Virucidal activity of Cactus on envelopped viruses

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Objectives

1. To evaluate the antiviral activity of Cactus (fruit and stem);

2. To isolate and identify the active compound if we find activity;

3. To study the action mechanism of this compound on viruses.

1 / stem and fruit

2 / stem and fruit

3 / stem and fruit

4 / stem

Anti HSV-2 and IFV-A activity of cactus samples

Solvent of extraction	Part of plant	Extract name	Antiviral activity							
			Tested viruses							
			HSV-2			IFV-A				
			CC_{50} (µg/ml)	IC_{50} (µg/ml)	SI (CC_{50}/IC_{50})	CC_{50} (µg/ml)	IC_{50} (µg/ml)		SI (CC_{50}/IC_{50})	
Ethanol extract	Stem	1CE	660	76	8.7	880	82	80	**10.7**	**11.0**
		2CE	960	98	**10.2**	1340	100	104	**13.4**	**12.9**
		3CE	800	36	**22.2**	1100	54	54	**20.4**	**20.4**
		4CE	960	30	**32.0**	1100	36	42	**30.6**	**26.2**
	Fruit	1FE	1200	> 1200	< 1	1160	> 1160	> 1160	< 1	< 1
		2FE	1360	> 1360	< 1	1380	> 1380	> 1380	< 1	< 1
		3FE	1500	1400	1.1	1800	1100	1340	1.6	1.3
Water extract	Stem	1CW	2540	1400	1.8	2620	1200	1280	2.2	2.0
		2CW	1620	> 1620	< 1	1800	1800	1720	1.0	1.1
		3CW	1840	880	2.1	2000	720	930	2.7	2.2
		4CW	1900	130	8.6	2100	190	180	7.9	9.7
	Fruit	1FW	2500	2200	1.1	2500	2240	2240	1.1	1.1
		2FW	2860	> 2860	< 1	2900	2900	2740	1.0	1.1
		3FW	2900	> 2900	< 1	3200	> 3200	> 3200	< 1	< 1

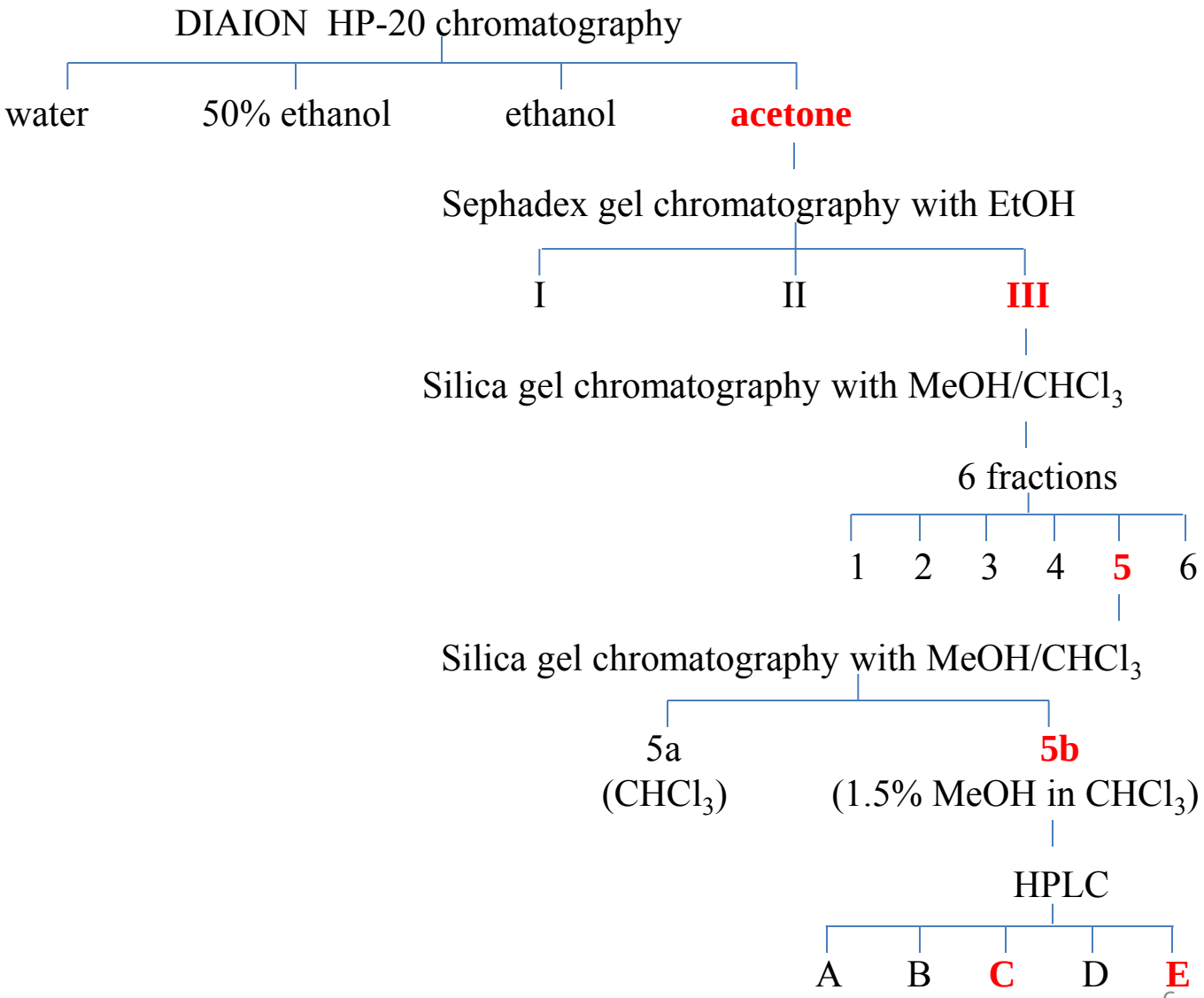
1-4: samples; C: cladode; F: fruit; E: ethanol; W: water.

Antiviral activity of fractions of ethanol extract obtained by DIAION HP-20 chromatography

- More than 3 kg of Cactus stem have been collected in Tunisia and freeze dried.
- The dried stem was diluted in ethanol and the extract was then evaporated.
- The ethanol extract was fractionated with DIAION HP-20 chromatography by using 4 solvents: water, 50% ethanol, ethanol and acetone.

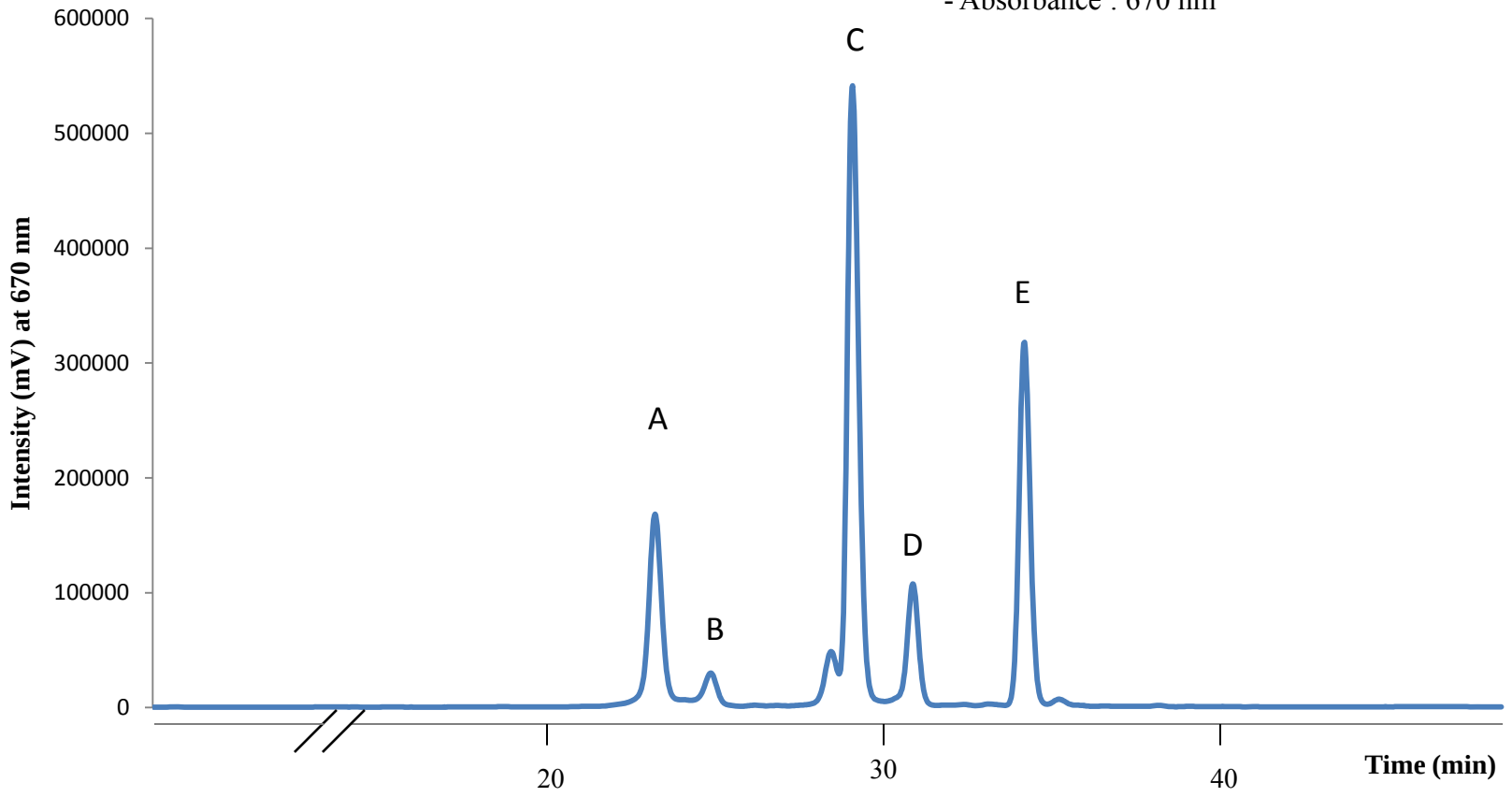
Sample	HSV-2					IFV-A				
	CC_{50} (µg/ml)	IC_{50} (µg/ml)		SI (CC_{50}/IC_{50})		CC_{50} (µg/ml)	IC_{50} (µg/ml)		SI (CC_{50}/IC_{50})	
		A	B	A	B		A	B	A	B
ethanol extract	960	32	30	**30.0**	**32.0**	1000	36	42	**30.6**	**26.2**
water fraction	2800	> 1000	>1000	< 2.8	< 2.8	5600	> 1000	> 1000	< 5.6	< 5.6
50% ethanol fraction	2000	> 1000	> 1000	< 2	< 2	4200	> 1000	> 1000	< 4.2	< 4.2
ethanol fraction	960	27	36	**36**	**29**	1600	74	67	**22**	**24**
acetone fraction	675	12	11	**56**	**61**	1100	22	24	**50**	**46**

Isolation of the active(s) compound(s)



HPLC chromatogram of the fraction 5b

- Chromatography reverse phase by using 2 solvents :
  - solvent A : 80% MeOH in 1 M CH_3COONa
  - solvent B : 80% MeOH in AceOH
- Absorbance : 670 nm

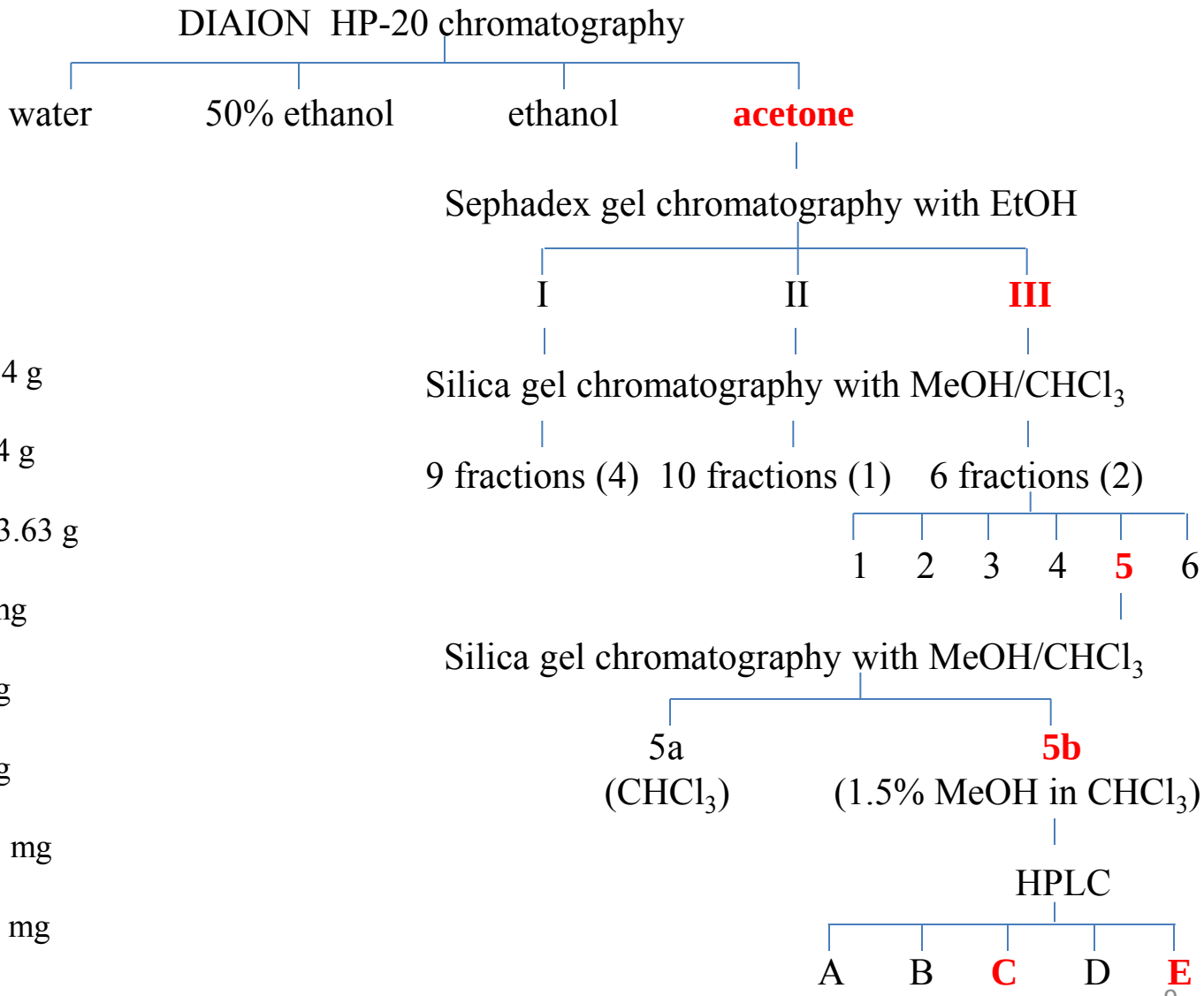


Antiviral activity of compounds C and E

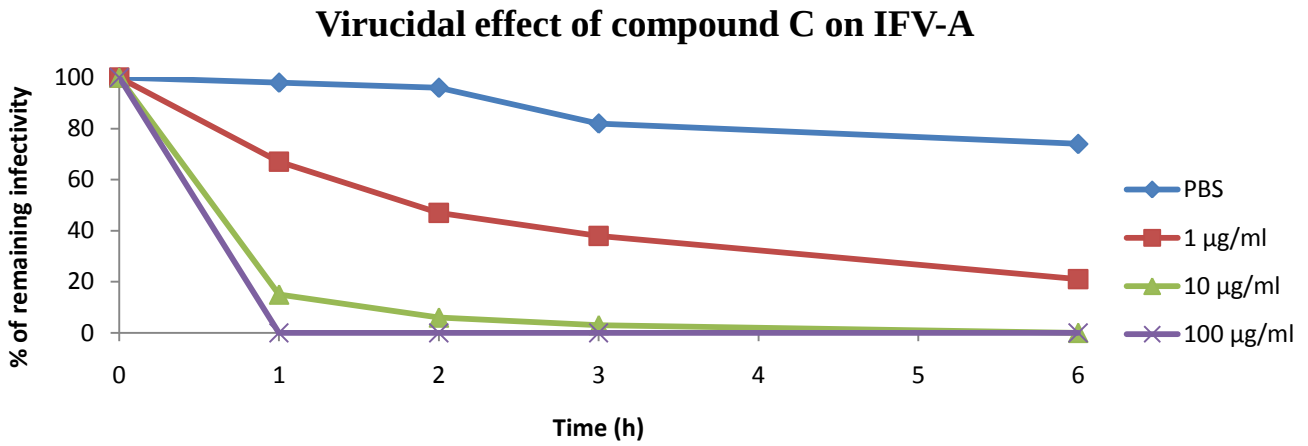
Sample	HSV-2			IFV-A			PV-1		
	CC_{50} (µg/ml)	IC_{50} (µg/ml)	SI (CC_{50}/IC_{50})	CC_{50} (µg/ml)	IC_{50} (µg/ml)	SI (CC_{50}/IC_{50})	CC_{50} (µg/ml)	IC_{50} (µg/ml)	SI (CC_{50}/IC_{50})
C	4.0	0.30	13	5.1	1	5.1	4.0	8.70	0.46
E	0.96	0.016	60	1.1	0.33	3.3	0.96	2.2	0.44

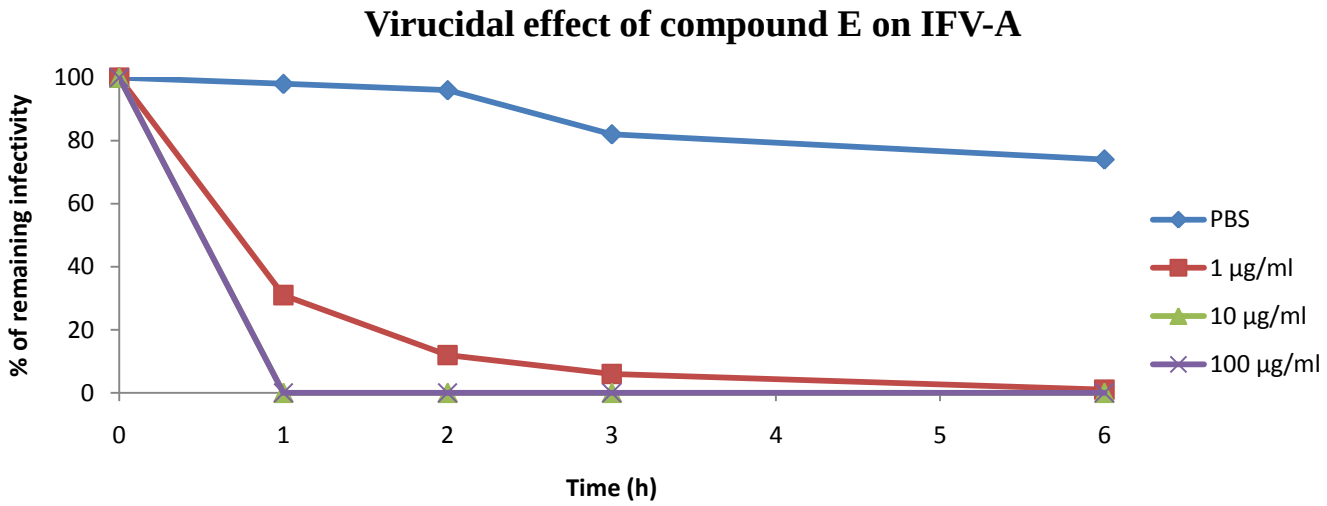
Isolation of the active(s) compound(s)

- Material : 3.6 kg
- Dried material : 264 g
- Ethanol extract : 34 g
- Acetone fraction : 3.63 g
- Fraction III : 630 mg
- Fraction 5 : 100 mg
- Fraction 5b : 24 mg
- Component C : 3.1 mg
- Component E : 2.1 mg

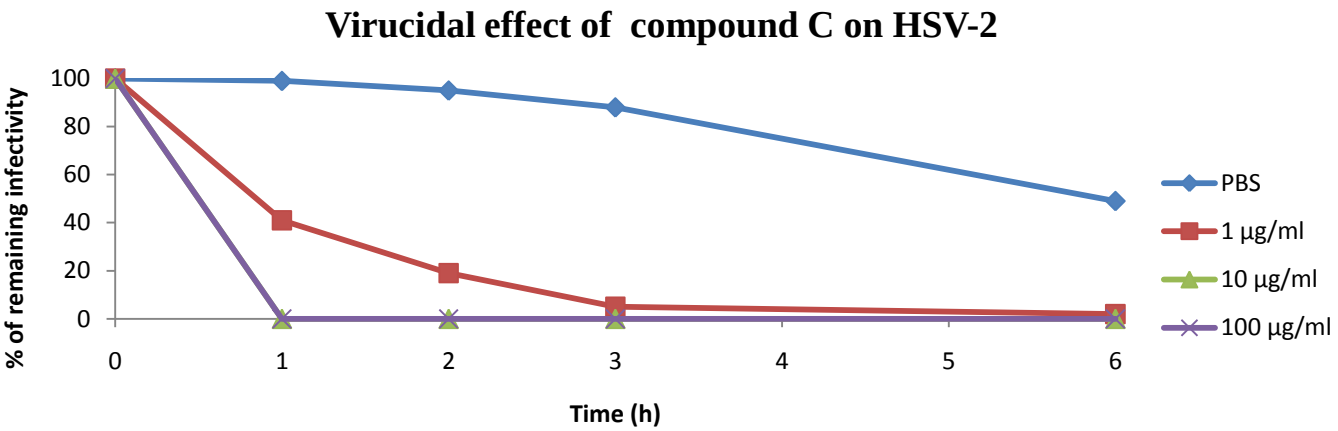


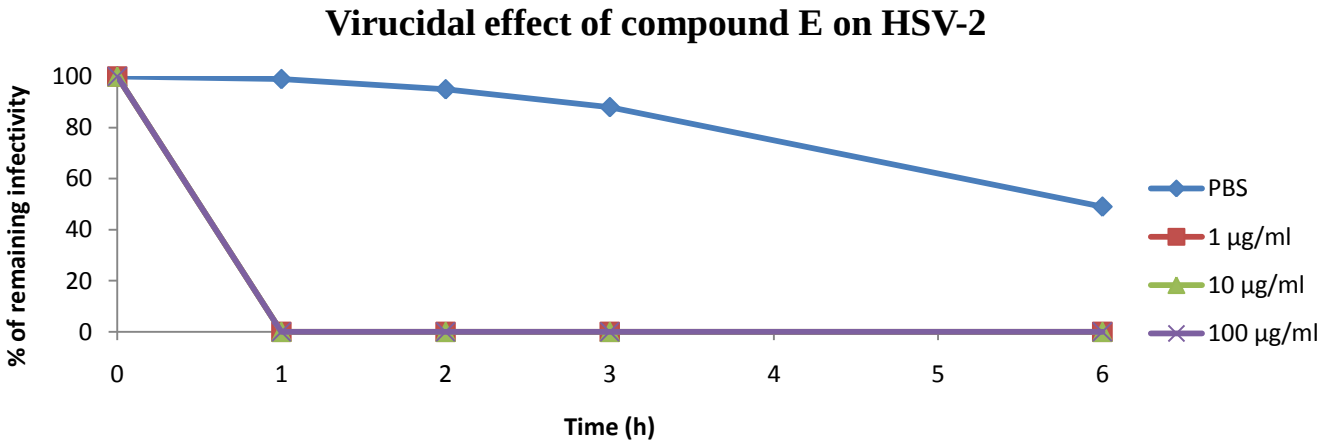
Virucidal effect of compounds C and E on IFV-A



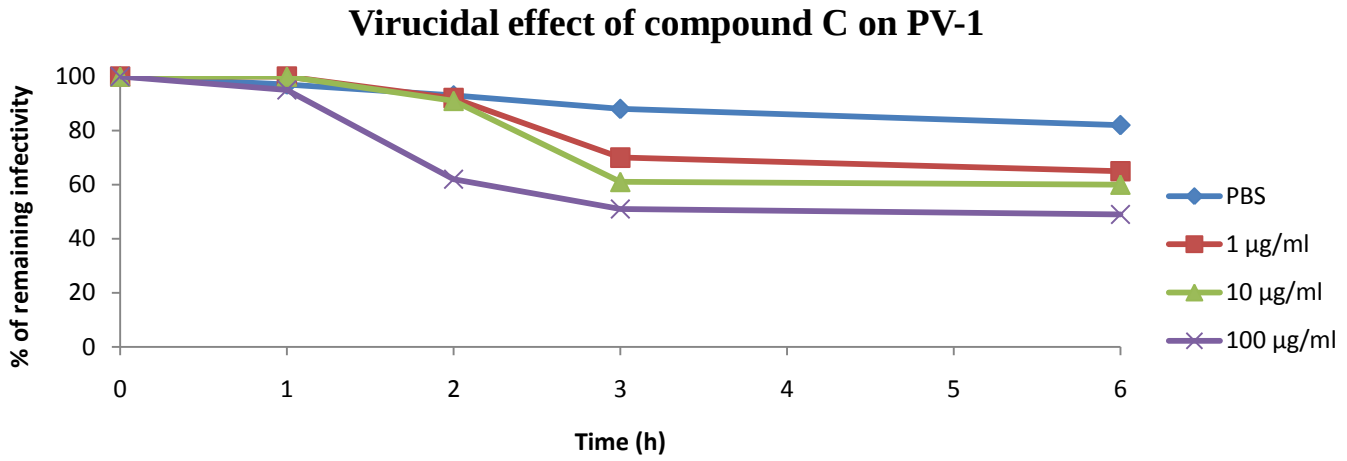


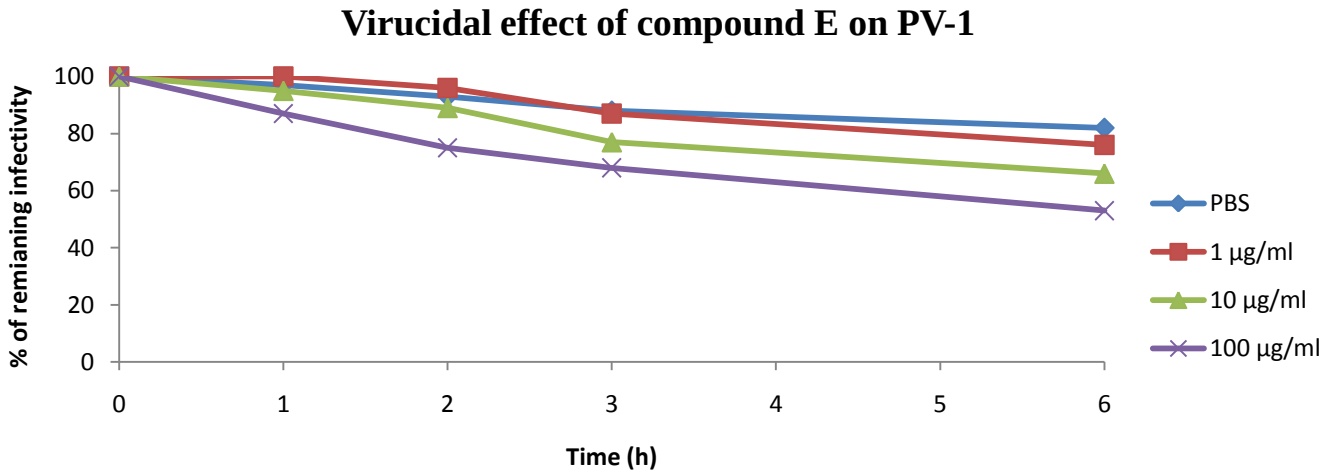
Virucidal effect of compounds C and E on HSV-2





Virucidal effect of compounds C and E on PV-1





Identification of compounds C and E

1. ^{1}H Nuclear Magnetic Resonance (NMR) spectral data

Proton	2^1	3^1	3^2 (E)	3^2 (Z)	5	7^1	8^1	8^2	10	12^1	13^2 - H	13^2 - OH	13^4 - OMe	17	18	18^1	20	21 - NH	23 - NH
Published data (pheophorbide a)	**3.38**	**8.22**	**6.40**	**6.21**	**9.73**	**3.26**	**3.75**	**1.71**	**9.90**	**3.69**	**6.90**		**3.93**	**4.59**	**4.66**	**1.86**	**8.86**	**-1.30**	**0.89**
Component C	3.36	8.20	6.39	6.20	9.69	3.22	3.67	1.68	9.86	3.70	6.89		3.93	4.57	4.66	1.86	8.84	-1.33	0.86
Component E	3.39	8.23	6.41	6.21	9.75	3.27	3.77	1.71	9.91	3.73	-	5.56	-	4.57	4.65	1.88	8.86	-1.40	0.87

2. Mass Spectrometry (MS) spectral data

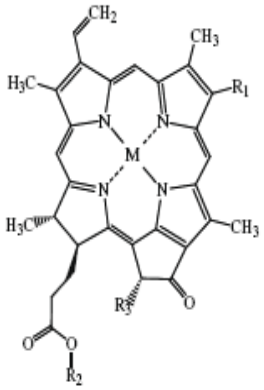
- ✓ Component C : m/z 592 (M^+) / $C_{35}H_{36}0_5N_4$ ⇨ **Pheophorbide a**
- ✓ Component E : m/z 534 (M^+) / $C_{33}H_{34}0_3N_4$ ⇨ **Pyropheophorbide a**

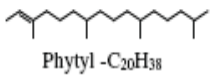
3. UV Spectral data

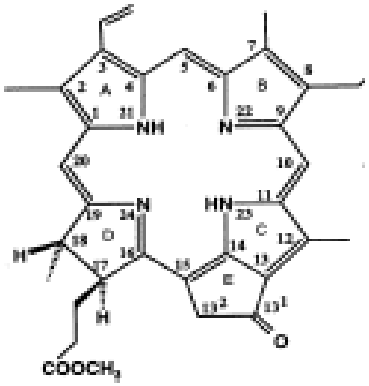
- ✓ Component C : 408.5, 504.5, 534.0, 609.0, 666.0
- ✓ Component E : 409.0, 506.0, 535.0, 608.5, 664.0

⇨ UV spectral data are similar to those of **Pheophorbide a** and **Pyropheophorbide a**

Molecular structure of compounds C and E







Pigment	M	R_1	R_2	R_3
Chlorophyll a	Mg	CH_3	$C_{20}H_{38}$	$COOCH_3$
Chlorophyll b	Mg	CHO	$C_{20}H_{38}$	$COOCH_3$
Chlorophyllide a	Mg	CH_3	H	$COOCH_3$
Chlorophyllide b	Mg	CHO	H	$COOCH_3$
Pheophytin a	2 H	CH_3	$C_{20}H_{38}$	$COOCH_3$
Pheophytin b	2 H	CHO	$C_{20}H_{38}$	$COOCH_3$
Pheophorbide a	2 H	CH_3	H	$COOCH_3$
Pheophorbide b	2 H	CHO	H	$COOCH_3$
Pyropheophorbide a	2 H	CH_3	H	H
Pyropheophorbide b	2 H	CHO	H	H

Conclusion & Perspectives

✓ Cactus cladode contains molecules possessing high antiviral activity.

✓ These molecules are **Pheophorbide a** and **Pyropheophorbide a**, 2 chlorophyll derivatives.

✓ These molecules exhibit **virucidal effect** on **enveloped viruses**, as HSV and IFV, but didn't show any activity on non enveloped virus: these active compounds may recognize **specific receptors** of enveloped virus.

✓ These molecules showed more potent activity on HSV-2 than on IFV-A: this phenomenon can be explained by the difference of the envelop structure and/or of the viral absorption and penetration on host cell between these two viruses.

⇨ In Perspective, we project:

- to study the characteristic of the specific receptors of enveloped virus recognized by the active molecules and to understand the action mechanism of these molecules on these receptors;

- to carry out an *in vivo* study to determine the cytotoxicity and the antiviral activity of Pheophorbide a and Pyropheophorbide a on animal system.