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PHYTOCHEMICAL STUDY OF *MAERUA CRASSIFOLIA* FORSSK. GROWING IN EGYPT

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ABSTRACT

Phytochemical study of the aerial parts of Maerua crassifolia Forssk. resulted in the isolation and identification of kaempferol, quercetin, quercetin-3-O-arabinopyranoside, kaempferol-3-O-galactorhamnoside, rutin, lyoniresinol-3-O-glucopyranoside and stachydrine. These compounds were isolated and identified from this plant for the first time. The identification was based on physical, chemical and spectral studies including UV, IR, ^{1}H-NMR, ^{13}NMR and MS spectra.

INTRODUCTION

The genus *Maerua* (Family Capparaceae = Capparidaceae), is represented in Egypt only by two species which grow wildly in deserts *viz., M. crassifolia* Forssk. and *M. oblongifolia* (Forssk.) A.Rich [1]. In folk medicines infusion of the leaves of *M.crassifolia* is used for intestinal diseases, decoction of the leaves and bark is used as febrifuge for cephalagia, toothache, infected hairy skin. Mixture of powdered leaves. with henna leaves and fat is used for rapid healing of wounds and sores; cataplasm of this mixture reduced pain of bone fracture [2]. The alcohol extract

of the herb has neuromuscular blocking action and anti-tumor activity [3,4].

The interest in the phytochemical study of M. crassifolia Forssk. arises on the basis of recorded biological effects and folk medical uses of the plant, in addition to the lack of information about its constituents.

EXPERIMENTAL

General Experimental Procedure:

Melting points were uncorrected, ^{1}H and ^{13}C-NMR spectra were carried out in DMSO-d_6 at 400 MHz and 100.5 MHz, respectively. For column chromatography Amberlite IR A-45 (weak anion exchange resin) and silica gel (E.Merck) or wakogel C-200 (Japan) were used. Silica gel 60 F 254 (E.Merck) and cellulose Art. 2331 Merck (Avicel) were used for TLC. UV spectra were carried out using Hitachi 550, double beam spectrophotometer (Japan). IR spectra were carried out using IR spectrometer, JASCO A-302 (Japan). ^{1}H and ^{13}C-NMR spectra were recorded by ^{1}H and ^{13}C-NMR Brüker Am-400 (West Germany) Mass spectra were carried out using MS spectrometer Hitachi-M-80 (Japan). All the spectra were carried out in Tokyo College of Pharmacy, Tokyo, Japan.

Plant Material

N. Crassifolia Forssk. was collected from El-Hafafit in the Eastern desert in April 1987. The plant was kindly indentified by Prof. Dr. Nabil El-Hadidy Professor of taxonomy, Faculty of Science, Cairo University. Aerial Parts were dried and reduced to No. 40 Powder. A voucher sample is kept in the Dept. of Pharmacognosy, Faculty of Pharmacy, Assiut University.

Extraction and Fractionation:

Two Kg of the dried powdered plant was extracted with methanol by percolation till exhaustion. The methanolic extract was evaporated under reduced pressure (132 g). TLC of the extract showed the presence of triterpenes and/or sterols, flavonoids, glycosinolates and nitrogenous substances.

Seven compounds were isolated according to the following flow sheet.

Flow Sheet for the Isolation of the Constituents of *Maerua crassifolia* Forssk.

Maerua crassifolia herb (2.0 kg)

Hot MeOH ext. (4 x 2.5 L)

evaporated

semisolid residue (132 g)

mix with 500 ml H_2O

shake with Hexane (5 x 1.5 L)

Hydro-methanolic Fr.

shake with $CHCl_3$ (5x1.5 L)

Hexane Fr. (28 g)

The evaporated hydro-methanolic Fr. (68 g)

Amberlite (IR. A 45) col. chr.

(1:25), eluted with

The evaporated $CHCl_3$ Fr. (35 g)

H_2O (6 L)	H_2O-MeOH (6 L) 3:2	H_2O-MeOH (6 L) 1:1	H_2O-MeOH (6 L) 1:2	MeOH (6 L)
(14 g)	(9 g)	(3 g)	(3.6 g)	(4.3 g)

Silica gel col. chr. (1:35)

eluted with

($CHCl_3$-MeOH-H_2O)

80 :20 :0 (600 ml)

70 :27 :3 (500 ml)

collected in Frs. each 100 ml

glucose glycosinolate MA F_5

(1.7 g) (640 mg)

sucrose

Silica gel col. chr. (1:35) eluted with

($CHCl_3$-MeOH-H_2O)

85 :15 :0 (500 ml)

70 :27 :3 (800 ml)

collected in Frs. each 100 ml

F_3 ML F_4 F_5

(135 mg) (57 mg) (38 mg) (660 mg)

Silica gel col. chr.

(1:25) eluted with

($CHCl_3$-MeOH)

100.0 (400 ml)

80:20 (500 ml)

collected in Frs.

each 100 ml

F_1 (22 mg)

F_2 (114 mg)

Acid Hydrolysis [5]

Each isolated glycoside (5 mg) was dissolved in 5.0 ml MeOH to which 20% H_2SO_4 solution was added and the mixture was refluxed on a boiling water bath for 8 hours. A sample of the hydrolysate was withdrawn every 30 minutes and subjected to TLC. After complete hydrolysis, the mixture was cooled and the aglycone was separated by successive extration with chloroform. The chloroform was concentrated under reduced pressure and subjected to TLC using systems I, II and III.

The aqueous phase was neutralized with barium carbonate, filtered. The filterate was examined by PC for the liberated sugars using systems (IV, V, VI and VII) and appropriate authentic sugars using thymol-H_2SO_4 as spray resgent.

Solvent Systems:

The following solvent systems were used:

System I chloroform-methanol (9:1).
System II chloroform-methanol (85:15).
System III chloroform-methanol (8:2).
System IV chloroform-methanol-water (75:23:2)
System V chloroform-methanol-water (65:35:5).
System VI n-butanol-acetic acid-water (6:3:1).
System VII acetone-pyridine-water (3:1:1).

Characters of the Isolated Compounds:

Compounds F_1-F_5: gave yellow colours with ammonia vapour and 5% methanolic aluminium trichloride. Physical and chemical properties are compiled in Table 1, and their UV spectral data are summarised in Table 2.

Compound ML (57 mg): obtained as amorphous powder, melted at 103-105°C (methanol) $[\alpha]^{25}$+38.9° (methanol, C=0.2) UV λ_{max}^{MeOH} 225 and 278 nm, IR (KBr) ν cm^{-1} 3550, 3100, 2900, 1612, 1515, 1500, 1312, 1210 and 1105. +ve FAB-MS 583(M+1), 605($M+Na^+$), 621($M+K^+$), CIMS showed M at m/z 582(50%) other significant peaks appeared at m/z 420(M-hexose) and the base peak at m/z 167.

^{13}C-NMR (100.5 MHz, pyridine-d_5) δ 33.76(t, C-1), 49.69(d, C-2), 65.45(t, C-2α), 46.09(d, C-3), 71.69(t, C-3α), 42.27(d, C-4), 148.19(s, C-5), 138.60(s, C-6), 147.98(s, C-7), 107.43(d, C-8), 129.40(s, C-9), 126.52(s, C-10), 139.38(s, C-1`), 107.22(d, C-2`), 148.19(s, C-3`), 135.21(s, C-4`), 148.19(s, C-5`), 107.22(d, C-6`), 105.36(d, C-1``), 75.19(d, C-2``), 78.67(d, C-3``), 71.15(d, C-4``), 78.43(d, C-5``), 62.82(t, C-6``), 58.62(q, OMe-5), 56.01(q, OMe-7), 56.42(q, OMe-3`), 56.42(q, OMe-5`).

Compound MA (1.7 g): obtained as crystalline prisms, soluble in water, methanol and ethanol, insoluble in chloroform and benzene, very hygroscopic, anhydrous crystals melted at 232-233°C, the hydrochloride melted at 234-236°C, gave orange colour with modified Dragendorff's reagent. The MS revealed M^+ at m/z=143 and other peaks at m/z 114, 98 and 84.

RESULTS AND DISCUSSION

The methanolic extract of the aerial parts of *M. crassifolia* Forssk. gave positive tests for flavonoids. When chromatographed over Amberlite column, followed by silica gel column flavonoids, being weak phenolics, could be eluted from weak anionic (Amberlite IR A 45)

by hydromethanol, five-flavonoids were isolated (F_1-F_5), in addition to an alkaloid and a lignan glucoside.

The UV absorption spectra of the isolated flavonoids showed band I absorption at more than 350 nm indicating that they are flavonols. With NaOAC bathochromic shift in band I (more than +9 nm) were obtained indicating free OH group at C-7. Green colour obtained with $FeCl_3$ indicated the presence of OH group at C-5.

UV data, ^{1}H-NMR and ^{13}C-NMR (Tables 2,3 and 4) of compounds F_1 and F_2 are identical with those reported for kaempferol and quercetin respectively [6,7]. Identity was confirmed by cochromatography and mixed melting point determination with authentic samples.

The UV data of compound F_3 (Table 2) showed that the compound is a flavonol glycoside. ^{1}H-NMR data (Table 3) revealed the characteristic signals for quercetin [8], in addition to sugar protons. ^{13}C-NMR of F_3 (Table 4) showed characteristic signals for quercetin-3-O-glycoside. In addition, the ^{13}C-NMR revealed five sugar carbons (pentose) at δ 101.4, 71.6, 70.7, 65.9 and 64.2 ppm. Acid hydrolysis followed by TLC of the hydrolysate proved that the sugar part is arabinose, while the aglycone part is quercetin. The compound was identified as quercetin-3-O-arabinopyranoside [6].

UV data (Table 2) of compound F_4 showed that it is a flavonol-3-O-glycoside (λ_{max}^{MeOH} 358 nm band I) having free OH group at C-7. ^{1}H-NMR (Table 3) shows characterestic pattern for kaempferol and two anomeric protons, indicating that it is bioside. The anomeric proton at δ 4.4 (s) and methyl group at δ 1.1 (d, J=6.2 Hz) are characteristic for rhamnose. The other anomeric proton appeared as a doublet at δ 5.3(J=7.7 Hz). ^{13}C-NMR (Table 4) exhibited the characteristic signals for kaempferol as well as 12 carbon signals for two sugar moieties. Six of them are attributed to rhamnose (δ 100.2, 72.1, 70.8, 70.6, 68.4 and 18.0). The other six signals are characteristic for galactose [8,9]. Partial acid hydrolysis yielded rhamnose then galactose (identified by PC and TLC using authentic sugars rhamnose and galactose). The aglycone was identified as kaempferol (mp, and co-chromatography with authentic kaempferol). The compound was identified as kaempferol-3-O-rhamnosyl-galactopyranoside.

UV data of compound F_5 (Table 2) shows that it is a flavonol glycoside containing ortho dihydroxy group and free OH group at C-7. ^{1}H-NMR (Table 3) shows the characterestic pattern for quercetin [7] and two anomeric protons for two sugar moieties, one of them is rhamnose (δ 4.4, s and CH_3 at δ 1.0 d, J=6.1 Hz) the other proton at δ 5.3(d, J=7.2 Hz) for β-D-glucose. ^{13}C-NMR showed the characteristic signals for quercetin and other 12 carbon signals for rhamnose and glucose [9]. Partial acid hydrolysis gave rhamnose followed by glucose (identified by PC and TLC using authentic sugars). The aglycone was identified as quercetin. The compound was thus identified as rutin.

^{1}H-NMR spectrum of compound ML revealed signals for four aromatic methoxyl groups at δ 3.7(3H, s), 3.75(6H, s) and 3.77(3H, s). The aromatic part of the spectrum revealed two singlets assigned to three aromatic protons at δ 6.74(1H, s) and 7.05(2H, s). The

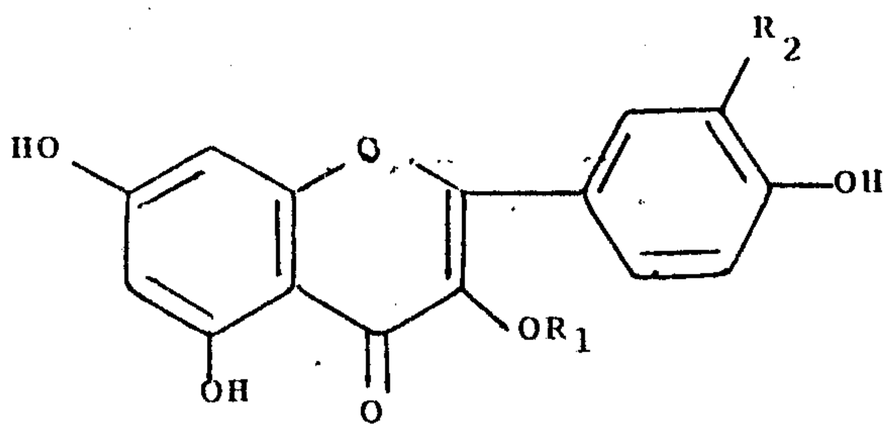
doublet at δ 4.98(1H, d, J=7.8 Hz) is referred to the anomeric proton of the β-D-sugar. The β-configuration of the glycosyl linkage was derived on the basis of the J value (7.8 Hz) of the anomeric proton in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum (pyridine-d5) showed 12 signals ascribable to two substituted benzene rings, 6 singals for glucopyranosyl residue, four aromatic methoxyls and two for carbinol carbons (2 and 3). This strongly suggests that the compound ML has lignan glycoside skeleton [10].

On acetylation (acetic anhydride + pyridine over night) it yielded hepta acetate melted at 85-87°C. Acid hydrolysis yielded an aglycone and a sugar identified as glucose (TLC, PC using authentic sample). CIMS exhibited a peak corresponding to (M^{+}-162) (M^{+}-hexose).

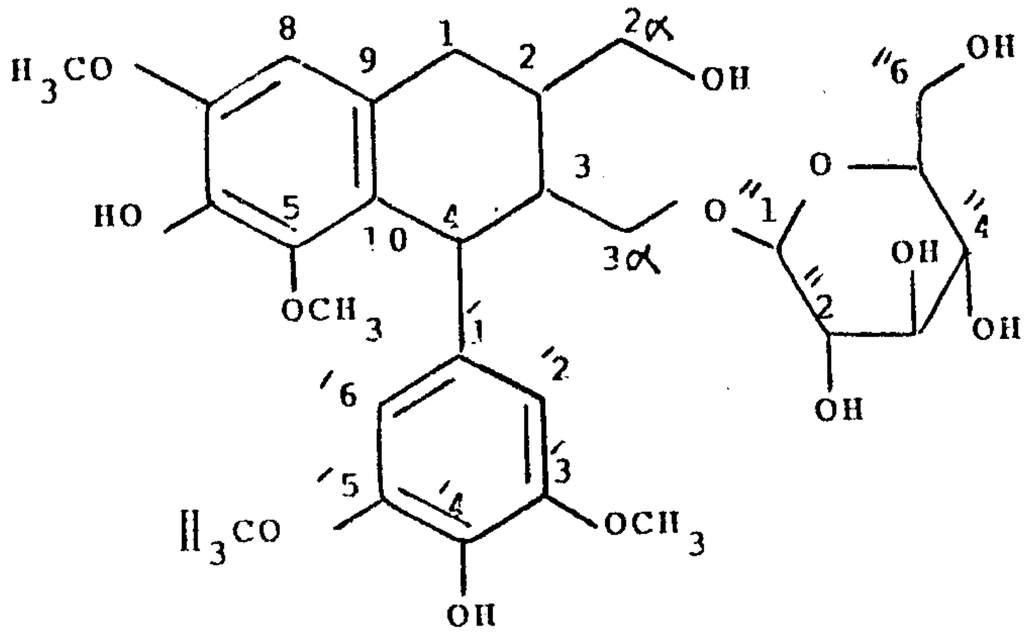
The above mentioned data favourably compare with those reported for lyoniresinol-3-O-β-glucopyranoside previously isolated from *Cinnamomum cassia* Blume [10].

The mass spectrum of compound MA (M^{+}=143) and the positive response with Dragendorff's reagent indicated its alkaloidal nature.

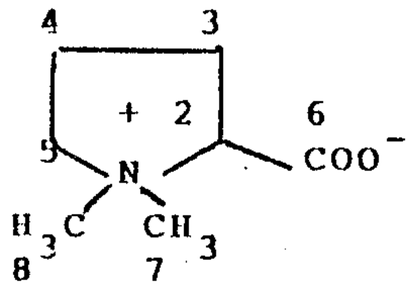
^{1}H-NMR spectrum (CD_3OD) showed two sharp singlets each integrating for three protons at δ 3.16 and 3.34 for N-$(CH_3)_2$. Other signals appeared at δ 2.1(2H, m, H_2-4), 2.3 and 2.5(m, H_2-3), 3.51 and 3.71(2m, H_2-5) and 4.04(1H, m, H-2). ^{13}C-NMR ($CDCl_3$) showed signals at δ 19.76(t, C-4), 26.62(t, C-3), 46.33(q, C-8), 52.73(q, C-7), 67.98(t, C-5), 77.63(d, C-2) and 170.69(s, CO). These data were found identical with those reported for the alkaloid stachydrine, which has been isolated from *Capparis tomentosa* [11] and *Courbnia glauca* [12].



Flavonoid	R2	R1
F1	H	H
F2	OH	H
F3	OH	arabinose
F4	H	galactose + rhamnose
F5	OH	glucose + rhamnose



compound ML



compound MA

Table 1: Physical and Chemical properties of the Isolated Flavonoids.

Compound Character	F_1 (22 mg)	F_2 (114 mg)	F_3 (135 mg)	F_4 (38 mg)	F_5 (1.3 g)
1-Melting point (°C)	285-286 (MeOH)	315-317 (MeOH)	230-231 (MeOH)	186-189 (MeOH-acetone)	190-192 (MeOH)
2-Condition	yellow needle crystals	yellow needle crystals	yellow long needles	yellow fine needles	yellow needle crystals
3-Solubility	soluble in most organic solvents insoluble in benzene and pet-ether		soluble in methanol, ethanol, butanol and H_2O		
4-Shinoda's colour test	orange colour in the organic layer (aglycones)		The colour in the aqueous layer (glycosides)		
5-$FeCl_3$ test	All give green colour, free hydroxyl group at C-5				
6-Colours of the spots under UV	yellow fluorescence		brownish	brownish	brownish
7-R_f Values	0.35 sys.I 0.49 sys.II	0.15 sys.I 0.32 sys.II	0.22 sys.IV 0.58 sys.V	0.15 sys.IV 0.45 sys.V	0.07 sys.IV 0.27 sys.V

Table 2: UV Spectral Data of the Isolated Flavonoids F_1-F_5

Compound	Band	Reagent: MeOH (λ_{max})	+$AlCl_3$		+$AlCl_3$ +HCl		+NaOAc		+NaOAc +H_3BO_3		NaOH	
F_1	I	371	432	+61	+432	+61	393	+22	378	+7	411	+50
		294	550				331				dec.	
	II	258	273	+5	272	+4	277	+9	264	+6	277	+9
		268							273			
F_2	I	372	466	+94	427	+55	397	+25	392	+20	415	+43
					361				327			
	II	256	259	+3	265	+9	276	+20	260	+4	278	+9
		269	268	+12	277	+21			287			
F_3	I	360	431	+71	401	+41	388	+28	375	+15	411	+51
		298			355		320		301			
	II	258	267	+9	273	+15	271	+13	174	+16	277	+19
		267										
F_4	I	352	378	+26	368	+16	384	+32	374	+22	403	+51
		296	356				314					
	II	257	261	+4	262	+5	266	+9	259	+2	263	+6
		268										
F_5	I	361	432	+71	404	+43	390	+29	378	+17	411	+50
		302	332		356		320		301			
			305									
	II	258	266	+8	272	+14	273	+15	266	+8	275	+17
		267			293							

Table 3: 400 MHz ^{1}H-NMR Spectral Data of the Isolated Flavonoids (DMSO-d $_6$).

Proton	F_1 (J Hz)	F_2 (J Hz)	F_3 (J Hz)	F_4 (J Hz)	F_5 (J Hz)
6	6.2,d(1.8)	6.5,d(1.5)	6.2,d(2)	6.2,d(2)	6.2,d(2)
8	6.5,d(1.8)	6.7,d(1.5)	6.4,d(2)	6.4,d(2)	6.4,d(2)
2`	8.1,d(8.8)	7.8,d(1.5)	7.5,d(2.2)	8.1,d(8.8)	7.6,d(2.1)
3`	6.9,d(8.8)	-,----	-,----	6.9,d(8.8)	-,----
5`	6.9,d(8.8)	7.2,d(8.4)	6.8,d(8.5)	6.9,d(8.8)	6.8,d(8.4)
6`	8.1,d(8.8)	7.8,dd(8.4,1.5)	7.7dd,(8.5,2.2)	8.1,d(8.8)	7.5,dd(8.4,2.1)
anomeric			5.3,d(5.2)	5.3,d(7.7)	5.3,d(7.5)
proton				4.4,s	4.4,s
other sugar				3.2-3.6,m	3.1-3.7,m
protons				1.1,d(6.2)	1.0,d(6.1)
				CH_3 of rhamnose	

Table 4: ^{13}C-NMR Spectral Data of the Isolated Flavonoids (DMSO-d $_6$)

Carbon No.	F_1	F_2	F_3	F_4	F_5
2	147.1	146.7	156.3	155.6	155.4
3	135.8	135.6	133.7	133.5	133.3
4	176.1	175.7	177.5	177.6	177.3
5	160.9	160.6	161.2	161.5	161.2
6	98.5	98.1	98.6	98.9	98.6
7	164.2	163.8	164.2	164.4	164.1
8	93.7	93.3	93.3	93.9	93.5
9	156.5	156.1	156.2	156.8	156.5
10	103.3	102.9	103.7	104.1	104.0
1`	121.9	121.9	120.9	121.0	121.2
2`	129.7	115.1	115.3	131.1	115.2
3`	115.7	145.0	144.9	115.2	144.7
4`	158.5	147.6	148.5	160.1	148.4
5`	115.7	115.5	115.7	115.2	116.2
6`	129.7	120.0	121.9	131.1	121.5
Sugar moiety			ar-1 101.4	gal-1 102.2	glc-1 101.2
			2 71.6	2 71.3	2 74.0
			3 70.7	3 73.2	3 76.4
			4 65.9	4 68.2	4 70.0
			5 64.2	5 73.7	5 75.9
				6 65.5	6 67.0
				rha-1 100.2	rha-1 100.7
				2 70.8	2 70.5
				3 70.6	3 70.3
				4 72.1	4 71.8
				5 68.4	5 68.2
				6 18.0	6 17.7

ar=arabinose, gal=galactose, glc=glucose, rha=rhamnose

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Phytochemical Study of Maerua crassifolia Forssk. Growing in Egypt.

دراسة كيميائية لنبات الموريا كراسيفوليا فورسك الذى ينمو فى مصر

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قسم العقاقير - كلية الصيدلة - جامعة اسيوط - اسيوط - مصر

* كلية الصيدلة بطوكيو - طوكيو - اليابان

نبات الموريا كراسيفوليا من النباتات الصحراوية التى تنمو فى مصر. وباستقصاء المراجع المتوفرة وجد انه يستخدم فى الطب الشعبى كعلاج لامراض المعده وآلام الاسنان ويساعد على سرعة شفاء الجروح والبثور.

ونظرا لاهمية هذا النبات فى الطب الشعبى وعدم وجود دراسة كيميائية كافية عليه فقد روى اجراء هذه الدراسة.

وقد امكن فصل سبعة مركبات منها خمسة فلافونيدات هى: الكامبيفرول ، الكوارسيتين الروتين ، كامبيفرول-٣- أ-جالكتورامنوزايد ، كوارسيتين-٣- أ- ارابينوزايد ودلك بالاضافة الى قلوانى الاستاكيدرين ومركب آخر هو ليونيريسينول-٣- أ-جلوكوبيرانوزايد.

وهذه المركبات التى تفصل لاول مرة من نبات الموريا كراسيفوليا تم التعرف عليها بواسطة الصفات الطبيعية والكيميائية لها ومقارنتها بعينات اصلية ، وكذلك باستعمال الطرق الطيفية المختلفة مثل الاشعة فوق البنفسجية ، دون الحمراء ، الرنين النووى المغناطيسى بنوعيه البروتونى والكربونى ومطياف الكتلة.

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