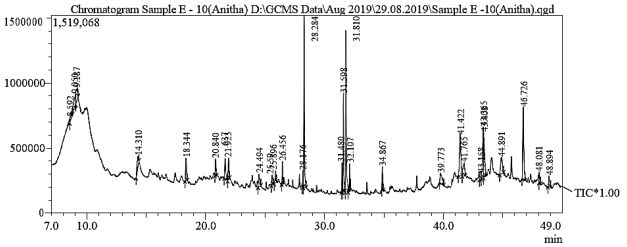

Figure 2: Chromatogram of *W. contracta* leaf methanol extract

Among the total twenty-eight phytocompounds as represented by the peaks, nineteen have well proven potential pharmacological and biological activities. But the pharmacological influence of Megastigmatrienone, Megastigmatrienone 4, Methyl stearate, 3-Cyclopentylpropionic acid, 2-dimethyl aminoethyl ester, valerenol, 4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4.1.0]heptane, and Oxatricyclo[20.8.0.0(7,16)]triaconta-1(22), 7(16), 9,13,23,29-hexaene are not available in literature, may

Table 2: Qualitative analysis observations of sequential extracts of *W. contracta* leaf

Phytochemicals	Petroleum ether extract	DCM extract	Acetone extract	Methanol extract	Aqueous extract
Gum and mucilage	+	+	+	+	+
Glycoside- Saponins	-	-	-	+	+
Tannins and phenolic compounds	+	+	+	+	+
Alkaloids	-	-	-	-	-
Steroids and triterpenoids	+	+	+	+	-
Flavonoids	+	+	+	+	+

Legend: (+) Present; (-) Absent

Table 3: GC-MS details of phytoconstituents of *W. contracta* methanolic leaf extract.

Peak #	Area%	R.T [min]	Name of phytoconstituents	Molecular formula	Molecular weight g/mol
1	1.18	8.592	Piperazine, 2,5-dimethyl-(2-Tert-butoxy-1-methylethyl) benzene	$C_6H_{14}N_2$	114.19
2	2.60	9.050			
3	2.47	9.187	N, N-Dimethylglycine	$C_4H_9NO_2$	103.12
4	2.11	14.310	p-Vinyl guaiacol	$C_9H_{10}O_2$	150.17
5	2.45	18.344	Dehydro-.beta.-ionone	$C_{13}H_{22}O$	194.31
6	1.56	20.840	Megastigmatrienone	$C_{13}H_{18}O$	190.28
7	2.51	21.637	3-Hydroxy-.beta.-damascone	$C_{13}H_{20}O_2$	208.3
8	1.93	21.923	Megastigmatrienone 4	$C_{13}H_{18}O$	190.28
9	1.84	24.494	trans-10,11-Epoxyfarnesenic acid methyl ester	$C_{16}H_{26}O_3$.	266.38
10	1.09	25.599	5-Ketoborneol	$C_{10}H_{18}O$	154.25
11	1.09	25.896	6-Methylphenanthridine	$C_{14}H_{11}N$	193.24
12	1.43	26.456	Neophytadiene	$C_{20}H_{38}$	278.5
13	0.93	28.176	Methyl palmitoleate	$C_{17}H_{32}O_2$	268.4
14	11.72	28.284	Methyl palmitate	$C_{17}H_{34}O_2$	270.5
15	1.79	31.480	Methyl 9,12-octadecadienoate	$C_{19}H_{34}O_2$	294.5
16	7.72	31.598	Linolenic acid, methyl ester	$C_{19}H_{32}O_2$	292.5
17	12.12	31.810	Phytol	$C_{20}H_{40}O$	296.5
18	2.17	32.107	Methyl stearate	$C_{19}H_{38}O_2$	298.5
19	1.41	34.867	3-Cyclopentylpropionic acid, 2-dimethyl aminoethyl ester	$C_{14}H_{27}NO_2$	213.32
20	2.47	39.773	Stigmast-5-en-3-ol, (3. BETA.)-	$C_{29}H_{50}O$	414.7
21	6.88	41.422	.beta.-Amyrin	$C_{30}H_{50}O$	426.7
22	2.57	41.765	Valerenol	$C_{15}H_{24}O$	220.35
23	1.23	43.158	Cyclopentanol, 3,3,4-trimethyl-4-p-tolyl-, (R, R)-(+)-	$C_{15}H_{22}O$	218.33
24	6.03	43.365	.alpha.-Amyrin	$C_{30}H_{50}O$	426.7
25	5.67	43.408	Lupeol	$C_{30}H_{50}O$	426.7
26	3.48	44.891	4,4-Dimethyl-3-(3-methyl but-3-enylidene)-2-methylenebicyclo[4.1.0]heptane	$C_{15}H_{22}$	202.33
27	9.11	46.726	3-Oxatricyclo[20.8.0.0(7,16)]triaconta-1(22), 7(16), 9,13,23,29-hexaene	$C_{29}H_{42}O$	406.6
28	1.06	48.081	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	430.7
29	1.37	48.894	Retinol	$C_{20}H_{30}O$	286.45
	100.00				

Table 4: GC-MS screened phytoconstituents with their documented pharmacological applications

S. No	Name of the compound	Compound nature	Pharmacological/Biological activity
1.	Piperazine, 2,5-dimethyl-(2-Tert-butoxy-1-methylethyl) benzene	heterocyclic amine	antimicrobial, antiproliferative, antioxidant, anti-inflammatory, antifilarial, neuroprotective [28]
2	N, N- Dimethyl glycine	derivative of amino acid glycine	Treatment of autism and pervasive developmental disorder [29].
3.	p-Vinyl guaiacol	phenolic acid metabolite	anticancer [30]
4.	Dehydro-.beta.-ionone	β-carotene derivative	antiproliferative [31]
5.	3-Hydroxy-.beta.-damascone	carotenoid-derived aroma compounds	anti-inflammatory, anti-cancer [32]
6.	trans-10,11-Epoxyfarnesenic acid methyl ester	terpene	antioxidant, anticarcinogenic, antibacterial, and antifungal,neuroprotective, juvenile and gonadotropic hormone activity [33]
7	Ketoborneol	monoterpenoid	acesodyne, sedation, antiinflammation, antibiosis, antiproliferative, antioedematogenic [34]
8	Methyl phenanthridine	phyto sterol	analgesic, antitussive, antimalarial, cytotoxic, anticonstipation [35, 36]
09	Neophytadiene	diterpenoid	anti-inflammatory, cardioprotective, antioxidant [35]
10	Methyl palmitate	fatty ester	anti-inflammatory, antifibrotic [37]
11	Linolenic acid, methyl ester	fatty ester	anti-proliferative, anti-diabetic, anti-inflammatory [38]
12	Phytol	diterpene	antimicrobial, anticancer, anti-inflammatory, diuretic [39]
13.	Stigmast-5-en-3-ol, (3.BETA.)-	sterols	anti-proliferative, cholesterol reducing, antidiabetic [40]
14.	Beta Amyrin	triterpenoid	analgesic, anti-inflammatory, antinociceptive, antiulcer, gastroprotective, hepatoprotective, larvicide, mosquitocide [39]
15.	Cyclopentanol, 3,3,4-trimethyl-4-p-tolyl-, (R, R)- (+)-	sesquiterpenoid	antibacterial, antifungal, anti-inflammatory, cytostatic and specific enzyme inhibition [41]
16.	Alpha Amyrin	triterpenoid	anti-inflammatory, antioxidant, anti-ulcer, anti-hyperlipidemic, anti-tumor, hepatoprotective [42]
17.	Lupeol	triterpenoid	antiangiogenic, antimalarial, antiviral, antiflu, antihyperglycemic, anti-inflammatory, antilithic, cytotoxic, antioxalate, antioxidant, antiperoxidant, antiprostaglandin, antirheumatic, antitumor, antiurethrotic, antiedemic, hypotensive [39]
18.	dl-.alpha.-Tocopherol	vitamin E	antialzheimeran, antianginal, antiarthritic, antiasthmatic, antiatherosclerotic, anticancer, anticataract, anticonvulsant, antidementia, antidiabetic, antiinfarctal, antiinfertility, antimutagenic, antioxidant, antineuropathic, antiparkinsonian, antirheumatic, antistroke, cancer-preventive, cardioprotective, immunomodulatory [30]
19.	Retinol	vitamin A	Normal body physiology growth regulation, vision, reproduction, cellular differentiation proliferation, bone and brain development, hematopoiesis [39]

be due to the absence of activity or the novel compounds yet be studied. This needs further investigation on the identification of beneficial active principles.

Among the screened metabolites Dehydro-.beta.-ionone, 3-Hydroxy-.beta.-damascone, trans-10, 11-Epoxyfarnesenic acid methyl ester, Megastigmatrienone, and Megastigmatrienone 4 are appreciated for their aromatic compounds used as flavouring agents, whereas Ketoborneol has camphor fragrance [34]. Out of the nineteen medicinally potent phytoconstituents twelve (66.66%) are reported to have natural anti-inflammatory activities, thirteen (72.22%) have antiproliferative, antitumor, anticancer activities, seven (38.88%) have antioxidant principles and six (33.33%) showed antimicrobial properties. Few of them exhibited hepatoprotective, analgesic, antiangiogenic, antidiabetic, cardioprotective and neuroprotective activity regularly, in addition to the diverse activities listed in Table 4.

The above metabolites based on their functional groups belong to sterols, amino acid derivatives, phenolic acid derivatives, sesquiterpenes, triterpenes, diterpenes, monoterpenes, methyl esters, vitamins A and E etc. Interestingly, the highest percentage of them belong to terpenoids and their derivatives, signifying their considerable pharmacological bioactivities.

The leaf of well-known anti-inflammatory ethnomedicinal herb Camellia japonica contained lupeol an anti-angiogenic, anti-cancer and anti-inflammatory component [40] . Borneol, a terpene derivative present in *W. contracta* leaf and also in Artemisia, *Blumea balsamifera*, *Kaempferia galanga* are reported to increase the delivery and distribution of compounds into the brain, including nanoparticles [34]. The traditional medicinal herbs of Orchidaceae, Dioscoreaceae, Combretaceae, and Betulaceae families contain considerable amounts of phenanthrenes, which possess cytotoxicity, antimicrobial, spasmolytic, anti-inflammatory, antiplatelet aggregation, and phytotoxicity [36]. Neophytadiene, a molecule isolated from the marine algae Turbinaria ornata showed both *in vitro* and *in vivo* anti-inflammatory activity [37]. The methanol extracts of Ceriops decandra leaves, an ethnomedicinal mangrove plant contained phytoconstituents Lupeol and Stigmast-5-en-3-ol. The phytol extracted from the leaves of Rhizophora mucronata showed cytotoxicity against Human gastric adenocarcinoma (AGS) cells. These reports provide evidence that the high potential bioactive compounds screened in the leaf methanolic extracts of *W. contracta* may also contain anticancer and other pharmacological properties.

Thus, the profound pharmacological influence of the identified phytoconstituents substantiates the potential therapeutic properties of *W. contracta*, as a new source of medicine. Consequently, being a close relative of predominant traditional medicinal genus Sida and Abutilon, *W. contracta* has been rightly proved to possess diverse medicinally valuable properties.

4 CONCLUSION

To validate the medicinal potency of *W. contracta* as a source for novel drug for future use, the prospecting of phytochemicals was undertaken. It can be concluded from the results that *W. contracta* contain a consortium of pharmacologically active compounds and may become a promising phytodrug for various ailments, owing to its fair resemblance with the related species of Malvaceae. The parameters selected for the study are apt in depicting the possession of a broad spectrum of SMs. The methanol extract emerged as a most potent one carrying diverse therapeutically active phytoconstituents. Further, the data of GC-MS screening disclosed the presence of nineteen pharmacologically active constituents out of twenty-eight phytochemicals, giving scope for the possession of some novel unidentified drug molecules. As per the literature presence of anti-inflammatory, antioxidant, anticancer, antimicrobial activities, antidiabetic and neuroprotective effects in addition to their acesodyne, sedation, antileishmanial, antifilarial, antibiosis, and cholesterol-lowering,antidiabetic, anxiolytic, antifibrotic, antinociceptive, immune-modulating activities can be expected from *W. contracta*, owing to the presence of terpenes as predominant group of phytoactive principles, followed by methyl esters, derivatives of carotenoids, phenolic acids, amino acids, tocopherols, and retinols. These phytoactive compounds and their derivatives could form a new basis for the development of novel drugs against diverse ailments.

The current investigation opens up further research work on the pharmacological, and phytochemical aspects to elucidate the medicinal value of *W. contracta*.

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