Egypt. J. Chem. **Vol. 67**, No. 7 pp. 183 - 188 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Insecticidal and Phytochemical Constituents of *Centaurea Aegyptiaca* L. against Cotton Aphid, *Aphis Gossypii* Glov.

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Abstract

The chromatographic separation of *Centaurea aegyptiaca* L. methanolic extract successively yielded four main fractions of different polarities. Among the tested fractions, butanol and ethyl acetate fractions were the most toxic against cotton aphid, *A. gossypii* using spraying method technique under laboratory conditions after 24 and 72 h of treatment. Application of optimal chromatographic separation of *C. aegyptiaca* butanol and ethyl acetate fractions afforded six secondary metabolites which were isolated and identified belonging to two main classes flavonol glycosides, the new 6-hydroxy-3-methoxykaempferol-8-*O*-*β*-D-glucopyranoside (centaegyin) **1** beside the three 6,8-dihydroxykaempferol-3-*O*-*β*-D-glucopyranoside **2**, astragalin **3** and populnin **4** those reported from the plant for the first time and two elemanolide sesquiterpenes, methyl 8*α*-(3,4-dihydroxy-2-methylene-butanoyloxy)-6*α*,15-dihydroxy-elema-1,3,11(13)-trien-12-oate **5** and methyl 8*α*,6*α*,15-trihydroxy-elema-1,3,11(13)-trien-12-oate **6**. Assessment of the toxic effect of the isolated compounds, from the active fractions, against *A. gossypii* revealed that flavonoids **1, 2** and **3** were the active principles in the butanol fraction after 24 and 72 h of treatment. The isolated compounds were structurally identified using various spectroscopic analyses (ESI-MS, ^{1}H-NMR, ^{13}C-NMR, H-H COSY, HSQC and HMBC).

Keywords: *Centaurea aegyptiaca*; flavonol glycosides; elemanolides; *Aphis gossypii*

1. Introduction

Aphis gossypii Glover (Homoptera: Aphididae) is the most prevalent agricultural insect that reduces cotton productivity and quality. Small and slow-moving, cotton aphids typically feed on the under surface of cotton leaves by sucking sap from the plant with their piercing mouthparts. In addition to causing feeding damage and spreading various viruses, cotton aphids are responsible for the reduction in cotton yields and quality. Additionally, *A. gossypii* produces honeydew, a sticky substance that sticks to leaves and encourages the formation of sooty mold, which lowers cotton fiber quality and output [1, 2].

The chemical control has been the main method used against *A. gossypii* in Egypt. The exaggeration of nonselective synthetic insecticides to suppress the pest infestations poses a various threats to the environment, non-targeted organisms and human health and increases production costs of up to 20 percent [2- 5].

Recently, natural products are considered to be an alternative approach and substitute in biological control that preserves the environment from pollution. Therefore, more attention and research trials are paid by several scientists all over the world for natural products to be used as natural pesticides replacing the synthetic substances [4].

The Egyptian Murrar Masry, *Centaurea aegyptiaca* L. (Asteraceae), is a perennial herb widely distributed along the Red Sea coastal strip, the Egyptian desert, Gebel Elba and Sinai [6]. Sesquiterpene lactones are the main distributed phytochemical [7], as well as flavonoids, phenolic acid derivatives [6, 8], sterols, triterpenes and lignins [9].

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EJCHEM use only: Received date 29 October 2023; revised date 10 December 2023; accepted date 18 December 2023

DOI: 10.21608/EJCHEM.2023.245267.8792

Ethyl acetate fraction of *C. aegyptiaca* displayed a potent acaricidal activity against adult females of *Tetranychus urticae* and its active principles were syringaresinol and methyl 8α, 6α, 15-trihydroxy-elema-1,3,11(13)-trien-12-oate using leaf-dipping technique [9].

Our study was carried out and directed to assess the effect of the Egyptian *C. aegyptiaca* extracts as natural insecticides against the cotton aphid, *A. gossypii* and to characterize the bioactive ingredient of these extracts using various chromatographic and spectral techniques.

2. Materials and methods

2.1 Instruments

NMR spectra were obtained by either JEOL 500 or Bruker AMX 400 MHz Mansoura University. Chemical shifts are expressed in δ (ppm). LC/MSD IQ Infinity II 1260 equipped with Agilent was used to perform ESI-MS spectra, Mansoura University.

2.2 Chemicals

Silica gel Merck grain size 0.2-0.063 mm was used for normal column chromatography (C.C.); silica gel Merck GF 254 percolated plates on aluminium sheets 20x20 cm were used for thin layer chromatography (TLC) and preparative TLC. Loba Company, India provided the solvents, methanol, butanol, ethyl acetate, methylene chloride and pet ether (60–80°C).

2.3 Plant material

A specimen of *Centaurea aegyptiaca* L. in April 2021, was collected in the vicinity of Mansoura. Prof. Ibrahim A. Mashaly, who specializes in Plant Ecology at the Plant Department of the Faculty of Science at Mansoura University, identified the species.

2.4 Processing of the plant material

The whole *Centaurea aegyptiaca* L. air dried parts was processed typically as previously reported by Mostafa *et al*., (2021) [9]. The ethyl acetate fraction was subjected to polyamide CC. to yield seventeen sub-fractions. Sub-fractions XI was purified using PTLC and a mixture of CH_2Cl_2 /MeOH (98:2 v/v) to afford compound **5** (R*f* 0.38, 60 mg). Sub-fraction X was separated on PTLC by EtOAc/ MeOH (95:5) mixture to give **6** (R*f* 0.76, 50 mg).

Butanol fraction was also subjected to polyamide CC and gave ten sub-fractions. Sub-fraction III has been applied on PTLC Silica gel plates using EtOAc–MeOH–H_2O (85:12:3) as a developing system to afford compound **1** (R*f* 0.33, 105 mg), **2** (R*f* 0.20, 35 mg), **3** (R*f* 0.54, 38 mg) and **4** (R*f* 0.63, 21 mg).

6-hydroxy-3-methoxykaempferol-8-*O*-*β*-D-glucopyranoside (centaegyin) 1. Yellow residue. (105 mg); ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, *J*, Hz): 8.04 (2H, d, *J* 8.8 Hz, H-2'' 6'), 6.88 (2H, d, *J* 8.8 Hz, H-3', 5'), 5.15 (1H, d, *J* 7.4 Hz, H-1''), 3.46 (1H, m, H-2''), 3.42 (1H, m, H-3''), 3.35 (1H, m, H-4''), 3.20 (1H, m, H-5''), 3.68 (1H, dd, *J* 2.2, 11.8 Hz, H-6''), 3.54 (1H, dd, *J* 5.3, 11.8 Hz, H-6''), 3.85 (3H, s, -OCH_3); ^{13}C NMR (100 MHz, δ, ppm): 157.87 (C-2), 134.97 (C-3), 178.50 (C-4), 152.58 (C-5), 135.31 (C-6), 155.16 (C-7), 126.50 (C-8), 144.78 (C-9), 109.46 (C-10), 122.90 (C-1'), 132.16 (C-2'), 116.4 (C-3'), 161.69 (C-4'), 116.4 (C-5'), 132.16 (C-6'), 105.02 (C-1''), 75.60 (C-2''), 78.06 (C-3''), 71.19 (C-4''), 78.28 (C-5''), 62.47 (C-6''), 60.53 (-OCH_3).

6,8-dihydroxykaempferol-3-*O*-*β*-D-glucopyranoside 2. Yellow solid**.** (95 mg); ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, *J*, Hz): 8.04 (2H, d, *J* 8.7 Hz, H-2'' 6'), 6.88 (2H, d, *J* 8.7 Hz, H-3', 5'), 5.10 (1H, d, *J* 7.4 Hz, H-1''), 3.46 (1H, m, H-2''), 3.42 (1H, m, H-3''), 3.34 (1H, m, H-4''), 3.20 (1H, m, H-5''), 3.68 (1H, dd, *J* 2.6, 11.8 Hz, H-6''), 3.54 (1H, dd, *J* 5.2, 11.8 Hz, H-6''); ^{13}C NMR (100 MHz, δ, ppm): 158.61 (C-2), 135.35 (C-3), 178.93 (C-4), 147.83 (C-5), 136.38 (C-6), 151.24 (C-7), 130.11 (C-8), 138.75 (C-9), 108.70 (C-10), 122.72 (C-1'), 132.20 (C-2'), 116.16 (C-3'), 161.79 (C-4'), 116.16 (C-5'), 132.20 (C-6'), 104.65 (C-1''), 75.60 (C-2''), 78.00 (C-3''), 71.24 (C-4''), 78.32 (C-5''), 62.48 (C-6'').

kaempferol-*3*-*O*-*β*-D-glucopyranoside (astragalin) 3. Yellow solid. (73 mg); ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, *J*, Hz): 6.12 (1H, br.s, H-6), 6.29 (1H, br.s, H-8), 8.04 (2H, d, *J* 8.8 Hz, H-2'' 6'), 6.88 (2H, d, *J* 8.8 Hz, H-3', 5'), 5.10 (1H, d, *J* 7.4 Hz, H-1'')**.**

kaempferol-7-*O*-*β*-D-glucopyranoside (populnin) 4. Yellow solid. (18 mg); ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, *J*, Hz): 6.35 (1H, br.s, H-6), 6.49 (1H, br.s, H-8), 7.80 (2H, d, *J* 8.5 Hz, H-2'' 6'), 6.90 (2H, d, *J* 8.5 Hz, H-3', 5'), 5.35 (1H, d, *J* 7.6 Hz, H-1'')**.**

2.5 Insect collection and rearing

A. gossypii was collected from naturally infested cotton plants which were unsprayed before with any pesticides in greenhouse of the farm of Faculty of Agriculture, Mansoura University, Egypt.

2.6 Bioassays

C. aegyptiaca L. extracts and isolated pure compounds were emulsified in water using 0.1% triton X-100. Five serial diluted concentrations were

obtained and applied immediately *via* spraying using a plastic hand atomizer for each tested treatment. 0.1% triton X-100 in water was sprayed as the control treatment. After the spraying procedure, the leaves of each treatment and control were placed separately on a 2% agar bed in 9 cm-diameter Petri dishes to preserve moisture. Under laboratory setting, Petri dishes with leaves were incubated at 24 ± 3 ºC, 75 ± 8% relative humidity, and 12:12 hours light-darkness.

On an infested cotton leaf a total of counted one hundred individuals of *A. gossypii* have been placed in a Petri dish with a diameter of 15 cm. Spraying method technique was performed to treat every affected leaf with the selected aphis number [1]. Three replicates for each treatment were obtained and after 24 and 72 hrs of treatment the mortality was observed.

2.7 Statistical analysis

Abbott's formula was used to correct the mortality percentages [10] and LC_{50}, LC_{90} and slope values were evaluated in accordance to Finney (1971) [11]. The toxicity index was computed using Sun's equation for various fractions and isolated chemicals [12].

3. Results and discussions

The potential of production of a prototype ecofriendly botanical insecticides with low mammalian toxicity and reducing the usage of synthetic chemical insecticides is a global demand. Herein, examination the insecticidal activity of *Centaurea aegyptiaca* fractions against cotton aphid, *A. gossypii* using spraying method technique under laboratory conditions after 24 and 72 h of treatment were performed.

Strong aphidicial activity were recorded for butanol and ethyl acetate fractions after 24 and 72 hrs of exposure with LC_{50} 16.76, 47.23 and 10.07, 20.81 ppm, respectively. Aphidicial activity- guided isolation of six naturally metabolites from the promising fractions, which were spectrally identified and examined for their insecticidal activity against cotton aphid in order to deduce the active principles.

Various chromatographic technique have been resulted in isolation of six compounds, which spectrally elucidated using different 1D and 2D NMR analyses. The identified metabolites were belonging to two main classes (Fig. 1) four flavonol glycosides, and two elemanolides sesquiterpenes.

Compound **1** was isolated from butanol fraction as a yellow residue (R*f* 0.33, 105 mg), It gives a yellow colour when treated with *p*-anisaldehyde spray reagent and heating. Examination the positive ESI-MS of **1** showed *quasi*-molecular ions $[M+H]^+$ at m/z 495.0 corresponding to the molecular formulas $C_{22}H_{22}O_{13}$.

^1H and ^{13}C NMR data of **1** (table 1) displayed signals characteristic for flavonol glycosides. The observation of AA'BB' system at δ_H 8.04 ppm (2H, d, *J* 8.8 Hz, H-2', 6') δ_C (132.16) and 6.88 ppm (2H, d, *J* 8.8 Hz, H-3', 5') δ_C (116.14) has suggested a 4'-monosubstituted ring B.

On the other hand, Ring A was suggested to be tetra substituted pattern from the absence of its proton signals and the down-fielded carbon resonances at δ_C, 152.58 (C-5), 135.31 (C-6), 155.16 (C-7) and 126.50 (C-8) [13].

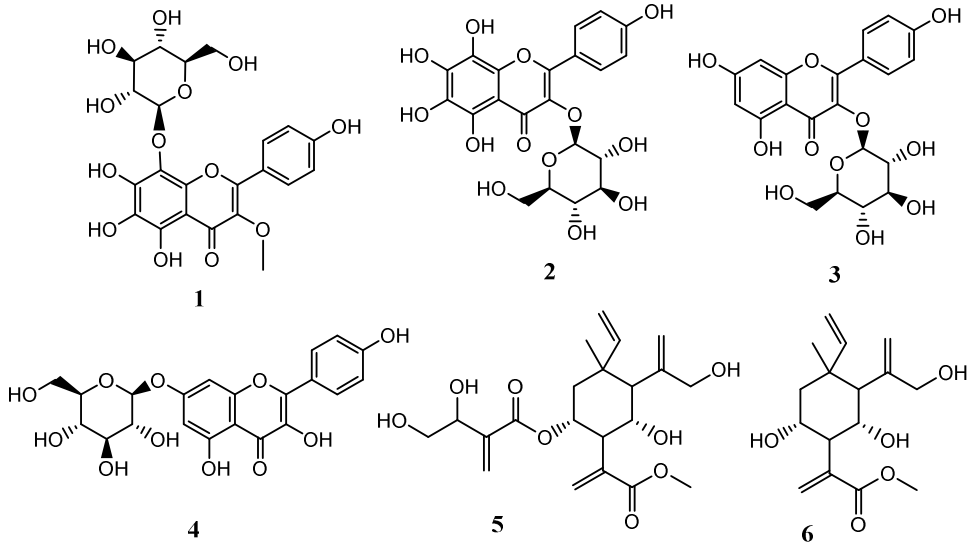
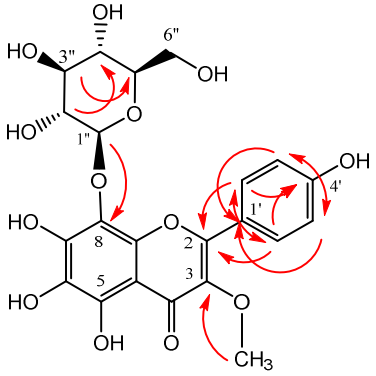


Fig 1: The isolated compounds from *Centaurea aegyptiaca* L.

The spectrum also showed a doublet appeared at δ_H 5.15 (1H, d, *J* 7.4 Hz, H-1"), in the aliphatic region which is belonging to the anomeric proton of the sugar moiety, whose configuration was determined to be *β*-configuration from its coupling constant value. ^{13}C NMR spectrum of **1** showed twenty two signals, six in the aliphatic region for the sugar unit and the remaining signals in the aromatic region for the flavonol unit. The presence of one *β*-glucopyranoside moiety was confirmed by HSQC experiment and by comparing its characteristic chemical shifts [14, 15]. Observation the long rang correlation HMBC of C-8 (Fig. 2) with the anomeric proton H-1", located the position of the attachment of the sugar moiety at C-8 carbon of the aglycone also, the methoxy protons was clearly correlated with C-3 so it confirmed its attachment at C-3. All the above mentioned data allow us to propose the structure of compound **1** to be 6-hydroxy-3-methoxykaempferol-8-*O*-*β*-D-glucopyranoside (centaegyin) which considered to be a new compound to the best of our knowledge.



1

Fig 2: Key HMBC correlations of compound 1

Butanol fraction also afforded three isolated flavonol glucosides different in their oxygenation and glycosidation pattern and was identified to be 6,8-dihydroxykaempferol-3-*O*-*β*-D-glucopyranoside **2** [16], kaempferol-*3*-*O*-*β*-D-glucopyranoside (astragalin) **3** [17] and kaempferol-7-*O*-*β*-D-glucopyranoside (populnin) **4** [18], these compounds were reported from this plant species for the first time.

Ethyl acetate fraction yielded two main elemanolides sesquiterpenes, were elucidated to be methyl 8*α*-(3,4-dihydroxy-2-methylene-butanoyloxy)-6*α*,15-dihydroxy-elema-1,3,11(13)-trien-12-oate **5** and methyl 8*α*,6*α*,15-trihydroxy-elema-1,3,11(13)-trien-12-oate **6**. These two sesquiterpenes were identified previously from *C. aegyptiaca* [9].

Table 1: ^{1}H and ^{13}C-NMR of compound 1

Position	δ_H (multiplicity, J)	δ_C
2	-	157.87
3	-	134.97
4	-	178.50
5	-	152.58
6	-	135.31
7	-	155.16
8	-	126.50
9	-	144.78
10	-	109.46
1'	-	122.90
2', 6'	8.04 (2H, d, 8.8 Hz)	132.16
3', 5'	6.88 (2H, d, 8.8 Hz)	116.14
4'	-	161.69
1"	5.15 (1H, d, 7.4 Hz)	105.02
2"	3.46 (1H, m)	75.60
3"	3.42 (1H, m)	78.06
4"	3.35 (1H,m)	71.19
5"	3.20 (1H, m)	78.28
6"a	3.68 (1H, dd, 2.2, 11.8 Hz)	62.47
6"b	3.54 (1H, dd, 5.3, 11.8 Hz)	
-OCH_3	3.85 (3H, s)	60.53

Insecticidal activity of *C. aegyptiaca* fractions and isolated compounds to cotton aphid, *A. gossypii*.

The crude extract as well as the four obtained fractions were assessed for their insecticidal activity under laboratory conditions against *A. gossypii* after 24 and 72 h of exposure (table 2). Among the tested fractions, butanol was the most toxic after 24 h of treatment followed by methanol, ethyl acetate, methylene chloride and the least one petroleum ether fractions. The LC_{50} values were 16.76, 41.61, 47.23, 73.21 and 214.19 ppm respectively. While after 72 h of treatment butanol was also the most toxic followed by ethyl acetate, methanol, methylene chloride and petroleum ether fractions. The LC_{50} values were 10.07, 20.81, 35.69, 51.49, and 130.42 ppm, respectively.

Tracking down the active principles in the most promising fraction was the main goal for this study. The activity of any extracts depends on the polarity and nature of its chemical constituents [1, 4, 9].

Assessment the insecticidal activity of the isolated compounds from the most promising fractions under laboratory conditions against *A. gossypii* after 24 h and 72 h of exposure was recorded in table 3. Compound **3** which was isolated from butanol fraction (most active fraction) exhibited a highly toxic activity on the basis of toxicity index followed by **1**, **2**, **6**, **4** and **5**, respectively after 24 h of treatment. While after 72 h were **3**, **2**, **1**, **4**, **6** and **5**, respectively.

Several studies reported the toxic properties of flavonoids [1, 4]. In addition, the cotton plant

synthesized flavonoids using specific defense-related enzymes through regulating phenylpropanoid metabolic pathway for defense response against cotton aphid [19].

Overall, the butanol fraction of *C. aegyptiaca* and its active flavonoids agents, centaegyin **1**, 6,8-dihydroxykaempferol-3-*O*-*β*-D-glucopyranoside **2**, astragalin **3**, could be a potential natural and renewable products in controlling of *A. gossypii* Glover.

Table 2. Toxicity of *C. aegyptiaca* extracts against cotton aphid, *A. gossypii* after after 24 and 72 h of treatment.

Plant extract	24 h of treatment				72 h of treatment			
	LC_{50} (ppm) and CL at 95%	LC_{90} (ppm) and CL at 95%	Slope	Toxicity index	LC_{50} (ppm) and CL at 95%	LC_{90} (ppm) and CL at 95%	Slope	Toxicity index
Methanol fraction	41.61 (23.55- 74.50)	1558.68 (373.31-535833.9)	0.81±0.26	40.27	35.69 (22.29-82.12)	861.64 (222.15- 131308)	0.93 ± 0.28	28.21
Pet. ether fraction	214.19 (145.84 - 402.33)	1722.82 (713.95-23119.47)	1.41±0.37	7.82	130.42 (80.97- 411.43)	2157.20 (578.40 -131430.69)	1.05 ± 0.28	7.72
Methylene chloride fraction	73.21 (28.73- 112.13)	702.11 (350.41-6399.07)	1.30±0.37	22.89	51.49 (22.42- 83.59)	691.13 (265.39- 30306.42)	1.14 ± 0.36	19.55
Ethyl acetate fraction	47.23 (29.08 - 112.47)	905.70 (260.95-35330.50)	1.00±0.26	35.47	20.81 (13.81-37.95)	246.22 (96.56- 2335.71)	1.19 ± 0.26	48.39
Butanol extract	16.76 (8.71 - 23.77)	93.54 (59.15-271.11)	1.71±0.40	100.00	10.07 (5.49- 14.31)	63.89 (37.44- 240.19)	1.60 ± 0.38	100.00

CL: confidence limits
Toxicity index = LC_{50} value of the most potent fraction's / LC_{50} value of other tested fraction's * 100

Table 3. Toxicity of *C. aegyptiaca* isolated compounds against cotton aphid, *A. gossypii* after after 24 and 72 h of treatment.

Fraction	Isolated compounds	24 h of treatment				72 h of treatment			
		LC_{50} (ppm) and CL at 95%	LC_{90} (ppm) and CLat 95%	Slope	Toxicity index	LC_{50} (ppm) and CL at 95%	LC_{90} (ppm) and CL at 95%	Slope	Toxicity index
Butanol	(1)	10.39 (2.50- 17.06)	231.63 (89.67- 8976.15)	0.95 ± 0.29	57.81	10.48 (6.85-15.24)	114.34 (52.97 -788.09)	1.23 ± 0.28	33.39
	(2)	19.43 (7.49-32.21)	426.82 (138.37- 45290.86)	0.96 ± 0.30	30.91	9.56 (4.92- 16.27)	313.50 (83.57-54897.07)	0.85 ± 0.26	36.60
	(3)	6.00 (0.62 -11.34)	75.78 (42.49- 546.08)	1.16 ± 0.36	100.00	3.50 (0.6 -6.06)	37.14 (21.4-185.81)	1.25 ± 0.35	100.00
	(4)	36.29 (23.10- 67.29)	1168.33 (318.47-56188.98)	0.85 ± 0.22	16.55	15.30 (8.95- 24.38)	208.28 (81.33-3899.69)	1.13 ± 0.30	22.87
Ethyl acetate	(5)	71.30 (52.71- 96.63)	312.82 (194.26- 837.45)	1.99 ± 0.39	8.42	44.93 (32.24-68.38)	258.26 (134.82-1238.67)	1.68± 0.38	7.79
	(6)	26.36 (8.87-41.27)	207.52 (120.20- 985.46)	1.43 ± 0.39	22.79	16.66 (6.60-25.29)	140.01 (75.64-854.43)	1.39 ± 0.38	21.00

4. Conclusion

Aphicidal activity directed fractionation of the methanolic extract of *Centaurea aegyptiaca* L to the most promising fractions ethyl acetate and butanol. Chromatographic and spectral analyses yielded six metabolites belonging to two main classes' flavonol glycosides and elemanolides sesquiterpenes, a new 6-hydroxy-3-methoxykaempferol-8-*O*-*β*-D-glucopyranoside was characterized from butanol fraction. Of the tested fractions and isolated compounds, butanol fraction of *C. aegyptiaca* and its active flavonoids, astragalin and centaegyin were the most potent against *A. gossypii* Glover. So, these active naturally principles are recommended for control of *A. gossypii* after investigation its field experiments.

5. Acknowledgment

The authors extend their appreciation to our collages at Plant Protection Research Institute, ARC.

6. Conflict of interests

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article."

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