Phytochemical Analysis and *in vitro* Antiviral Activities of the Essential Oils of Seven Lebanon Species

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The chemical composition of the essential oils of *Laurus nobilis*, *Juniperus oxycedrus* ssp. *oxycedrus*, *Thuja orientalis*, *Cupressus sempervirens* ssp. *pyramidalis*, *Pistacia palaestina*, *Salvia officinalis*, and *Satureja thymbra* was determined by GC/MS analysis. Essential oils have been evaluated for their inhibitory activity against SARS-CoV and HSV-1 replication *in vitro* by visually scoring of the virus-induced cytopathogenic effect post-infection. *L. nobilis* oil exerted an interesting activity against SARS-CoV with an IC_{50} value of 120 μg/ml and a selectivity index (SI) of 4.16. This oil was characterized by the presence of β-ocimene, 1,8-cineole, α-pinene, and β-pinene as the main constituents. *J. oxycedrus* ssp. *oxycedrus* oil, in which α-pinene and β-myrcene were the major constituents, revealed antiviral activity against HSV-1 with an IC_{50} value of 200 μg/ml and a SI of 5.

Introduction. – In the past decades, besides a variety of synthetic antiviral drugs with different molecular targets, a large number of phytochemicals have been recognized to control infections caused by viruses. Recently, the anti-herpesvirus activity of several essential oils of different plant sources as well as of various constituents of essential oils was demonstrated [1][2].

The severe acute respiratory syndrome (SARS) is a febrile respiratory illness primarily transmitted by respiratory droplets or close personal contact. The causative organism has been identified as a novel coronavirus, *i.e.*, SARS-CoV [3]. The overriding clinical feature of SARS is the rapidity with which many patients develop symptoms of acute respiratory distress syndrome (ARDS). Currently, there are no approved or universally recommended therapies for SARS. Treatment for the disease is mainly supportive.

Herpes simplex virus type 1 (HSV-1) is a common human pathogen that causes localized skin infections of the mucosal epithelia of the oral cavity, the pharynx, the oesophagus, and the eyes. The virus may establish an acute primary infection, followed by the development of a latent, lifelong infection [4]. Presently, the only aspect of the

HSV life-cycle for which antiviral therapy has been successfully developed is the process of DNA replication, which is targeted by a small group of nucleoside analogues that include acyclovir (ACV), valaciclovir, and famciclovir. However, ACV-resistant strains of HSV and drug toxicity have been recently reported [5].

Laurus nobilis L. was used as folk remedies in different countries to treat numerous diseases. Laurel essential oil was reported to be used in the preparation of hair lotion for its antidandruff activity and for the external treatment of psoriasis [6]. *Thuja orientalis* L. tree was used in various herbal remedies and aromatherapy preparation. In traditional Chinese medicine, the leaves and stems of *T. orientalis* are used to treat nervous disorders, insomnia, and heart palpitations, as well as to stop hemorrhages and reduce fever. Recently, the polysaccharide fraction isolated from *T. occidentalis* was reported to demonstrate an anti-human immunodeficiency virus (HIV) activity [7]. *Juniperus oxycedrus* L. ssp. *oxycedrus* was used in folk medicine for the treatment of various infection diseases [8]. *Salvia officinalis* L. is a medicinal plant well-known for its reputation of being a panacea. The inhibitory activity against HSV-1, HSV-2, and an ACV-resistant strain of HSV-1 (ACV (res)) of an aqueous extract of *S. officinalis* was recently reported [9]. The most common *Satureja* specimen is *S. thymbra* L., which is known as an herbal home remedy, due to its antimicrobial, gastrosedative, and diuretic properties [10]. *Pistacia palaestina* Boiss. and *Cupressus sempervirens* ssp. *pyramidalis* L. essential oils have not been investigated so far for their chemical composition and biological activity.

The present study aimed at examining the effects of essential oils obtained from several plants from Lebanon on HSV-1 and SARS-CoV replication *in vitro*.

Results and Discussion. – *Compositions of the Oils.* The yields of essential oils ranged from 1.5 to 3.5% (*Table 1*). To identify putative active compounds present within the essential oils, gas-chromatography (GC) systems were employed. The chemical composition of the oils was reported in *Table 2*. *L. nobilis* berry oil was characterized by the presence of β-ocimene (21.83%), 1,8-cineole (9.43%), α-pinene (3.67%), and β-pinene (2.14%) as major constituents. Two interesting sesquiterpenes, *i.e.*, eremanthin (3.65%) and dehydrocostuslactone (7.57%), were also identified. *T. orientalis* oil was characterized by 43 constituents (86.68% of the total oil) in which the main components were α-pinene (35.72%), δ-3-carene (9.48%), and α-cedrol (9.55%).

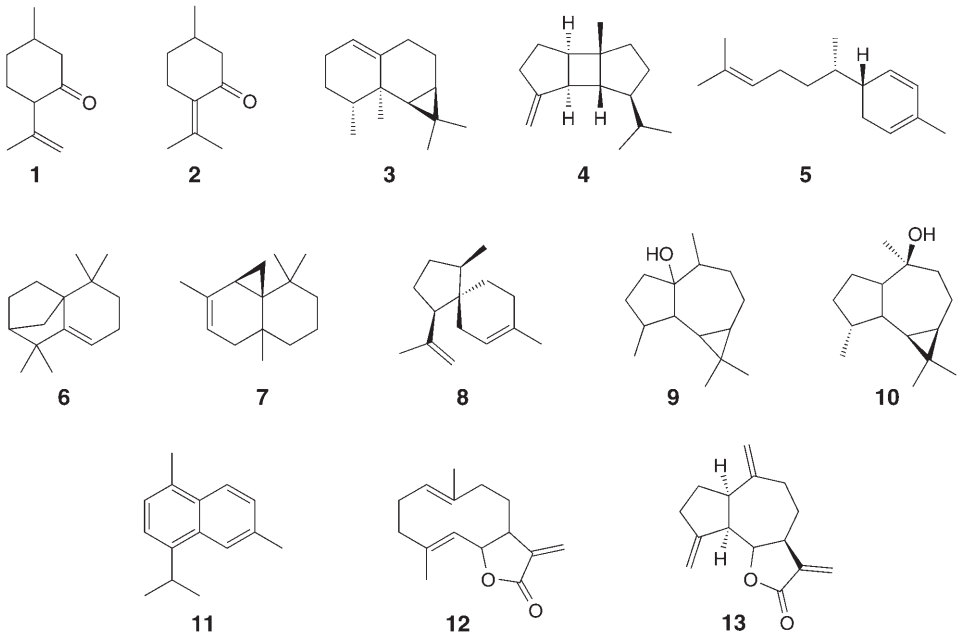
Table 1. *Sources of the Samples of the Studied Essential Oils and Their Voucher Specimen Numbers*

Plant	Location	Voucher specimen No.	Studied material	% *w*/*w*[a])
L. nobilis L.	Nahr-Ibrahim	1224	Berry	3
J. oxycedrus L. ssp. *oxycedrus*	Baskinta	1267	Berry	2.5
T. orientalis L.	Ayoun-kourkoush	1265	Fruits	3.5
C. sempervirens L. ssp. *pyramidalis*	Ajaltoun	1248	Fruits	3
P. palaestina Boiss.	Ain-Saadé	1268	Fruits	2
S. officinalis L.	Ain-Saadé	1271	Leaves	2.25
S. thymbra L.	Ayoun-kourkoush	1273	Leaves	1.5

[a]) % *w*/*w:* Hydrodistillation yield.

A total of 48 compounds (82.39% of the total oil) were identified in *J. oxycedrus* ssp. *oxycedrus* berry oil. α-Pinene (27.4%) and β-myrcene (18.9%) were the major constituents. Other identified compounds were α-phellandrene (7.1%), limonene (6.7%), epibicyclosesquiphellandrene (2.3%), and δ-cadinene (2.2%). Forty-one components, representing 80.91% of the total, were identified in *S. thymbra* oil, in which *p*-cymene (10.76%), α-pinene (10.15%), thymol (9.92%), sabinene (8.64%), γ-terpinene (7.56%), carvacrol (4.98%), *trans*-caryophyllene (3.67%), β-pinene (2.90%), and linalool (2.81%) were the main abundant compounds.

C. sempervirens ssp. *pyramidalis* oil was characterized by 19 components, representing 90.45% of the total oil. The main components were α-pinene (53.56%), α-terpinene (18.90%), thymol (3.84%), and terpinolene (3.15%). Twenty-six compounds were identified in *S. officinalis* (94.39% of the total oil) in which 1,8-cineole (43.62%), α-thujone (12.99%), sabinene (6.97%), camphor (5.71%), α-pinene (4.72%), α-humulene (3.41%), α-terpineol (3.18%), and β-pinene (3.01%) were the major components.



1 2 3 4 5 6 7 8 9 10 11 12 13

P. palaestina oil was characterized by 29 components, representing 79.82% of the total oil. The main components were sabinene (17.08%), limonene (8.56%), β-pinene (6.48%), γ-terpinene (6.33%), *p*-cymene (6.01%), and aromadendrene (3.99%).

Antiviral Activities. In this study, we report the antiviral activity of seven essential oils obtained from berry, fruits, and leaves of different species collected in Lebanon. Results are summarized in *Table 3*. Our results demonstrated how *L. nobilis* berries oil exhibited an IC_{50} value of 120 μg/ml against SARS-CoV with a selectivity index (SI; TC_{50}/IC_{50}) of 4.2. An interesting activity with an IC_{50} value of 60 μg/ml was found when *L. nobilis* berry oil was incubated with HSV-1 virus. *Armaka et al.* reported the ability

Table 2. *Composition* [%] *of Essential Oils Obtained from* L. nobilis (*Ln*), J. oxycedrus *ssp.* oxycedrus (*Joo*), T. orientalis (*To*), C. sempervirens *ssp.* pyramidalis (*Csp*), P. palaestina (*Pp*), S. thymbra (*St*), *and* S. officinalis (*So*)

Compound[a])	t_R[b])	*Ln*[c])	*Joo*[c])	*To*[c])	*Csp*[c])	*Pp*[c])	*St*[c])	*So*[c])	Method[d])
Tricyclene	6.78	–	–	0.28±0.01	0.17±0.01	–	–	0.10±0.02	GC/MS
α-Thujene	6.97	0.10±0.01	–	0.25±0.02	0.18±0.01	0.92±0.05	0.89±0.11	0.16±0.01	GC/MS
α-Pinene	7.13	3.67±0.03	27.40±0.05	35.72±0.63	53.56±0.32	6.81±0.12	10.15±0.32	4.72±0.11	GC/MS
α-Fenchene	7.34	–	–	1.21±0.11	0.72±0.018	–	–	–	GC/MS
Camphene	7.38	1.69±0.04	0.10±0.02	0.19±0.04	0.24±0.01	0.39±0.01	0.08±0.009	2.55±0.08	GC/MS
m-Cymene	7.82	–	–	0.24±0.02	–	–	–	–	GC/MS
Sabinene	7.94	1.64±0.03	4.51±0.01	0.85±0.02	1.01±0.034	17.08±0.25	8.64±0.15	6.97±0.21	GC/MS
β-Pinene	7.96	2.14±0.01	0.40±0.05	–	1.78±0.076	6.48±0.09	2.90±0.18	3.01±0.14	GC/MS
β-Myrcene	8.21	0.56±0.01	18.90±0.07	1.64±0.07	–	–	0.68±0.03	tr	GC/MS
α-Phellandrene	8.49	0.11±0.07	7.10±0.002	0.17±0.01	–	1.13±0.03	tr	–	GC/MS
δ-3-Carene	8.62	–	–	9.48±0.12	–	tr	–	–	GC/MS
α-Terpinene	8.71	0.15±0.01	–	0.15±0.01	18.9±0.14	3.60±0.06	1.10±0.12	0.17±0.01	GC/MS
p-Cymene	8.86	0.12±0.05	0.51±0.08	1.23±0.02	0.82±0.02	6.01±0.09	10.76±0.53	1.08±0.04	GC/MS
Limonene	8.94	0.10±0.01	6.70±0.05	2.90±0.02	1.95±0.08	8.56±0.11	0.57±0.09	1.20±0.05	GC/MS, CoI
1,8-Cineole	8.98	9.43±0.07	–	–	–	–	0.28±0.06	43.62±0.44	GC/MS, CoI
β-Ocimene	9.09	21.83±0.18	–	0.18±0.03	–	–	–	–	GC/MS
α-Ocimene	9.24	–	–	0.12±0.01	–	–	–	–	GC/MS
γ-Terpinene	9.45	0.10±0.01	0.10±0.01	0.87±0.10	0.31±0.01	6.33±0.12	7.56±0.11	0.39±0.08	GC/MS
Terpinolene	9.94	–	0.22±0.04	2.94±0.22	3.15±0.13	2.86±0.06	0.62±0.15	tr	GC/MS
Fenchone	9.96	0.12±0.02	–	–	–	–		–	GC/MS
Linalool	10.08	–	0.40±0.02	–	–	–	2.81±0.11	–	GC/MS, CoI
α-Thujone	10.32	–	–	–	–	–	0.08±0.11	12.99±0.13	GC/MS
Fenchol	10.35	–	–	0.28±0.01	–	–	–	–	GC/MS
δ-Isothujone	10.39	–	–	–	–	–	–	1.48±0.02	GC/MS
cis-p-2-Menthen-1-ol	10.45	–	–	–	–	0.32±0.05	–	–	GC/MS
α-Campholene aldehyde	10.52	–	0.10±0.06	0.22±0.01	–	–	0.25±0.11	–	GC/MS
trans-Pinocarveol	10.74	–	0.10±0.03	0.57±0.07	–	–	–	–	GC/MS
Camphor	10.81	0.35±0.04	–	0.84±0.09	–	–	–	5.71±0.12	GC/MS
trans-Pinocamphone	11.03	–	–	0.36±0.01	–	–	–	–	GC/MS
Isopinocamphone	11.23	–	–	0.44±0.01	–	–	–	–	GC/MS

Table 2 (cont.)

Compound[a])	t_R[b])	*Ln*[c])	*Joo*[c])	*To*[c])	*Csp*[c])	*Pp*[c])	*St*[c])	*So*[c])	Method[d])
Terpinen-4-ol	11.28	–	0.10 ± 0.05	–	–	–	–	–	GC/MS
α-Terpineol	11.42	0.40 ± 0.02	0.30 ± 0.08	0.20 ± 0.01	1.08 ± 0.02	2.43 ± 0.08	1.53 ± 0.16	3.18 ± 0.32	GC/MS
Myrtenol	11.52	–	–	0.16 ± 0.03	–	–	–	–	GC/MS, CoI
Isoborneol	11.58	0.31 ± 0.01	–	0.15 ± 0.01	–	–	–	–	GC/MS
cis-Piperitol	11.65	–	–	–	–	–	0.10 ± 0.06	–	GC/MS
Verbenone	11.69	–	0.10 ± 0.01	0.19 ± 0.01	–	–	–	–	GC/MS
Fenchol acetate	11.80	–	–	0.30 ± 0.02	–	–	–	–	GC/MS
Isopulegone (**1**)	11.82	–	–	–	–	–	0.10 ± 0.02	–	GC/MS
trans-Carveol	11.93	–	0.20 ± 0.03	–	–	–	–	–	GC/MS
Methyl thymyl ether	12.05	–	–	–	0.14 ± 0.01	0.38 ± 0.02	–	–	GC/MS
Pulegone (**2**)	12.07	–	–	–	–	–	tr	–	GC/MS
Citronellol	12.18	–	0.30 ± 0.05	–	–	–	–	–	GC/MS
Carvone	12.25	–	0.10 ± 0.09	–	–	–	–	–	GC/MS
Geraniol	12.32	–	0.10 ± 0.02	–	–	–	tr	–	GC/MS
Neryl acetate	12.57	–	–	–	–	–	0.26 ± 0.03	–	GC/MS
Bornyl acetate	12.59	0.23 ± 0.01	0.62 ± 0.04	0.86 ± 0.04	–	–	–	0.24 ± 0.06	GC/MS
α-Terpinyl acetate	12.62	–	0.10 ± 0.03	–	–	–	–	–	GC/MS
Thymol	12.63	–	–	–	3.84 ± 0.11	–	9.92 ± 0.07	–	GC/MS
α-Fenchyl acetate	12.65	–	–	–	–	2.05 ± 0.05	–	–	GC/MS
Carvacrol	12.90	–	–	–	–	–	4.98 ± 0.08	–	GC/MS
Bornylene	13.19	–	–	–	0.72 ± 0.03	–	–	–	GC/MS
1-p-Menthen-8-yl acetate	13.31	–	–	–	–	–	0.40 ± 0.08	–	GC/MS
α-Cubebene	13.35	–	0.53 ± 0.07	–	–	0.52 ± 0.03	–	tr	GC/MS
Eugenol	13.41	–	–	–	–	–	–	–	GC/MS
α-Ylangene	13.67	0.23 ± 0.08	0.40 ± 0.05	0.46 ± 0.02	–	0.22 ± 0.01	–	–	GC/MS
Calarene (**3**)	13.69	–	–	–	0.31 ± 0.01	–	–	–	GC/MS
α-Copaene	13.71	0.17 ± 0.01	0.30 ± 0.04	0.44 ± 0.01	–	0.20 ± 0.01	1.67 ± 0.13	–	GC/MS
α-Bergamotene	13.75	0.10 ± 0.04	–	0.10 ± 0.01	–	–	–	–	GC/MS
β-Bourbonene (**4**)	13.79		–	–	–	–	0.24 ± 0.05	–	GC/MS
β-Elemene	13.81	1.0 ± 0.06	–	–	–	0.10 ± 0.01	0.21 ± 0.01	–	GC/MS
Methyl eugenol	13.86	–	–	–	–	–	–	–	GC/MS
Zingiberene (**5**)	13.90	–	–	0.11 ± 0.01	–	0.48 ± 0.01	–	–	GC/MS

Table 2 (cont.)

Compound[a])	t_R[b])	*Ln*[c])	*Joo*[c])	*To*[c])	*Csp*[c])	*Pp*[c])	*St*[c])	*So*[c])	Method[d])
Longifolene	13.97	–	0.20±0.02	–	–	–	–	–	GC/MS
Isolongifolene (**6**)	14.05	–	–	–	1.35±0.02	–	–	–	GC/MS
α-Gurjunene	14.06	–	–	–	–	–	0.51±0.06	–	GC/MS
trans-Caryophyllene	14.17	0.32±0.04	1.60±0.09	3.43±0.02	–	0.63±0.01	3.67±0.11	1.05±0.07	GC/MS, CoI
Widdrene (**7**)	14.24	–	–	0.81±0.01	–	–	–	–	GC/MS
Aromadendrene	14.30	–	–	–	–	3.99±0.01	–	0.99±0.04	GC/MS
cis-Thujopsene	14.32	–	0.30±0.02	–	–	–	–	–	GC/MS
β-Gurjunene	14.36	–	0.41±0.01	–	–	–	–	–	GC/MS
trans-β-Farnesene	14.38	0.13±0.01	0.32±0.01	0.71±0.02	–	–	–	–	GC/MS
α-Humulene	14.43	0.10±0.01	1.01±0.05	0.42±0.03	–	0.29±0.01	0.34±0.03	3.41±0.45	GC/MS
β-Acoradiene (**8**)	14.63	–	–	0.62±0.01	–	–	–	–	GC/MS
Epibicyclosesquiphellandrene	14.79	–	2.30±0.02	1.10±0.07	–	2.40±0.03	1.68±0.14	–	GC/MS
α-Guaiene	14.85	–	–	–	–	–	–	–	GC/MS
β-Bisabolene	14.95	–	–	2.19±0.12	–	–	–	–	GC/MS
β-Himachalene	14.98	–	–	0.89±0.06	–	–	–	–	GC/MS
β-Selinene	15.01	–	0.80±0.08	–	–	–	0.11±0.02	–	GC/MS
α-Muurolene	15.09	–	0.90±0.02	–	–	–	0.37±0.04	–	GC/MS
γ-Cadinene	15.15	0.36±0.02	0.61±0.07	–	–	–	–	tr	GC/MS
δ-Cadinene	15.21	0.14±0.01	2.20±0.08	2.86±0.02	0.22±0.01	1.51±0.07	3.11±0.12	0.1±0.01	GC/MS
Cadina-1,4-diene	15.38	–	0.10±0.04	–	–	–	–	–	GC/MS
Palustrol (**9**)	15.67	–	–	–	–	–	0.99±0.08	–	GC/MS
Spathulenol	15.73	–	–	–	–	–	0.61±0.07	–	GC/MS
γ-Gurjunene	15.88	–	–	–	–	0.33±0.11	–	1.16±0.05	GC/MS
Viridiflorol (**10**)	15.90	–	–		–	–	0.78±0.04	0.11±0.04	GC/MS
α-Cedrol	16.03	–	0.31±0.05	9.55±0.07	–	–	–	–	GC/MS
β-Guaiene	16.14	–		–	–	0.16±0.03		–	GC/MS
β-Maaliene	16.44	–	–	–	–	3.08±0.04	–	–	GC/MS
Eremophilene	16.52	–	0.20±0.03	–	–	–	–	–	GC/MS
Cadalene (**11**)	16.79	–	0.10±0.02	–	–	–	–	–	GC/MS
(*E*,*E*)-Farnesol	17.02	–	0.60±0.06	–	–	–	–	–	GC/MS

Table 2 (cont.)

Compound[a])	t_R[b])	*Ln*[c])	*Joo*[c])	*To*[c])	*Csp*[c])	*Pp*[c])	*St*[c])	*So*[c])	Method[d])
4-Oxo-α-ylangene	17.37	–	–	–	–	–	0.15±0.07	–	GC/MS
Methyl palmitate	18.46	–	0.10±0.04	–	–	–	–	–	GC/MS
Biformene	18.64	–	–	–	–	0.56±0.01	0.10±0.05	–	GC/MS
Palmitic acid	18.74	–	0.21±0.08	–	–	–	–	–	GC/MS
Manoyl oxide	19.26	–	0.20±0.03	–	–	–	–	–	GC/MS
Eremanthin (**12**)	19.31	3.65±0.09			–	–	–	–	GC/MS
Rimuene	19.39	–	–	–	–	–	0.71±0.24	–	GC/MS
Ethyl palmitate	19.56	–	0.11±0.07	–	–	–	–	–	GC/MS
Methyl stearate	19.95	–	0.12±0.06	–	–	–	–	–	GC/MS
Ethyl linoleate	20.29	–	tr	–	–	–	–	–	GC/MS
Ethyl stearate	20.47	–	tr	–	–	–	–	–	GC/MS
Dehydrocostuslactone (**13**)	20.82	7.57±0.12	–	–	–	–	–	–	
Identified compounds		56.82	82.39	86.68	90.45	79.82	80.91	94.39	

[a]) Compounds listed in order of elution from *SE30 MS* nonpolar column. [b]) t_R: Retention time [min]. c) Relative area percentage (peak area relative to total peak area in %). tr: trace, *i.e.*, <0.05%. [d]) CoI: co-injection with authentic compound.

of isoborneol to completely inhibit HSV-1 replication, without affecting viral adsorption [11]. The content of this monoterpene in *L. nobilis* berry oil was found in low percentage, and probably, therefore, it is not able to exert antiviral activity against HSV-1.

Table 3. *Antiviral Activities of Lebanon Essential Oils*[a])

Essential oil	Vero cells	HSV-1		SARS-CoV	
	TC_{50} [μg/ml]	IC_{50} [μg/ ml]	SI	IC_{50} [μg/ml]	SI
L. nobilis	500 ± 1.02	60 ± 0.5	8.3	120 ± 1.2	4.2
T. orientalis	> 1000	> 1000	> 1	130 ± 0.4	3.8
J. oxycredrus ssp. *oxycedrus*	1000 ± 1.7	200 ± 1.2	5	270 ± 1.5	3.7
C. sempervirens ssp. *pyramidalis*	> 1000	> 1000	> 1	700 ± 2.3	1.5
P. palaestina	500 ± 0.8	500 ± 2.2	> 1	> 1000	> 1
S. officinalis	> 1000	> 1000	> 1	870 ± 1.5	> 1
S. thymbra	> 1000	220 ± 1.6	4.5	–	–
Acyclovir	> 22.5 (100 μM)	0.85 (3.77 μM)	26.5	–	–
Glycyrrhizin	783.4 (952 μM)	–	–	641.0 (779 μM)	1.2

[a]) Results are shown as mean ± SD, $n = 3$. IC_{50}: concentration required to inhibit 50% of virus growth: TC_{50}: drug concentration that reduces the cell growth by 50% (cellular toxicity); SI = selectivity index (TC_{50}/IC_{50}); – : not tested.

A certain activity against SARS-CoV was found for *T. orientalis* and *J. oxycedrus* ssp. *oxycedrus* oils with IC_{50} values of 130 and 270 μg/ml, and a SI of 3.8 and 3.7, respectively. Interestingly, *J. oxycedrus* ssp. *oxycedrus* oil exhibited the highest activity against HSV-1 with a IC_{50} value of 200 μg/ml and a SI of 5. On the other hand, *T. orientalis* oil did not show any antiviral activity against HSV-1 when it was incubated under the same conditions. HSV-1 Growth was inhibited also when *S. thymbra* oil was used (IC_{50} of 220 μg/ml and SI of 4.6). The *C. sempervirens* oil did not exhibit any activity against HSV-1 ($IC_{50} > 1000$ μg/ml). This results may be related to the inactivity of the main component α-pinene as we have previously demonstrated [2]. A weak activity against SARS-CoV was found when *C. sempervirens* ssp. *pyramidalis* and *S. officinalis* essential oils were applied in virus culture (IC_{50} 700 and 870 μg/ml, resp.). *P. palaestina* essential oil was inactive against SARS-CoV ($IC_{50} > 1000$ μg/ml) and less active against HSV-1 (IC_{50} 500 μg/ml). Interestingly, *L. nobilis*, *T. orientalis*, and *J. oxycedrus* ssp. *oxycedrus* oils exhibited higher potencies to inhibit SARS-CoV and a great margin of safety compared to the positive control glycyrrhizin (IC_{50} 641 μg/ml; SI 1.2).

Cytotoxic Activity. Cytotoxic effects of the essential oils were tested in confluent layers of Vero cells by MTT assay. Assessment of cytotoxicity is clearly an important aspect of the evaluation of a potential antiviral agent, because a useful oil should be selective for virus-specific processes with no or only few effects on cellular metabolism. In Vero cells the TC_{50} value of tested samples was in a range of 120 to 1000 μg/ml.

Conclusions. – Severe acute respiratory syndrome (SARS) is an emerging disease that created international anxiety because of its relatively high infectious, rapid

progression and relatively high death rate. The fact that no conventional medicine was used for the treatment of SARS was based on the evidence that natural products from plants exhibited antiviral activity to other coronaviruses although the mechanism of action of these herbal products is mainly through inhibition of viral replication [12].

In this paper, we presented the first evidence for a strong antiviral activity of *L. nobilis* oil against SARS-CoV, and we also reported the interesting anti-herpetic activity of *J. oxycedrus* ssp. *oxycedrus* and *S. thymbra* oils providing a potential use of these oils for treatment of viral infectious diseases.

Experimental Part

Plant Materials. Berries of *Laurus nobilis* L. (Lauraceae) and *Juniperus oxycedrus* L. ssp. *oxycedrus* (Cupressaceae), fruits of *Thuja orientalis* L. (Cupressaceae), *Cupressus sempervirens* L. ssp. *pyramidalis* (Cupressaceae), and *Pistacia palaestina* Boiss. (Labiatae), *Salvia officinalis* L. (Labiatae), and leaves of *Satureja thymbra* L. (Labiatae) were collected from June to November 2003 in Lebanon (*Table 1*). A voucher specimen of each plant was authenticated botanically by Prof. *S. Safi*, Biology Department, Faculty of Sciences II, Lebanese University, and deposited with the Herbarium of Faculty of Sciences II, Lebanese University.

Isolation of Essential Oils. The fresh aerial parts (200 g of each of the above mentioned species) were submitted to hydrodistillation for 3 h using a *Clevenger*-type apparatus as described in [2], yields in percent are listed in *Table 1*. The white-yellow essential oils were dried (anh. Na_2SO_4) to remove traces of moisture and stored at 4–8° in bottles covered with aluminium foil to prevent the negative effect of light.

GC/MS Analysis. To determine the essential oils composition, analyses were carried out using a GC system (*Hewlett-Packard Co.*, model *6890*) with a fused cap. column (30 m length; 0.25 mm i.d.; 0.25-μ film thickness; static phase methylsilicone *SE-30*) directly coupled to a selective mass detector (*Hewlett Packard 5973*). Electron impact ionization was carried out at an energy of 70 eV. He was used as carrier gas. Injector and detector were maintained at 250° and 280°, resp. The anal. conditions were as follows: oven temp. was 5 min isothermal at 60°, then 60–280° at a rate of 16°/min, then held isothermal for 10 min. The mass range from 50 to 550 amu was scanned at a rate of 2.9 scans/s. Identification of the components was based on the comparison of the MS data on computer matching against *Wiley 138* and those described in literature (*Table 2*) [13].

Cytotoxicity Assay. Cytotoxicity of the essential oils towards Vero cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction in active mitochondria using monkey kidney cell line Vero (*ATCC*, Manassas, VA) as described in [2]. The drug concentration that reduced the cell growth by 50% is expressed as TC_{50} (Tissue $Culture_{50}$).

Antiviral Assay. Antiviral action of essential oils against HSV (F Strain ATCC VR733) and SARS-CoV (isolate FFM-1 obtained from the sputum of a patient hospitalized with a diagnosis of SARS in the Isolation Unit of Frankfurt University Hospital, Germany) replication was tested as follows. Vero cells were seeded in 96-well plates and infected with HSV-1 or SARS-CoV at multiplicity of infection (MOI) of 0.01. The viruses were propagated in Vero cells as described in [2][14]. In accordance with WHO recommendations, all work involving infectious SARS-CoV was performed under biosafety level (BSL)-3 conditions in a BSL-3 facility. Acyclovir (ACV) was used as a control for antiviral activity against HSV-1, while glycyrrhizin (GLZ) was used against SARS-CoV. Both positive control compounds were purchased from *Sigma-Aldrich*, D-Munich. Virus and the tested essential oils of different concentrations were added at the same time in MEM supplemented with 2% FBS. For each dilution step, 8 wells were used in parallel. Virus infection was assessed by visually scoring of the virus-induced cytopathogenic effect (CPE) 72 h (HSV-1) or 48 h (SARS-CoV) post-infection. The effective concentration inhibiting 50% of virus growth (IC_{50}) was determined as concentration of compound required to inhibit the CPE effect to 50% of the control value. The selective index (SI), also known as a therapeutic ratio or margin of safety, is the ratio of the amount of drug that causes a therapeutic effect to the amount that causes a toxic effect, *i.e.*, TC_{50}/IC_{50}.

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