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GC/MS Analysis of Lipid Constituents and Antimicrobial Activity of *Arum cyrinaicum* Extracts

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RUM CYRENAICUM belonging to family *Araceae*, it is a perennial herb native to Europe, northern Africa, and western Asia, with the highest species diversity in the Mediterranean region. The main objective of the paper is to isolate, identify lipid constituents and also to evaluation of the potency of different extracts of *Arum cyrinaicum* (AC) as antimicrobial agents. Different parts of the plant (roots and seeds) were extracted with n-hexane in a Soxhlet for 24 hours till exhaustion. The extracts were fractionated to acetone insoluble fraction, unsaponifiable fraction and fatty acids fraction which were identified using GC/MS.

The results of GC/MS analyses of seeds acetone insoluble fraction gave nonacosane (43.3%) and nonacosanol (37.74%) as predominants, while of roots showed the presence of nonacosane (36.1%) as the major hydrocarbons. The unsaponifiable matters of seeds revealed the presence of hexadecane (n- C_{16} , 44.11%) and 9,12-octadecadiene (40.04%). While of roots revealed the presence of a mixture of hydrocarbons and sterols. The GC/MS analysis of fatty acid methyl esters of seeds revealed the presence of myrestic acid (62.9%) as the main component, while of roots showed the margaric acid (29.2%) and myrestic acid (23.1%) as major acids respectively. The results of antimicrobial activity of different extracts (using disk diffusion method with determination of inhibitory zone (I.Z.)) against of some microorganisms including G +ve, G – Ve bacteria and fungi proved that, the chloroform and petroleum ether extracts of stem, seeds and roots in addition to the butanol fraction of roots showed high effect against Gram -ve bacteria (*St. aureus*).

Keywords: Arum cyrenaicum, Lipid fraction, Fatty acids, GC/MS, Antimicrobial activity.

Introduction

Araceae family is a herbaceous, monocotyledonous flowering plants in which flowers are borne on a type of inflorescence called a spadix. it is known as the *Arum* family due to the presence of *Arum* genus in this family. It is native to Europe, northern Africa, and western Asia, with the highest species diversity in the Mediterranean region[1]. It includes about 125 genera and over 3750 species, among the largest inflorescence

in the world is that of the *Arum*[2]. In Libya, it represented by three genera with three species in which *Arum cyrenaicum* is the most common and endemic one which grow at Al-Jabal Al-Akhdar region[3]. Historically, some of the plants in the *Arum* family have been used to treat respiratory illnesses due to some properties as an expectorant, and to treat dermal corns due to its irritant properties. Acute and short-term toxicity of *Arum* species include some symptoms on animal results

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in central nervous system excitability, seizures, renal failure, and encephalitis[4]. *A. palaestinum* used as anti-inflammatory, analgesic in kidney stones and sand, Also it is used in alternative medicine for diseases of the airways such as laryngitis, pharyngitis, bronchial catarrh, coughs, for gastrointestinal diseases such as chronic gastritis, colitis, analgesic for liver disease, bladder, neurosis, and treatment of hemorrhoids [5, 6].

El-Desouky et al[7, 8] isolated piperazine, isoorientin (luteolin 6-C-glucoside) and vitexin (apigenin 8-C-glucoside), luteolin, vicenin II. chryseriol and chryseriol-7-glucoside from the *n*-butanol fraction of *A. palaestinum* leaves. While caffeic acid, isoorientin, 3,6,8trimethoxy- 5,7,3',4'-tetrahydroxy flavone and 3,4,5-trihydroxy-1*H*-pyrrol-2(5*H*)-one were isolated from the ethyl acetate extract. Farid et al in 2017[9] identified Five compounds; vitexin, isovitexin, isoorientin, chrysoeriol 7-Oneohesperidoside, chrysoeriol 7-O-(β-apiosyl)-βglucopyranoside from the butanol fraction of A. palaestinum. Two glucomannans (A and B) and an attendant polysaccharide (C) have been isolated from the tubers of A. orientale by gel chromatography [10]. Three flavonoids were isolated from A. cyrenaicum by Abdelkarim et al [11] in 2018 which were identified as: chrysoeriol-7-rhamnoglucoside, 5-methoxy-quersetin-3-Oglucoside and 6-acetyl-5'-methoxyluteolin.

Afifi et al., (5) isolated glikomanini, (glycosidic saponin) and nicotine from A. palaestinum. The analyses of the odour produced by inflorescences of A. macularurn proved the presence of 2heptanone, indole and germacrene B, p-Cresol as major components amongst the 56 compounds present. In other species of Arum like A. italicum and A. dioscorids, a simple amines and diamines together with skatole have been found in the condensed vapours produced by cut and enclosed appendices. The floral parts contained β -carotene, diepoxy- β -carotene in fairly large quantities and sometimes lutein. [12, 13], while Fatam et al [14] identified the main constituents of the unsaponifable fraction and fatty acids present in A. cyernaicum flowers and they proved the presence of hydrocarbons, sterols and triterpenes with major sterols, stigmasterol (14.57%), cholesterol (7.63%) and campasterol (6.71%), in addition to the main acids are: palmitic acid (20.09%), arachidic acid (15.30%), stearic acid (13.4%) and erucic acid (13.03%). This study aims to isolate, identify lipid constituents and also to evaluate the potency of different extracts of *AC* (roots and seeds) as antimicrobial agents.

Materials and Methods

Plant Material

The plant was collected from Wadi El-Husaien, along the coastal of Ras El-Hilal to Shahat city road, Algebel Alakhdar city, in April 2015, during the flowering stage. The plant was kindly identified by Dr. AbdesalamAL-Maqasabi at Botany Department, Faculty of Science, Binghazi University. A voucher specimen has been deposited at the Herbarium of Biology department, Faculty of science, Sirte University, Sirte, Libya. The seeds and roots were separated, air dried and ground till it became as a fine powder.

Instruments

GC/MS analysis of the acetone insoluble fraction

The fatty alcohols mixture was subjected to GC/MS analysis using the following conditions; *Gas chromatography:* Aglient 6890 gas chromatograph equipped with an aglient mass spectrophotometer with a direct capillary interface and fused silica capillary column Agilent 19091S-433, HP-5MS Phenyl Methyl Siloxane (30m x 250µm x 0.25µm film thickness).

Helium was used as a carrier gas at about 20ml/min, pulsed spitless mode, the solvent delay was 4min. and the injector size was 10.0µL.

The mass spectral detector was operated in an electron impact mode with an ionizing energy of 70 eV scanning from m/z 50 to 700.

The ion source temperature was 230° C and the quadruple temperature was 150° C.

The GC temperature program was started at 190° C (15min.), then elevated to 280° C at rate 4° C/min. The detector and injector temperature set at 280° C and 250° C respectively.

Gas chromatographic analysis of unsaponifiable matters

1-Instrument, 6890 GC method, 2- Oven, Initial, temperature: 70°C.

3- Rate	: Final temp	Final time
1.00	220	10 min.
2.00	280	5 min.

4- Initial temperature (injector temp.): 250°C Spitless. 5- Detector temp.: 280°C

6- flow rate : 1 mL/min., Column: Capillary

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Column, Model Number: Agilent 19091S-433, HP-5MS 5% Phenyl Methyl Siloxane (length: 30m, diameter: 250µm Film- thickness 0.25µm). Max temperature: 325°C, Mode: constant flow Carrier gas : N_2 30ml/min., Hydrogen: H_2 30ml/min., Air : 300ml/min.

GC/MS chromatography of the fatty acid methyl esters

1-Instrument, 6890 GC Method, 2-Oven, Initial temperature: 170°C, Initial time: 0min. 3- Rates, Rate 40, final temp. 120°C, final time 2min.

Rate	Final temperat	ture final time
1	220	10.00
2	280	5.00

4- Inlet temp., 250° C, 5- Flow rate : 1ml/min., 6- Column: Capillary column, Model Number: Agilent 19091S-433, HP-5MS 5% Phenyl Methyl Siloxane (length: 30m, diameter: 250µm, Film thickness 0.25µm), Max temperature: 325°C, Mode: constant flow, Initial flow: 1.0mL/min.7-Flow Rates, N₂: 30ml/min., H₂: 30ml/min., Air: 300mL/min.

Isolation of Lipids

About 400g of the roots and 125 g of the seeds (dried powder of AC) were extracted separately with *n*-hexane in a soxhlet apparatus for 24 hours till exhaustion. The combined n- hexane extract was dried over anhydrous sodium sulphate and evaporated in vacuo at 40°C till dryness to give a pale yellow residue 2g of the roots, 2g of the seeds respectively. The residue was dissolved in boiling acetone 150 ml and left overnight at room temperature. The amorphous precipitate was filtered, washed with coldace tone and recrystallized from chloroform/methanol to give bright white crystals 0.13g of the roots and 0.17g of the seeds of Arum cyrenaicum respectively of acetone insoluble fraction. The filtrate (acetone soluble fraction) was evaporated till dryness 1.0g of the roots and 1.2 g of the seeds of AC respectively were saponified by refluxing with 100 ml N/2 alcoholic KOH for four hrs.. The alcoholic solution was concentrated to about 25 ml and diluted with cold distilled water. The unsaponifilable matters were extracted with successive portions of diethylether (3×100ml). The combined ether extract was washed with distilled water, dehydrated over anhydrous sodium sulphate and evaporated in vacuo till dryness to give a yellowish brown semi solid residue of unsaponifiable matters 0.62g of the roots and 1.07g of the seeds respectively.

Extraction of the total fatty acids: The hydroal coholic soap solution after saponification was rendered acidic (pH=2) with 5 % sulphuric acid (H_2SO_4). The liberated fatty acids were thoroughly extracted several times with diethylether. The combined ether extract was washed with distilled water till free from acidity and dehydrated over anhydrous sodium sulphate. The solvent was evaporated in *vacuo* at about 40°C till dryness 0.56g of the roots, 1.06g of the seeds.

Preparation of the fatty acid methyl esters: The total fatty acids of the roots and of the seeds (300 mg and 500 mg, respectively) were dissolved in 75ml dry methanol containing 4-5% dry HCl and refluxed on a boiling water bath for four hours. The solvent was concentrated by evaporation till 25ml and diluted with 100 ml distilled water. The reaction mixture was extracted with successive portions of diethylether (3×100 mL). The combined ether extract was washed with distilled water till free from acidity, dried over anhydrous sodium sulfate, filtered and the solvent was evaporated in *vacuo* at 40°C to afford the total fatty acid methyl esters of roots and seed.

Antimicrobial activity study:

Preparation of the plant extracts for biological evaluation: About 50g of the air dried powdered plant parts (herb, roots and seeds) were first extracted (defatted) with pet. ether(b.r. 40-60°C) by maceration for 24 hours to afford pet ether extract followed by extraction with chloroform, ethyl acetate, butanol, methanol (70%) and water, filtered, dried over anhydrous sodium sulphate and evaporated *in vacuo* at 40°C to give the different extracts of different organs. Five concentrations were prepared (50, 100, 150, 200 and 250 mg/ml) for testing as antimicrobial agent.

Antimicrobial activity: The antimicrobial activity was determined using disk diffusion method of Kirby-Bauer and determination of inhibitory zone (I.Z.) [15].

Tested extracts: water, methanol, petroleum ether, chloroform, ethyl acetate, butanol extracts of herb, seeds and roots in addition to fatty alcohols of seeds and roots.

Tested microorganisms: *Staphylococcus aureus, Bacillus cereus, S. typhimurim, Escherichia coli, Pseudo. Aeroginosa, Bacillus subtills* and *Sa. sarcina. Aspergillus niger* and *Aspergillus flavus* and *Candida albicans* were obtained from the stock culture of Chemistry of natural and microbial products, National research center, Cairo, Egypt.

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Results and Discussion

The lipid constituents of different organs of AC were fractionated by dissolving in hot acetone to isolate the fatty alcohols and hydrocarbons mixture, after then saponification of acetone soluble fraction to isolate the fatty acids and the unsaponifiable matters. The GC/MS results of the acetone insoluble fraction of roots resulted in the identification of the fatty alcohols and hydrocarbons mixture which proved presence of heptacosane (39.6%) and nonacosane (36.1%) as the major hydrocarbons table (1), also the seeds were found to contain nonacosane (43.3%) and nonacosanol (37.74%) as predominant (table 2). The GC/MS of the roots unsaponifiable matters revealed the presence of a series of hydrocarbons (99.25%) in which 12-octadecadiene C H (51.73%) and hexadecane { C_{16} H34 , 43.18%)³⁴

the major compounds, in addition to two sterols (0.75%) (table 3), while of the seeds have a mixture of n-hydrocarbons (92.48%) in which hexadecane $(C_{16}H_{24}, 44.11\%)$ and 9,12- octadecadiene (40.04%), two compounds of sesquiterpenoid are nerolidol (0.2%) and farnesol (2.56%), a sterols fraction (4.76%), with lanosterol (2.39%) as a main constituents (table 4). The results of the GC/MS of fatty acid methyl esters of the roots of AC (table 5) showed that, it comprises saturated and unsaturated fatty acids in percentages of 91.60% and 8.40% in which margaric acid (29.2%) and myrestic acid (23.1%) are the major acids respectively, while the seeds (table 6) revealed the presence of six acids including saturated fatty acids (87.96%) and unsaturated fatty acids (12.04%) with myrestic acid (62.9%) as a main acid. It was found that, there is some differences between these findings and that reported by Fatma et al in 2018(14), where they identified the unsaponifiable constituents and fatty acids in the aerial parts, flowers and tuber of AC. The unsaponifiable fraction of flowers was found to contain hydrocarbons, sterols and triterpenes with stigmasterol (14.57%), cholesterol (7.63%) and campasterol (6.71%). While, the unsaponifiable fraction of the aerial parts comprises some hydrocarbons and sterols in which stigmasterol (13.02%) and β -sitosterol (7.36%). The fatty acids for flowers are: palmitic acid (20.09%), arachidic acid(15.30%), stearic acid(13.4%) and erucic acid (13.03%). While that of the tubers are: linoleic acid (46.36%), palmitic acid (22.53%) and arachidoic acid (10.25%). So we can conclude that there is a similarity between these data and that reported for the other organs of the same species with differences in the percentages of each constituent, these data were reported for the first time.

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Many efforts have been made to discover new antimicrobial compounds from a variety of sources such plants. One such source is folk medicine. Systematic screening of them may result in the discovery of novel effective compounds. The results of the antimicrobial evaluation (table7) showed different effects on the tested microorganisms (bacteria, yeast and fungi) as follow: petroleum ether extract of the aerial parts was found to have certain activity against S. aureus, S. typhimurim E. coli and Sa. sarcina at two concentration, petroleum ether extract of seeds showed moderate effect against only S. typhimurim (10mm), While roots extract showed effect against Sa. sarcina (10, 8, 6mm). The chloroform extract of the aerial parts, seeds and roots exhibited good effects against S. aureus only at different concentration in a concentration defendant manner. Additionally the Butanol extract of the roots exhibited the highest inhibition activity against one type of bacteria S. aureus at all concentrations (12,10,8,6,4mm).

Kirby-Bauer test results are interpreted using relates zone diameter to the degree of microbial resistance, also the chemical constituents of different extracts were identified through the chromatographic investigation of different extracts by paper chromatography and thin layer chromatography which revealed the presence of many classes of compounds such as tannins, phenolics, saponnins and flavonoidal glycosides. These data are disagree with that reported by Çolak, et al in 2009[16] where they reported that, the extracts (petroleum ether, methanol and ethyl acetate) of Arum maculatum showed good activity against some type of bacteria (Bacillus cereus, Micrococcus luteus, Pseudomonas phaseolicola, Yersinia *enterocolitica* and *Enterobacter* aerogenes) and a mold (Aspergillus niger). While Reza et al[17] demonstrated that Arum maculatum leaves extracts (especially ethanol : water (50:50) solvent) were effective against all tested bacteria, and Listeria monocytogenes was the most sensitive bacterium with lowest MIC value (12.5 mg/ml) and biggest diameter of growth inhibition zone (13.77 mm). Hatim et al in 2020 [18] reported that water and methanol extracts of Arum hygrophilum had a significant antibacterial activity against P. aeruginosa. Methanol extract further revealed an inhibitory effect on E. Faecalis, whereas ethanol extract displayed no antibacterial influence against all the tested microorganisms.

Peak no.	Ret. Time (min.)	Rel. %	Mole cular formula	Mol. Wt.	Compounds
1	31.4	4.99	C ₂₅ H ₅₂	352	Pentacosane
2	33.8	1.06	$C_{26}H_{54}$	366	Hexacosane
3	36.2	38.99	C ₂₇ H ₅₆	380	Heptacosane
4	38.1	3.21	C ₂₈ H ₅₈	394	Octacosane
5	40.14	35.63	C ₂₉ H ₆₀	408	Nonacosane
6	41.7	1.33	$C_{30}H_{62}$	422	Tricontane
7	43.4	5.19	C ₂₉ H ₅₈ O	408	Nonacosanol
8	43.6	3.9	C ₃₀ H ₆₀ O	436	Tricontanol
9	46.7	4.3	C ₃₂ H ₆₄ O	478	Dotricontanol

TABLE 1. GC-MS data of acetone insoluble fraction of AC roots.

TABLE 2. GC-MS data of acetone insoluble fraction of AC seeds.

Peak no.	Ret. Time (min.)	Rel. %	Molecular formula	Mol. Wt.	Compounds
1	31.3	2.8	C ₂₅ H ₅₂	352	Pentcosane
2	36.07	9.22	C ₂₇ H ₅₆	380	Heptacosane
3	38.09	2.63	C ₂₈ H ₅₈	394	Octacosane
4	40.05	43.3	$C_{29}H_{60}$	408	nonacosane
5	41.75	4.31	C ₃₀ H ₆₂	422	Tricontane
6	43.50	37.74	C ₂₉ H ₅₈ O	408	nonacosanol

TABLE 3. GC-MS da	a of the unsaponifiable	fraction of AC roots.
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Peak no.	Ret. Time (min.)	Rel. %	Compounds
1	55.34	0.13	DodecaneC ₁₂
2	76.12	0.63	TetradecaneC _{1 4}
3	85.94	0.98	PentadecaneC ₁₅
4	102.03	43.18	HexadecaneC _{1 6}
5	104.07	0.16	HeptadecaneC ₁₇
6	118.42	51.73	9,12-octadecadieneC ₁₈ H ₃₄
7	122.43	0.25	Tricosane C ₂₃
8	137.96	0.65	Pentacosane C ₂₅
9	145.16	0.11	Hexacosane C ₂₆
10	152.52	1.09	Heptacosane C ₂₇
11	168.50	0.34	Nonacosane C ₂₉
12	181.44	0.62	Campasterol
13	182.75	0.13	Stigmasterol

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Peak no.	Ret. Time (min.)	Rel. %	Compounds
1	56.33	0.89	Dodecane
2	69.79	0.2	Tetradecane
3	86.30	2.8	Pentadecane
4	95.70	2.56	FarnesolC ₁₅ H ₂₆ O
5	102.91	44.11	Hexadecane
6	118.74	40.04	9,12-octadecadiene
7	122.91	0.98	Tricosane
8	138.41	1.16	Pentacosane
10	145.45	0.18	Hexacosane
11	152.75	0.78	Heptacosane
12	153.58	0.19	1 -tricosene $C_{23}H_{46}$
13	161.19	0.18	Octacosane
14	168.79	0.97	Nonacosane
15	181.61	1.18	Campesterol
16	182.28	0.2	Nerolidol
17	185.27	1.19	Stigmasterol
18	185.87	2.39	Lanosterol

TABLE 4. GC-MS data of the unsaponifiable fraction of AC seeds.

TABLE 5. GC-MS data of fatty acid methyl esters of AC roots.

Peak no.	Ret. Time (min.)	Rel.%	Compounds	Molecular formula
1	11.41	23.1	Myrestic acid	$C_{14}H_{28}O_2, C14(0)$
2	13.52	22.5	Palmitic acid	$C_{16}H_{32}O_2, C16(0)$
3	21.15	29.2	margaric acid	$C_{17}H_{34}O_2, C17(0)$
4	21.33	4.5	Olic acid	$C_{18}H_{34}O_2, C18(1)$
5	22.43	3.9	Linoleic acid	$C_{18}H_{32}O_2, C18(2)$
6	33.83	3.2	Arachidic acid	$C_{20}H_{40}O_2, C20(0)$
7	47.14	12.1	Tricosanoic acid	$C_{23}H_{46}O_2, C23(0)$
8	74.21	1.5	Lignoceric acid	$C_{24}H_{48}O_2, C24(0)$

 TABLE 6. GC-MS data of fatty acid methyl esters of AC seeds.

Peak no.	Ret. Time (min.)	Rel. %	Compounds	Molecular formula
1	9.21	12.5	Lauric acid	$C_{12}H_{24}O_2, C12(0)$
2	12.12	62.9	Myrestic acid	$C_{14}H_{28}O_2, C14(0)$
3	12.74	4.95	Pentadecanoic acid	$C_{15}H_{30}O_{2}, C15(0)$
4	13.53	7.61	Palmitic acid	$C_{16}H_{32}O_2, C16(0)$
5	17.41	1.98	Palmitoleic acid	$C_{16}H_{30}O_{2}, C16(1)$
6	21.06	10.06	Oleic acid	$C_{16}H_{28}O_2, C16(2)$

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Microbes				Inhibition zone in mm						Funai		Voasts
		Gm+ Bacteria			Gm- Bacteri	a				1'u	ngi	Teasis
Ext.	Conc.		S. aureus	B. cereus	Salm. typhimurim	E. coli	Pseudo. Aeroginosa	B. subtillis	Sa. sarcina	Asp. niger	Asp. flavus	C. albicans
, r	Seeds	1 2 3 4 5	- - - -	- - - -	- - - -	- - - -	- - - -		- - -	- - -	- - -	- - -
wate	Roots	1 2 3 4 5	- - - -	- - - -	- - - - -	- - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
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methar	Roots	1 2 3 4 5	- - - - -	- - - -	- - - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -

TABLE 7. Antimicrobial activity of different extracts of AC

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TABLE 7.Cont.

Egy	TABLE 7.Cont.									
pt. J. Chem. 63,		Herb	1 2 3 4 5	- - 7 10	- - - -	- - 8 10	- - 6 8	- - - -	- - - -	- - 7 10
No. (2020)	Pet. Ether	Seeds	1 2 3 4 5	- - -	- - -	- - - 10		- - - -	- - -	
		Roots	1 2 3 4 5	- - - -		- - - -	-			- 6 8 10
		Herb	1 2 3 4 5	- - 12 15	- - - -	- - - -	- - -	- - - -	- - - -	- - -
	Chloroform	Seeds	1 2 3 4 5	6 8 10 12 14	- - - -	- - - -	- - -	- - - -	- - - -	- - -
		Roots	1 2 3 4 5	6 8 10 12					- - - -	-

1=50mg/ml, 2=100mg/ml, 3=150mg/ml, 4=200mg/ml, 5=250mg/ml, - = No Inhibition.

Standard Tetracycline: I.Z. = 19 mm Standard Miconazole: I.Z. = 22 mm.

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Conclusion

The lipid constituents (fatty alcohols, nhydrocarbons, fatty acids and unsaponifiable matters) of different organs of *Arum cyrenaicum* were isolated and identified using GC/MS. Also all extracts of herb, roots, seeds (water, methanol, petroleum ether, chloroform, ethyl acetate and butanol) were evaluated as antimicrobial agents

Conflicts of Interest: The authors declare that there is no conflict of interest.

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