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PHYTOCONSTITUENTS, ANTIOXIDANT AND ALLELOPATHIC PROPERTIES OF *SUAEDA AEGYPTIACA* (HASSELQ.) ZOHARY EXTRACT ON *CHENOPODIUM MURALE*

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Abstract

Suaeda (seep weeds) is one important halophytic genus of the Chenopodiaceae family and it consists of 100 species worldwide, distributed in coasts, deserts, lakeside and saline and alkaline land all over the world. This study aimed to screen the chemical constituents of *Suaeda aegyptiaca* collected from from Shbani, Rawa City, Al Anbar Governorate, Iraq and evaluate its the antioxidant and allelopathic potential. The methanolic extract of *S. aegyptiaca* was analyzed via GC/MS. Antioxidant activity was evaluated based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicle scavenging. The main active components in *S. aegyptiaca* were: Benzene,1,1'-(3-methyl-1,3-butadienylidene)bis-(CAS); 2-[2-[(p-Chlorophenyl) oxy]-5-methylphenyl]-1-methoxy-1,2-diphenyl-ethene; Pregnane-11,20-dione,3,17,21-tris [(trimethylsilyl)oxy]-, 20-[O-(phenylmethyl) oxime], (3à,5à)-. In the DPPH test system, the IC_{50} value of the antioxidant inhibition for *S. aegyptiaca* was 40.64 mg mL^{-1}. The methanolic extract from *S. aegyptiaca* revealed significant allelopathic activity against the weed, *C. murale* in a concentration-dependent manner, at the high concentration of extract (1 mg mL^{-1}), the germination, root and shoot growth of *C. murale* were reduced by 76.73, 79.34 and 82.33%, while the lowest concentration (0.2 mg L^{-1}) inhibited the germination by 8.68%, 15.96% and 27.66%, respectively, compared to control. The present study revealed that the methanolic extract of the *S. aegyptiaca* could be considered as promising, eco-friendly natural resources for antioxidants as well as weed control particularly the nuisance weed *C. murale*.

Key words: *Suaeda*, bio-herbicides, phytotoxicity, antioxidant, GC/MS, weed control.

Introduction

Plants are the oldest friends of mankind; they not only provided food and shelter, but also, served the humanity to cure different aliments. According to the World Health Organization (WHO), about three-quarters of the world population relies upon traditional remedies (mainly herbs) for health care (Calixto, 2005). Natural plant products could also prove useful in minimizing the adverse effects of various chemotherapeutic agents as well as in prolonging longevity. The global interest in the biological assessment of plants during the last few decades is therefore quite logical (Kaushik and Dhiman, 2000).

Research on plant secondary metabolism has been reported to possess various biological activities including antioxidant, allelopathic (Abd El-Gawad *et al*., 2018a&b; Elshamy *et al*., 2019), antifungal, antibacterial, antiviral (El-Amier *et al*., 2014a; El-Amier and Abo Aisha, 2019), anti-inflammatory (Tohidi *et al*., 2017), drugs (Zaki *et al*., 2016a & b, 2017, 2018) and insecticidal (Castillo *et al*., 2017) activities. In addition, the wild plant is a good source of food preservation industries, livestock fodder (Zahran and El-Amier, 2013), fibers (Zahran and El-Amier, 2014), fragrance industries and agro-industrial (Zuin and Ramin, 2018; Alzuaibr *et al*., 2020) and phytoremediation (El-Amier *et al*., 2018a & b; El-Alfy *et al*., 2020)

Medicinal plant in Iraq can be traced back to the Sumerian period (3000-1970 B.C.) and then to the Babylonian and Assyrian period of culture and civilization about two thousand B.C. Inrecent years, the world's efforts have directed towards the utilization of renewable resources of the cultivated and non-cultivated areas to produce more food and forage. Such efforts would be more successful and fruitful if they are based on previous knowledge of the environmental characteristics comprising soil, climate, vegetation, animal and human interference (Clark, 2000; Zahran and El-Amier, 2013;

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El-Amier and El-Hayyany, 2020). Halophytic plants have been used for medicinal purposes because of the presence of heath promoting bioactive compound (Ksouri *et al*., 2012).

Suaeda is one important halophytic genus of the family Chenopodiaceae, known as seep weeds or sea blite. The genus *Suaeda* comprises of 100 species worldwide, distributed in coasts (El-Amier *et al*., 2014b), deserts (El-Amier and Abdul-Kader, 2015), lakeside and saline and alkaline land all over the world (Boulus, 2002). *S. aegyptiaca* (Hasselq.) Zoharyis an annual herb, 60-100 cm herb, variable in shape, ascending and sub woody at the base (Ali and El-Gady, 1989; Boulus, 2002). Plants of *S. aegyptiaca* are distributed throughout Arabia in saline habitats especially on coasts and it is grow in rather different plant communities and even as weed in irrigated gardens and fields (Waisel, 1994).

Some species of *Suaeda* possess hypoglycemic, antiinflammatory, hypolipidaemic, cardiotonic, antioxidant, antimicrobial and anticancer activity (Bennani-Kabchi *et al*., 1999, Benwahhoud *et al*., 2001; Al-Ani *et al*., 2011; Oueslati *et al*., 2012a). Being a halophyte, *Suaeda* grow in stressful conditions that encourage the biosynthesis of a wide variety of bioactive metabolites. Halophytes are able to overcome the oxidizing stressful agents due to their powerful antioxidant system which includes enzymatic and non-enzymatic components (Ksouri *et al*., 2008; Oueslati *et al*., 2012b).

No previous study in Iraq conducted on *S. aegyptiaca* extract as allelopathic effect on nuisance weeds, but some studies were conducted to evaluate the antimicrobial activity of *S. aegyptiaca* extract. Therefore, this research aims to determine the phytochemical constituents and antioxidant activity of aerial parts of *S. aegyptiaca* collected from Shbani, Rawa City, Al-Anbar Governorate, Iraq, as well as to assess the allelopathic potential of the crude extract against the noxious weed *Chenopodiummurale* as potential green eco-friendly bioherbicide.

Materials and Methods

Plant material

The aerial parts of S*uaeda aegyptiaca* (Hasselq.) Zoharywere collected during the flowering stage from different sites near Shbani, Rawa City, Al-Anbar Governorate, Iraq. The identification of species was identified according to Boulos, (1999) by Dr. Yasser A. El-Amier, Lecturer of Plant Ecology, Faculty of Science, Mansoura University, Mansoura, Egypt. The aerial plant parts of this plant were washed with distilled water several times and were dried at room temperature. The dried sample was ground into a powder using a blender and kept in plastic bags at 4°C until use.

Gas Chromatography/Mass Spectrometry (GC/MS)

The methanolic extract obtained from wild halophyte *S. aegyptiaca* was subjected to Gas Chromatography and Mass Spectroscopy for the determination of bioactive compounds. Some of the important features are summarized below.

GC-MS analysis of the sample was carried out using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30m, 0.251mm, of 0.1 mm film thickness). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used, Helium gas was used as the carrier gas at a constant flow rate of 1mL/min. The injector and MS transfer line temperature were set at 280ºC. Vol. oil: The oven temperature was programmed at an initial temperature of 40ºC (hold 3 min) to 280ºC as a final temperature at an increasing rate of 5ºC /min (hold 5 min). Sap: The oven temperature was programmed at an initial temperature of 150ºC (hold 4 min) to 280ºC as a final temperature at an increasing rate of 5ºC /min (hold 4 min). unsap: The oven temperature was programmed at an initial temperature of 50ºC (hold 2 min) to150ºC at an increasing rate of 7ºC /min. then to 270 at an increasing rate of 5ºC /min (hold 2min) then to 310 as a final temperature at an increasing rate of 3.5ºC /min (hold 10 min). The quantification of all the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC/MS system.

Antioxidant activities

Antioxidant activity was determined in methanolic extract of the dried plant as described by Kosem *et al.,* (2007) with slight modifications as follows: about 20 g of powdered samples were extracted with 200 ml of methanol 70% for a week at room temperature. The extract was then collected and filtered through Whatman No.1 filter paper in a Buchner funnel under vacuum. Antioxidant activity was determined by using a stable free radical (1,1-diphenyl-2-picrylhydrazyl) DPPH (Miguel, 2010). Briefly, 2 ml of 0.15 mM DPPH was added to 2 ml of plant extracts in different concentrations (100-1000 ppm). A control was prepared by adding 2 ml of DPPH to 2 ml solvent. The mixture was kept in the dark at 37ºC for 30 min. The absorbance was recorded at 517 nm and the IC_{50} was calculated graphically. The antioxidant activity was expressed as:

$$\%\ Radical\ scavenging\ activity\ \% = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

Allelopathic activity

The seeds of *Chenopodium album* were collected from cultivated fields in Haditha City, Al-Anbar Governorate, Iraq. Uniform and ripened seeds were sterilized by soaking for 3 min in NaOCl (0.3%), then washed by sterile-distilled water three times and dried on sterilized Whatman cellulose filter paper and kept in sterilized bottles until further use.In order to test the phytotoxic activity, two layers of Whatman No. 1 filter paper were placed in 7 cm diameter glass Petri dishes. In each petri-dish 20 seeds were placed and 4 ml of each plant extract added in a concentration of 0.2, 0.4, 0.6, 0.8 and 1 mg mL^{-1} and incubated in the growth chamber at 27°C (Abd El-Gawad and El-Amier, 2015 & 2018b). The experiment was designed with three replications for each treatment and was repeated two times. After seven days of incubation, the germinated seeds were counted as well as the root and shoot lengths of all seedlings were measured. The inhibition of either germination or seedling length was calculated as follows:

$$\text{Inhibition (\%)} = 100 \times \frac{(\text{No/Length of control} - \text{No/Length of tretamnet})}{\text{No/Length of control}}$$

Results and Discussion

Phytoconstituents (GC/MS in the methanolicextract)

The methanolic extract of *S. aegyptiaca* was analyzed via GC/MS. The chromatogram exhibiting the main compounds of overall identified compounds in studied sample was presented in fig. 1. The results of chemical analysis of *S. aegyptiaca* active components by GC-MS are presented in table 1. The main active components in *S. aegyptiaca* were: Benzene,1,1'-(3-methyl-1,3-butadienylidene)bis-(CAS) (8.55%), 2-[2-[(p-Chlorophenyl) oxy]-5-methylphenyl]-1-methoxy-1,2-diphenyl-ethene (7.81%), Pregnane-11,20-dione,3,17,21-tris [(trimethylsilyl)oxy]-, 20-[O-(phenylmethyl) oxime], (3à,5à)- (6.94), 3-Hydroxy -1- (4- {13-[4-(3-hydroxy-3-phenylacryloyl) phenyl] tridecyl}-phenyl)-3-phenylprop-2-en-1-one (5.65%), 1,2-Benzenedi carboxylic acid, bis(2-ethylhexyl) ester(CAS) (5.26%) and Hexadecanoic acid, methyl ester (5.04%).

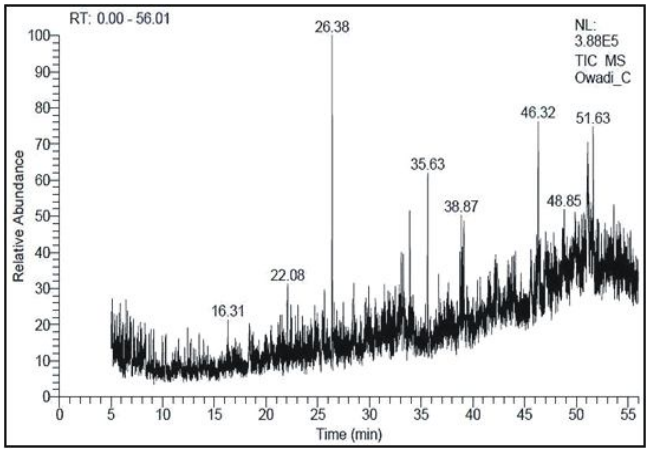

Fig. 1: GC-MS chromatogram of methanolic extract of *Suaeda aegyptiaca*.

Antioxidant activity

The methanolic extract from *Suaeda aegyptiaca* showed significant antioxidant activity referred to the ascorbic acid as standard (Table 2). The scavenging activity was significantly increased in a dose-dependent manner. At 50 mg mL^{-1}, the extract showed scavenging activities of 55.23% while, the lowest concentration (10 mg mL^{-1}) showed the lowest antioxidant activity (15.79%). The IC_{50} value of *S. aegyptiaca* extract was 40.64 mg mL^{-1} compared to standard ascorbic acid (13.84 mg mL^{-1}). Based on the data of IC_{50} values, the ascorbic acid (standard antioxidant) revealed 3-fold of the antioxidant compared to the MeOH extract of *S. aegyptiaca* (Table 2).

The coastal *S. aegyptiaca* is grown in a habitat with high salinity (salt-tolerant plant). Habitats and environmental conditions such as salinity inducelead to the production of phenolic compounds as a mechanism of acclimatization to stressful conditions. These bioactive compounds act as antioxidants that mitigate the effect oxidative stress and scavenge the ROS (Abd-ElGawad *et al*., 2019a & b; Abd-El-Gawad, 2016; Elshamy *et al*., 2019).

In addition, the antioxidant activity of the halophytes could be ascribed to the high content phenolic compound. It was reported that the total phenolic content of *Thymus vulgaris* and *Thymus daenensis* was induced by 20% after the application of 60mM NaCl and in consequence improved the antioxidant capacity (Bistgani *et al*., 2019).

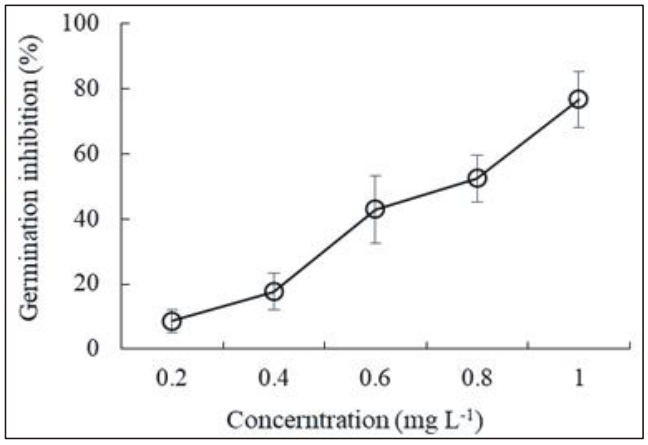

Fig. 2: Allelopathic effect of different methanol extracts from *Suaeda aegyptiaca*aerial parts on the seed germination inhibition percentage of *Chenopodium album*.

Table 1: GC/MS analysis of the active components of *Suaeda aegyptiaca* methanolic extract.

No.	Retention time	Compound	Area %	Molecular Formula
1	26.38	Benzene,1,1'-(3-methyl-1,3-butadienylidene)bis-(CAS)	8.55	$C_{17}H_{16}$
2	28.36	Pregnane-11,20-dione,3,17,21-tris [(trimethylsilyl)oxy]-, 20-[O-(phenylmethyl) oxime], (3à,5à)-	6.94	$C_{37}H_{63}NO_5Si_3$
3	33.07	11,23-Di-tert-butyl-5,17-diethoxy carbonyl-25,26,27,28-tetra hydroxycalix[4] arene	4.06	$C_{42}H_{48}O_8$
4	33.27	Ygrkkrrqrrrgpvkrrlfg/5	4.13	-
5	33.91	Hahnfett	4.44	-
6	35.63	Hexadecanoic acid, methyl ester	5.04	$C_{17}H_{34}O_2$
7	38.74	9-Desoxo-9-xihydroxy-3,7,8,9,12-pentaacetateingol	3.45	$C_{30}H_{42}O_{11}$
8	38.87	2,2'-Dibromo5,5'-di(4-methoxyphenyl)-4,4'-di-tert-butylbiphenyl	3.31	$C_{34}H_{36}Br_2O_2$
9	38.99	Cyclopropanebutyric acid,2-[(2-nonyl cyclopropyl) methyl]-, methyl ester	3.45	$C_{21}H_{38}O_2$
10	39.12	Phytol	4.47	$C_{20}H_{40}O$
11	39.44	Octadecanoic acid, methyl ester (CAS)	3.15	$C_{19}H_{38}O_2$
12	42.35	03027205002 FLAVONE 4'-OH,5-OH,7-DI-O-GLUCOSIDE	3.31	$C_{27}H_{30}O_{15}$
13	45.62	Cantaxanthin	4.82	$C_{40}H_{52}O_2$
14	46.32	1,2-Benzenedi carboxylic acid, bis(2-ethylhexyl) ester(CAS)	5.26	$C_{24}H_{38}O_4$
15	47.04	3-Hydroxy-1-(4-{13-[4-(3-hydroxy-3 phenylacryloyl) phenyl] tridecyl}-phenyl)-3-phenylprop-2-en-1-one	5.65	$C_{43}H_{48}O_4$
16	47.22	Dipyridamole	2.48	$C_{24}H_{40}N_8O_4$
17	48.76	2,2'-Dibromo5,5'-diphenyl-4,4'-di-tert-butylbiphenyl	3.14	$C_{32}H_{32}Br_2$
18	49.89	4-(4-Chlorophenyl)-1-(phenanthren-9-yl) isoquinoline	4.73	$C_{29}H_{18}ClN$
19	50.75	Epinephrinetetratms	3.83	$C_{21}H_{45}NO_3Si_4$
20	51.10	2-[2-[(p-Chlorophenyl) oxy]-5-methylphenyl]-1-methoxy-1,2-diphenyl-ethene	7.81	$C_{28}H_{23}ClO_2$
21	51.45	diacetyldeuteroporphyrin-XIIIdimethyl ester	3.18	$C_{36}H_{38}N_4O_6$
22	51.64	Cyclohexane,1,4-dimethyl-2-octadecyl-	4.32	$C_{26}H_{52}$

Similar results were reported by Amin and Musa, (2016) on same species in Al-Jouf Area, KSA, El-Amier and Abo Aisha, (2019) on *Fagonia*; Alzuaibr *et al.,* (2020) on *Diplotaxis harra* and El-Amier and Al-hadithy, (2020) on *Aizoon canariense* and Abd-ElGawad *et al*., (2020a &b) on coastal *Heliotropium curassavicum* and *Reichardia tingitana*. At present, wild plants are gaining popularity in developing countries as a medicinal food. Although medicinal plants may cause many biological activities in humans, very few are known. Although previous studies have shown the presence of active biomaterials in methanol extract (Akowuah *et al.*, 2002; El-Amier *et al*., 2014).

Table 2: Percentage of DPPH radical scavenging activity and IC_{50} values of methanolic extracts of *Suaeda aegyptiaca*.

Treatment	Concentration (mg mL^{-1})	Scavenging activity (%)	IC_{50} (mg mL^{-1})
Suaeda aegyptiaca	50	55.23±1.81	40.64
	40	52.61±1.20	
	30	45.34±0.67	
	20	23.71±0.35	
	10	15.79±0.23	
Ascorbic acid			13.84

Values are means ± standard error of triplicates. IC_{50}: the amount of sample necessary to decrease the absorbance of DPPH by 50%.

Allelopathic effect of the MeOH extract

The methanolic extract from *S. aegyptiaca* revealed significant allelopathic activity against the weed, *C. murale* in a concentration-dependent manner (Fig. 2-4). The shoot was more sensitive to the allelopathic effect compared to root, at the high concentration of extract (1 mg mL^{-1}), the germination, root and shoot growth of *C.*

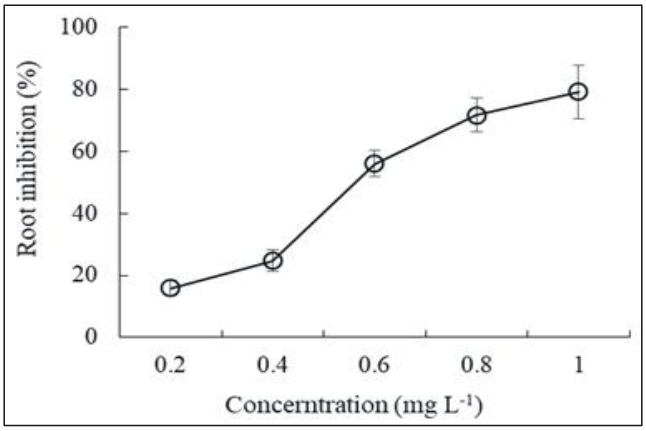

Fig. 3: Allelopathic effect of different methanol extracts from *Suaeda aegyptiaca* aerial parts on the root growth inhibition percentage of *Chenopodium album.*

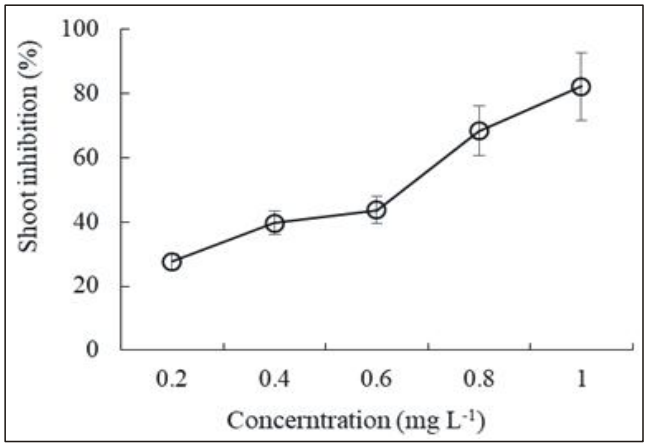

Fig. 4: Allelopathic effect of different methanol extracts from *Suaeda aegyptiaca* aerial parts on the shoot growth inhibition percentage of *Chenopodium album*.

murale were reduced by 76.73, 79.34 and 82.33%, while the lowest concentration (0.2 mg L^{-1}) inhibited the germination by 8.68%, 15.96% and 27.66%, respectively, compared to control (Fig. 2-4). The allelochemicals may have phytotoxicity or stimulation depend on the nature and concentration of the compound, the assay species, edaphic factors and physical/climatic factors (Abd El-Gawad *et al.,* 2018a&b). Similar results were reported by Abd El-Gawad, (2016), El-Amier and Abo Aisha, (2019) and Alzuaibr *et al.,* (2020) on some desert plants.

Theallelopathic activity of the coastal wild plants may be due to the high content of gallic and chlorogenic acids. Phenolic acids are considered as the most common and effective allelochemicals in the ecosystem (Li *et al*., 2010; Abd-El-Gawad *et al.*, 2019b; Elshamy *et al*., 2019). Phenolics diffuse into the environment and inhibit germination and growth when absorbed by plants (Inderjit, 1996; Abd-El-Gawad *et al*., 2020 b & c). In general, the extracts of coastal wild plants showed more allelopathic effect than the inland samples. This may be attributed to the effect of habitats, particularly the coastal habitat is stressful than the inland habitat due to the effect of salinity, where the plants produce more bioactive compounds in order to tolerate the harsh conditions (Vafadar Shoshtari *et al*., 2017; Abd-El-Gawad *et al*., 2019b & 2020b).

Conclusions

The GC-MS analysis of methanolic extracts of *Suaeda aegyptiaca* collected from Shbani, Rawa City, Al-Anbar Governorate, Iraq, revealed the presence of 22 compounds. The main active components in *S. aegyptiaca* were: Benzene,1,1'-(3-methyl-1,3-butadienylidene)bis-(CAS); 2-[2-[(p-Chlorophenyl) oxy]-5-methylphenyl]-1-methoxy-1,2-diphenyl-ethene; Pregnane-11,20-dione,3,17,21-tris [(trimethylsilyl)oxy]-, 20-[O-(phenylmethyl) oxime], (3à,5à)-. Based on *S. aegyptiaca* attained high content of various secondary metabolites and therefore can be considered a good source of natural antioxidant. In our study, germination of *C. murale* was inhibited under treatment of *S. aegyptiaca* methanolic extracts at 1 mg mL^{-1}. Moreover, both radicle and plumule were strongly inhibited under the same treatment. Therefore, this species can be used in the method of biological control of weeds. Also, further studies are required to identify and characterize the proper allelochemicals and demonstrate their modes of action.

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