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Original Article

Phytochemical Analysis, Antioxidant, and Anti-Microbial Activities of Suaeda vermiculata n-Hexane Extract in Comparison to the Plant's Hydrodistilled Volatile Oil

Mohsen S. Al-Omar[1,2,*]

ABSTRACT

Background: *Suaeda vermiculata*, a halophyte found in central Saudi Arabia, used as a remedy for jaundice, liver diseases, and viral infection. **Study aim:** Chemical identification and biological evaluation for the n-hexane extract of *S. vermiculata*. **Methods:** An assay of the antimicrobial and antioxidant potentials and contents of the volatile oil and n-hexane extract of the plant's aerial parts were analyzed through GC-MS analysis. **Results:** A total of 24 constituents representing 73.74 % of the *n*-hexane extract were identified wherein bornyl acetate, γ-elemene, and phytol were major ratio. The *n*-hexane extract inhibited DPPH-radicals inhibitions at 27% efficiency at 10 mg/ml concentration which was lower than the volatile oil DPPH-reactivity. The antimicrobial activity of *n*-hexane extract was relatively weaker than the volatile oil, except against *Candida albicans* that showed IZD values of 19 mm with MIC value at 5.2 mg/ml for the volatile oil while IZD value of 26 mm and MIC at 4.7 mg/ml was observed for the *n*-hexane extract. **Conclusion:** Despite high extractive value, the *n*-hexane as a solvent is not recommended for extraction as the oxygenated-terpenic components, considered responsible for antioxidant and antimicrobial activities were not fully extracted. The *n*-hexane extract which showed potent inhibition of *C. albicans* can be a source for further investigation for bioactivities of its constituents.

Key words: *Suaeda vermiculata*, *n*-hexane extract, Volatile oil, Antimicrobial activity, Antioxidant activity.

Mohsen S. Al-Omar[1,2,*]

[1]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, Qassim University, Buraydah, 51452, KINGDOM OF SAUDI ARABIA.
[2]Medicinal Chemistry and Pharmacognosy Department, Faculty of Pharmacy, JUST, Irbid 22110, JORDAN.

Correspondence

Mohsen S. Al-Omar

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, Qassim University, Buraydah, 51452, KINGDOM OF SAUDI ARABIA; Medicinal Chemistry and Pharmacognosy Department, Faculty of Pharmacy, JUST, Irbid 22110, JORDAN.

E-mail: m.omar@qu.edu.sa

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INTRODUCTION

Volatile oils are considered as the main odoriferous components in the plants and are mainly composed of mixtures of different compounds of monoterpenic and/or sesquiterpene hydrocarbons as well as oxygenated hydrocarbons[1]. Due to their important applications in medicinal and cosmetic preparations[2], volatile oils have been studied from varieties of several thousands of plant species. The extraction, componential analysis, structure, and identification of volatile oil components from their natural sources have been frequently studied (Abad, *et al.* 2012; Giacometti, *et al.* 2018). Traditionally, volatile oils are also medicinally used as a remedy for the treatment of several conditions, such as bacterial infections, drowsiness, flatulence, gastritis, body-ache, productive cough, nasal congestion, and urinary tract infections[5-8]. These oils are important components of cosmetics, and pharmaceutical preparations of toothpaste and mouthwashes[9,10], and are also used as part of salads, food preservatives, and flavoring agents[11].

Volatile oils have been extracted from several plants by employing different methods which include direct solvent extraction, normal distillation, distillation under vacuum, scarification, and microwave-assisted extractions[12-14]. Also, there have been several other studies conducted to compare the yields and componential efficiencies of the volatile oil extractions by different methods of procurement[15-17]. Some of these studies are focused on the volatile oil production by solvent-based isolation procedures which details the effects of volatile oil constituents of a particular plant[14,18], as well as changes in the biological activities following the particular isolation procedures[19-26]. According to several other reports, significant changes in the productivity, compositions, and biological activities are well-documented for the volatile oils isolated from particular plants by different isolation procedures. *S. vermiculata* Forssk. ex. J.F.Gmel. is a halophytic plant growing in the central areas of Saudi Arabia (Mohammed, *et al.* 2019) is traditionally used as a remedy for liver diseases, jaundice, and infections (Mohammed, *et al.* 2019; Mohammed 2020a; Mohammed 2020b). Broad-spectrum biological activities have been discovered for *S. vermiculata*, which includes antibacterial, antifungal, and antioxidant[24,26]. Also, *S. vermiculata* contains high proportions of pheophytin, a pigment, which had been known for their significant antioxidant and mild cytotoxic activities[22]. The plant also has economic value as it is used as a camel-feed by nomadic Bedouin[22].

S. vermiculata volatile oil was investigated in a previous study, and the chemical and biological activity comparisons between two halophytic plants; *S. vermiculata* and *S. cyclophylla* were reported[23]. However, due to low oil yield extraction

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by hydrodistillation process from the aerial parts of the *S. vermiculata* plant, another method employing solvent extraction was planned to maximize the yield and avoid the oil loss during the hydrodistillation process. The current study was planned to investigate the differences in oil quality, contents, and bioactivity of the oils obtained between hydrodistillation and solvent extraction processes of oil procurements from *S. vermiculata*. It employed *n*-hexane as a non-polar solvent for direct extraction in a continued and comparative investigation of the previous report.

MATERIALS AND METHODS

Plant materials and extraction procedure

The plant material was collected in September 2017 from Buraydah, Qassim region of Saudi Arabia (Figure 1). The plant was identified by Prof. Dr. Ahmed El-Oglah as *S. vermiculata* Forssk and the plant sample as number 78 was deposited in the herbarium of the College of Pharmacy, Qassim University. The plant materials were dried in shade for three weeks and grinded to a coarse powder using a mechanical grinder. Accurately, 200 gm of the plant powder were macerated overnight with gentle shaking on a mechanical shaker with 500 ml of *n*-hexane. The *n*-hexane extract was double-filtered using Whatman™ filter paper and evaporated under vacuum using Rotary evaporator at room temperature. The residue was stored in a -20 °C freezer.

Gas chromatography-mass spectroscopy (GC-MS) analysis

n-Hexane extract was analyzed by GC-MS instrument (Shimadzu GC-MS-QP2010, Koyoto, Japan) equipped with Rtx-5MS fused bonded column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) (Restek, USA). The analysis conditions for column temperature and flow rate in addition to injector temperature and flow rate of the helium carrier gas were identical to the literature [23]. The mass spectra were recorded under these conditions: filament emission current, 60 mA; ionization voltage, 70 eV; ion source, 200° C. Diluted samples (1% v/v) were injected with split mode (split ratio 1: 15). The data available in the library of the National Institute of Standards and Technology (NIST LIB) was matched to the mass fragmentation spectra of the components. Also, the retention time recorded for the volatile oil constituents in addition to experimental retention index carried out for a series of *n*-alkanes of C_{10} to C_{40} hydrocarbons under similar conditions for the volatile oil analysis was used in the volatile oil constituents elucidations in comparison with retention time (RT) and retention indexes (RI) recorded in the literature under similar conditions of the experiments.

Antioxidant activity of *n-hexane* extract against 1, 1-diphenyl-2-picrylhydrazyl (DPPH)

The potential ability of n-hexane extract to quench free radicals was evaluated against DPPH stable free radicals[27]. Serial dilution from n-hexane extract and positive controls quercetin and Trolox (100 µl) were mixed with 1900 µl of DPPH solution (300 µM). The mixtures were then incubated at room temperature for 20 min. The reduction in the DPPH color was measured at 517 nm by the spectrophotometer. The method was conducted in triplicate and the scavenging activity of extract and standard were calculated to form the following equation:

$$\text{Scavenging activity } \% = \left(1 - \frac{\text{Ab (test)}}{\text{Ab (blank)}}\right) \text{X } 100$$

The Ab (test) is referring to absorbance obtained from the mixture of the test samples with the DPPH solution, Ab (blank) is the absorbance obtained from the DPPH solution only at 517 nm.

Determination of antimicrobial activity

Microorganisms

Gram-positive bacteria; *Staphylococcus aureus,* Gram-negative bacteria *Escherichia coli,* and fungal microorganism; *Candida albicans* were used in the antimicrobial assay.

Agar well-diffusion method

Agar well diffusion method was used according to the literature [28]. The prepared microbial suspensions were incubated till showed turbidity comparable to the reference, 0.5 McFarland tube. The agar surface was smeared with sterile swaps and incubated for a few minutes. Accurately,

Figure 1: *Suaeda vermicuata* Frossk.

50 µl of the n-hexane extract (10 mg/ml in DMSO) was micropipette to the designed cups. Positive control drug discs (10 µg/ml amoxicillin, gentamycin and clotrimazole) were used as positive controls. Plates were then incubated (Sheldon Manufacturing INC., USA) for 24-72 hours and were checked every 24 h for the growth of microorganisms. Inhibition zone diameters (IZD) were visualized and measured to the nearest millimeter.

Minimal inhibitory concentration (MIC) measurement

MIC was measured similarly to the method described in the antimicrobial assay of *S. vermiculata* volatile oil[23]. The bacterial microorganisms were incubated at 37° C for 24 h in Mueller Hinton broth supplemented with Tween 80 detergent (final concentration of 0.5 % (v/v), while *Candida albicans* was incubated at 30° C for 24 h in Sabouraud dextrose broth-SDB + Tween 80. Serial dilutions of volatile oils were prepared in a 96-well microplate. Also, one growth control (MHB + Tween 80) and one sterility control (MHB + Tween 80 + test oil) were prepared. Plates were incubated at 37° C for 24 h for bacteria and at 30° C for 48 h for the *Candida albicans*. The white turbidity in the well bottom indicates bacterial growth[29].

RESULTS AND DISCUSSION

Due to volatile oil's importance and applications in the medical, culinary, and industrial fields, researchers have been interested in volatile oil studies (Böhme, *et al.* 2014; Hanif, *et al.* 2019). Comparison between extraction procedures aimed to enhance the yields and quality of the volatile oils were one of the major investigational interests[30-33]. *S. vermiculata* is an important halophytic plant used in traditional medicine in inflammation, pain, and liver disorders as well as is used as camels' feed[22]. The antimicrobial and antioxidant activities of *S. vermiculata* volatile oils isolated by hydrodistillation technique was investigated earlier (Mohammed, *et al.* 2019). Also, the antimicrobial activity of *S. vermiculata* volatile oil against *P. aeruginosa* and *E. coli* Gram-negative bacteria in addition to *Candida albicans* was confirmed (Mohammed, *et al.* 2019). The study also confirmed that *S. vermiculata* volatile oil prepared by hydrodistillation was inactive against Gram-positive bacteria *S. pyogenes, S. epidermidis,* and *S. aureus*[23].

The volatile oil constituents of *S. vermiculata* were extracted by *n*-hexane non-polar solvent, analyzed by GC-MS, and compared to the plant volatile constituents, obtained as oil, isolated by hydrodistillation procedure. The odor of *n*-hexane extract of *S. vermiculata* after complete dryness was similar to the odor of the volatile oils obtained from the plant by hydrodistillation. However, the color of the n-hexane extract was yellowish-green in contrast to the volatile oil yellowish-brown color. The greenish color of the *n*-hexane extract might have been retained due to the presence of a high proportion of the dark-green pheophytin, a pigment found in the plant (Mohammed, *et al.* 2019). The higher lipophilic character of the pheophytin has potential for its partial solubility in the *n*-hexane extract[22] and thus retained in the extract. The yields obtained from the *n*-hexane extraction of *S. vermiculata* was comparatively higher than that obtained from the hydrodistillation procedure. The yield percentage from the hydrodistillation was reported as 0.11 % (225 mg oil out of 200 gm of the plant dried powder)[23] in comparison to 0.87% (1756 mg extract out of 200 gm of the dried plant powder) obtained from the *n*-hexane extraction of the plant. The higher yield obtained from *n*-hexane extract is mainly attributed to the presence of the volatile and the non-volatile substances that have been extracted by the *n*-hexane.

The results of GC-MS analyses of *n*-hexane extract of *S. vermiculata* hydrodistillation (Table 1) revealed that *n*-hexane as a solvent has a better capability to extract more compounds that can affect the biological activity. The total identified compounds in *n*-hexane extract of *S. vermiculata* by GC-MS analysis were twenty-four which represented 73.74 % of the extract weight. In contrast to the reported compounds in the oil obtained by the hydrodistillation process, more products were identified in the n-hexane extract. The presence of hexenal, camphene, hexanoic acid, methyl decanoate, elemol, nonadecane, phytol, tricosane, pentacosane, hexacosane, heptacosane, β-sitosterol, and β-amyrin were exclusive to the n-hexane extract composition. However, some of these compounds were identified with a high proportional percentage such as nonadecane and phytol which were represented by 4.51 % and 10.14 %, respectively. The presence of phytol, β-amyrin, and β-sitosterol in the *n*-hexane extract of *S. vermiculata* is also compatible with the reported phytochemicals isolated from the plant (Mohammed, *et al.* 2019). The results in table 1 for the relative percentage of some monoterpenes also reflected the differences between the non-polar extraction and hydrodistillation process of *S. vermiculata* to yield the volatile oil.

For instance, linalool, fenchol, β-terpineol, borneol, and terpinen-4-ol were only represented in the hydro-distillate of the plant. Also, some of the volatile constituents were more represented in the plant hydrodistillation product such as eucalyptol, camphor, and α-terpineol that showed 4.49, 28.74, and 22.78 % in the plant distillate in contrast to 0.85, 2.76, and 5.47 % in *n*-hexane extract of *S. vermiculata*, respectively. These results reflected the inability of *n*-hexane and a non-polar solvent to extract alcoholic monoterpenes which could be more extracted by the more polar organic solvents such as ethyl acetate and methanol[30,31]. This proposition was also supported by the relative percentage of α-terpinene and bornyl acetate which were more represented in *n*-hexane extract (Table 1). Also, the relative percentage of total oxygenated monoterpenes and sesquiterpenes showed in Table 1 indicated that the hydrodistillation process is preferred to recover monoterpenes while non-polar solvents such as *n*-hexane are recommended for the sesquiterpenes extractions. The overall results in Table 1 reflected the ability of *n*-hexane to dissolve some of the plant's volatile oil constituents and the potential capability of the solvent to recover the higher non-volatile constituents of the plant such as phytol and β-sitosterol.

Anti-oxidant activity of *S. vermiculata n*-hexane extract and volatile oils distillate

The *n*-hexane extract of *S. vermiculata* was compared to the published data of the plant volatile oil (Mohammed, *et al.* 2019). The results in Figure 2 revealed that *S. vermiculata* volatile oil was more active in quenching DPPH free radicals at higher concentrations than the *n*-hexane extraction product. The differences in the antioxidant activity of *n*-hexane extract and volatile oil distillate of *S. vermiculata* are mostly related to the presence of high proportions of oxygenated monoterpenes in the volatile oil distillate of the plant compared to the non-polar extract obtained by *n*-hexane[30,31]. The presence of high proportions of α-terpineol, camphor, and borneol in the volatile oil distillate is also one of the reasons for the activity of *S. vermiculata* volatile oil distillate over *n*-hexane extract[34]. Figure 2 also showed weak DPPH-radicals quenching activity for the *n*-hexane extract of *S. vermiculata* compared to BHA, only at the higher concentrations 2.5 to 10 mg/ml) while volatile oil distillate of the plant comparatively quenched DPPH-free radicals better than BHA but significantly lower than quercetin.

Antimicrobial activity of *S. vermiculata n*-hexane extract and volatile oils distillate

The antimicrobial activity of *n*-hexane extract of *S. vermiculata* was tested by two different methods against microbial pathogens of Gram-positive, Gram-negative, and *C. albicans* fungal microorganisms. The results were compared to the reported data of the volatile oil distillate of the plant. Results in Table 2 revealed higher antimicrobial activities for the volatile oil distillate over the *n*-hexane extract of the plant, e.g., *n*-hexane extract was inactive against *S. pyogenes,* S. *epidermidis* which

Table 1: Chemical composition of the *n*-hexane extract of *S. vermiculata* Forssk herb.

Name	RI[E]	RI[R]	%RP (n-hexane extract)	%RP (hydrodistillation)*
Hexenal	805	806	0.09	
Camphene	943	943	0.33	
α-Terpinene	1016	1016	2.44	0.35
Hexanoic acid			0.23	
Eucalyptol	1029	1031	0.85	4.49
Linalool	1100	1082		0.89
Fenchol	1114	1120		0.44
trans-Pinocarveol	1140	1140	0.11	0.74
Camphor	1145	1149	2.76	28.74
β-Terpineol	1149	1158		0.5
Isocamphopinone (mono ketone)	1161	1170	0.82	0.24
Borneol	1167	1170		33.77
Terpinen-4-ol	1178	1178		2.38
Neoisomenthol	1186	1188		0.34
α-Terpineol	1191	1197	5.47	22.78
Dodecane	1196	1199	3.68	1.18
Bornyl acetate	1288	1286	11.04	0.68
Methyl decanoate		1325	3.15	
β-Damascenone	1387	1383	2.66	0.49
γ-Elemene	1431	1432	9.55	1.17
Elemol		1545	7.12	
trans-Longipinocarveol	1667	1651	1.72	0.82
Nonadecane	1902	1900	4.51	
Phytol	2130	2129	10.14	
Tricosane	2312	2300	2.11	
Pentacosane	2509	2500	0.94	
Hexacosane	2605	2600	0.71	
Heptacosane	2702	2700	0.16	
β-Sitosterol	3327	3327	2.21	
α-Amyrin	3390	3386	0.94	
	Total %		73.74	100
	Oxygenated Monoterpenes		21.37	95.99
	Monoterpene Ketones		3.76	28.98
	Monoterpene Alcohols		17.70	61.84

*Reported results obtained from Mohammed *et al.* 2019[23]
[E]Experimental Retention index (*RI*) using a series of n-alkanes (C_{10}–C_{40}) under identical experimental conditions.
[R]Reported retention index according to the NIST library and published literature data calculated under identical experimental condition

Table 2: Antimicrobial Activity of the n-hexane extract of *S. vermiculata* Forssk Herb.

Extract	Volatile oil distillate *		n-hexane extract		Gentamycin	Amoxicillin	Clotrimazole
Microorganism	IZD**	MIC[b]	IZD	MIC		IZD	
Streptococcus pyogenes	7	na	na	nd	nd	nd	nd
Staphylococcus epidermidis	9	na	na	nd	16	nd	nd
Staphylococcus aureus	nd	na	na	nd	20	9	nd
Pseudomonas aeruginosa	15	66±3.1	10 mm	105±4.15	9	15	nd
Escherichia coli	10	85±2.2	7 mm	197.5±12.8	14	13	nd
Candida albicans	19	5.2±2.7	26 mm	4.7±2.1	nd	nd	16

*Reported results obtained from Mohammed et al, 2019 [23] **IZD were conducted in triplicate and calculated to the nearest mm inhibition including the well or disk (6 mm). na= not active at the higher dose, nd= not determined.

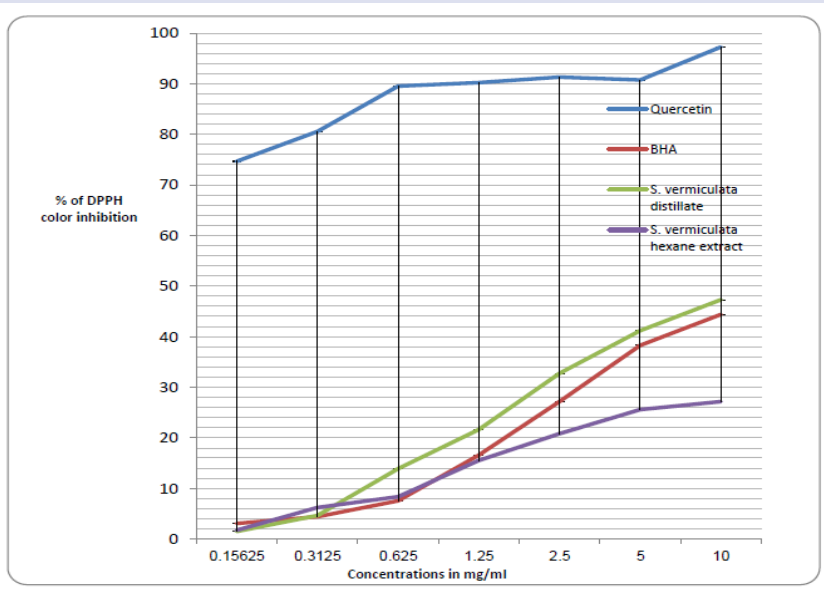

Figure 2: Scavenging Effect of the *S. vermiculata n*-hexane extract and volatile oils distillate.

were weakly inhibited by the volatile oil distillate with IZD values of 7 and 9 mm, respectively. Similar results were also shown for the effects of the plant on *P. aeruginosa* and *E. coli* where volatile oil distillate was moderately inhibiting their growth with IZD values of 15 and 10 mm and MIC values at 66 and 85 mg/ml, respectively. However, *n*-hexane extract weakly inhibited the growth of these bacterial microorganisms with IZD values of 19 and 26 mm and MIC values 105 and 197 mg/ml, respectively. Furthermore, Both plant products, *i.e.*, volatile oil distillate and *n*-hexane extract actively inhibited the growth of fungal strain, *C. albicans* with IZD values of 19 and 26 mm and MIC values 5.2 and 4.7 mg/ml, respectively.

CONCLUSION

The current study provides in-depth chemical and biological activity comparisons between the *n*-hexane extract and volatile oil distillate of *S. vermiculata*. More constituents were identified in the *n*-hexane extract of *S. vermiculata* than the plant's volatile oil distillate. Oxygenated monoterpenes were abundant in the volatile oil distillate while sesquiterpenes and aliphatics were more encountered in the *n*-hexane extract. Overall results concluded that *n*-hexane extract of the plant was less active than the volatile oil distillate as an antioxidant and antimicrobial agents. The better activities of the volatile oil distillate are considered mostly related to the higher abundance of oxygenated monoterpenes in the oil.

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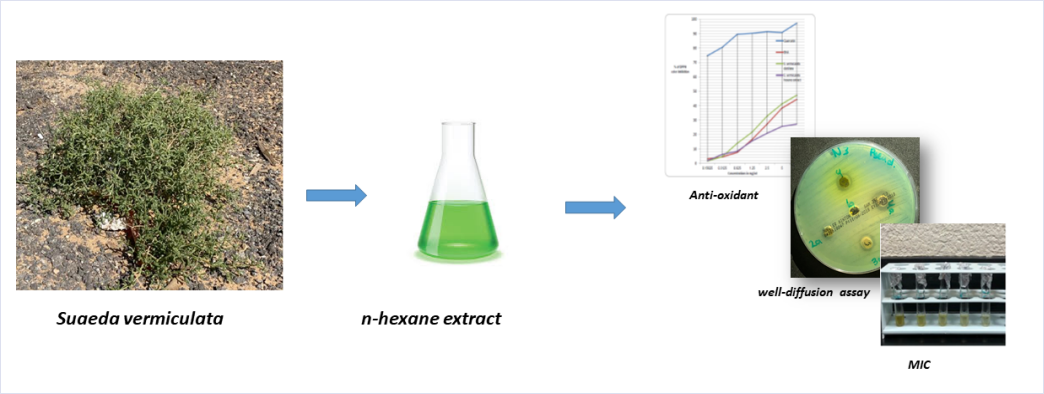
GRAPHICAL ABSTRACT



ABOUT AUTHORS

PhD Degree in Pharmacy (Med. and Nat. Prod. Chemistry), Univ. of Iowa, USA, 1994. Assistant Dean, 1996-1998; Chairman of Medicinal Chemistry and Pharmacognosy Department, Faculty of Pharmacy, JUST, Jordan, 2003-2006; Associate Professor, Faculty of Pharmacy, Applied Sciences University, Amman, Jordan 2009- 2010; Associate Professor, Faculty of Pharmacy, Umm Ul-Qura University, Mecca, KSA 2010- 2012; Associate Professor & Dept. Supervisor, Faculty of Pharmacy, Qassim University, KSA, 2013-2019.
The research interest focused on chemistry of natural products and their biological evaluation (0000-0001-8134-9623). Patent registration in the United States patent and trademark office with a serial No. 08/413,797.

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