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Chemical Composition and Antiviral Activity of The Essential Oils of Hybrid Mandarin *Citrus Reticulata* Blanco Cultivars; Merav and Murcott Cultivated in Egypt; Comparative Study

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Abstract: Merav and Murcott are different cultivars of *Citrus reticulata* Blanco (family Rutaceae). They are hybrid mandarin oranges cultivated in Egypt. This study aimed to compare the chemical compositions of the volatile oils from the two cultivars, which were extracted from the ripe fruit rind and fresh leaves utilizing the hydrodistillation procedure. The oil components were identified and quantified using gas chromatography-mass spectrometry analysis. The most important components of the leaves of Merav cultivar were: sabinene (21.19%), caryophyllene (11.98%) and linalool (10.89%), while D-limonene (96.02%) was the predominant active constituent in the oil of fruits rind. In comparison, the main constituents of the leaves of Murcott oil were linalool (31.63%), sabinene (23.40%) and β-trans ocimene (11.47%), while D-limonene (96.39%) was the highest concentration in the fruits' rind oil. In addition, antiviral activity was investigated against Hepatitis A virus (HAV) and Herpes simplex type-1 (HSV-I) viruses. It was assessed using MTT assay and the antiviral activity (%) was determined and compared with standard antiviral agents (Acyclovir and Amantadine). The oil of Murcott fruit rind showed excellent antiviral activity against HAV and HSV-1 (97.11 % and 99.48%) with selectivity index (SI) 10.86 and 12.79 respectively. While the oils of Merav fruit rind and leaves possess moderate antiviral activity against HAV (94.37% and 85.22 %) with SI 6.57 and 6.77 respectively. Also, they displayed valuable antiviral efficacy against HSV-1 (99.04% and 93.73%) with SI 8.64 and 5.39 respectively. These results paved the way for the discovery of new remedies for HAV and HSV-1 infection.

Keywords: *Citrus reticulata*; Merav; Murcott; Volatile oils; Rutaceae; Antiviral activity.

1. INTRODUCTION

Citrus peels and leaves are considered to be a waste from agriculture and industrial processes. These waste materials may have the ability to produce secondary metabolites, particularly essential oils (EO) and flavonoids. Monoterpenes are a distinctive component of citrus essential oils such as limonene, and sabinene, in addition to their oxygenated derivatives including; linalool alcohol, citral aldehydes, acids, ketones and sesquiterpenes[1]. They stand out as promising candidates for screening potential antiviral, anticancer, antibacterial, antioxidant, and free radical scavengers. Moreover, their versatile applications extend beyond medicinal purposes; they are utilized in pharmaceuticals, perfumery, and cosmetics, as well as in the food industry for their preservative qualities. From enhancing confectionery and desserts to enriching beverages, their utilization spans a broad spectrum of products[2,3]. Global fruit production increased by 63% between 2000 and 2022, exceeding a total production volume of 933 million tonnes during that year. Mandarin orange *Citrus reticulata* L. (*C. reticulata* L.), is native to tropical and subtropical Asia, it has many hybrids which vary in size and

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color even some types being seedless. One of the most frequently cultivated and consumed fruit in the Rutaceae family. Mandarins account for 76 million tonnes of its yearly production[4]. The Murcott mandarin pertain to the Rutaceae family, it is presumably a tangor, a hybrid resulting from the crossbreeding of *Citrus reticulata* and *Citrus sinensis*. While it is known as "Honey Tangerine," its official name remains Murcott. It is notable for their large size, intense orange color, rich in juice, and seed-abundant flesh within easily peelable, these fruits have substantial economic value. Murcott mandarin maturation period spanning from December to March, it distinguishes itself as one of the later maturing varieties within the mandarin family, further enhancing its market desirability[5]. Another tangor is the Merav mandarin, a hybrid between a sweet orange and a willow-leaf mandarin orange. Merav has 7 to 14 splits and a deep orange color with a lustrous and smooth texture. Like tangerines, they are commonly effortless to peel. Usually, their flesh is seedless, smaller than other mandarins but sugary in flavor, juicy, sweet, and less acidic than oranges[6].

Due to the presence of certain distinguished non-terpenoid aldehyde compounds, such (E,E)-2,4-heptadienal or (Z)-2-dodecenal, the majority of the volatile oil composition found in *C. reticulata* L. appears to differ from the composition found in the other Citrus species[7]. In addition, several forms of other nonterpenoid aldehyde compounds have been identified mainly in the peel of *C. reticulata* L. and rarely in the peel of other Citrus species. This is the case of (Z)-4-decenal , (E)-2-octenal, (Z)-5-dodecenal, (E)-2-nonenal, (E)-2-octenal[7], heptanal[8] and (Z)-6-dodecenal[9]. One notable component found in the rinds of *C. reticulata* is the sesquiterpene hydrocarbon β-copaene, which has only been observed in the five mentioned species: *C. reticulata, C. paradisi, C. grandis, C. sinensis* and *C. aurantium*[10]. From a quantitative standpoint, the most prevalent components in *C. reticulata* EO are monoterpene hydrocarbons, even if non-terpenoid aldehyde chemicals might aid in distinguishing this species from others. The most important of these is limonene, which typically makes up around 95% of the entire EO. Limonene (the most significant constituent of citrus oils) is notable due to its pharmacological and therapeutic benefits, including antiviral, anti-inflammatory, digestive, and cytotoxic effects. Additionally, it enhances the absorption of vitamin C, aiding in the battle against colds and flu. Also in food production, it serves as a flavoring agent, while in cleaning products, it is incorporated to impart a refreshing orange- or lemon-like scent[11].

Essential oils are comprised of multiple volatile components, so they possess considerable antiviral properties, such as those against HIV, avian influenza, influenza, yellow fever virus, and influenza virus, and anti-human herpesviruses[12]. Citrus clementine peel EO was reported to have a strong antiviral effect against COVID, which is explained by limonene's ability to bind to nsp16 and a different type of spike protein, which suppresses and targets SARS-COV-2 infection[13]. In Egypt, the most common cause of acute viral liver diseases is the hepatitis A virus (HAV)[14]. An investigation of Egyptian patients with acute viral hepatitis from 2014 to 2017 found that 93% of cases were caused by HAV infection, whereas 2.7-8% of cases were due to HBV and HCV infection[15]. After a national HBV vaccination program for infants and an intensive HCV mass screening campaign that treated over 4 million patients receiving direct antiviral therapy, the incidence of infection with HBV and HCV among Egyptians dropped by 93% and 20%, respectively, in 2024 compared to 2015. The lack of a national HAV vaccination program is one potential reason for the rising incidence of HAV infection among Egyptians[16]. Human pathogen herpes simplex virus (HSV) develops and invades the mucocutaneous membrane at the point of entrance of the cells. In some cases, this leads to repeated infections as the virus can reactivate spontaneously. As a result, antiviral drugs including amantadine, valacyclovir, famciclovir, and acyclovir suppress the virus or shorten its duration and decrease the risk of developing clinical symptoms. In contrast, other antiviral drugs had harmful adverse effects, while the virus spread, it became resistant to these drugs. These factors make it challenging to find new natural substances that act as antiviral agents[17]. Considering the published data about both varieties (Merav and Murcott), there wasn't previous data regarding the chemical composition and biological properties of Merav mandarins grown in Egypt. Also, we observed that the antiviral activity of the volatile oils from the leaves and fruit rind of the hybrid mandarin grown in Egypt had not previously been studied. So, this study evaluates the antiviral potential of the essential oils against HAV and HSV-I, in addition, compares the chemical components of the essential oils extracted from the leaves and fruit rind of the Egyptian *C. reticulata* Blanco cultivars, Merav and Murcott

2. METHODS

2.1. Plant material

Leaves and ripe fruits rind of *C. reticulata* Blanco Cultivars; Merav and Murcott were collected from El-Batoul Farm for Citrus fruits, Nubariah City, El-Beheira Governorate, Egypt in June 2023 for the

leaves and in February 2024 for the ripe rind. Professor Dr. Wafaa Amer, who specializes in taxonomy at Cairo University in Egypt, confirmed their identities. In the herbarium of the Department of Pharmacognosy and Medicinal Plants, Faculty of Pharmacy (Girls), Al-Azhar University, four samples with vouchers (CRL23, CML23, CRF24 and CMF24) have been placed for the Merav leaves, Murcott leaves, Merav fruit rind and Murcott fruit rind, respectively.

2.2. Extraction of the essential oils

Hydro-distillation was performed using Clevenger's apparatus on specimens (1 kg, each) of the mature fruit rind of *C. reticulata* cv. Merav and Murcott (MVF and MTF) and the leaves of *C. reticulata* cv. Merav and Murcott (MVL and MTL)[18]. According to the Egyptian Pharmacopoeia, for further examination, the essential oils were stored in dark ampoules in the refrigerator with anhydrous sodium sulfate[19.] The specific gravity and refractive index of each tested oil sample were evaluated.

2.3. GC-MS analysis of the essential oils

L. Han et al., (2024)[20] experimental conditions were applied to perform GC-MS analysis of the volatile oil specimens from MVL, MTL, MVF, and MTF, which utilizes the features of a GC-MS system from Agilent Technologies that has a mass spectrometer detector (5977A) and a gas chromatograph (7890B). With a solvent delay of five minutes and a spectral band of m/z 50-550, mass spectra were obtained using electron ionization (EI) at 70 eV. The mass fragmentation pattern of the oils was compared to standards documented in the Adams Library to identify the various components[21]. Using GLC analysis of a series of standard n-alkanes under identical experimental conditions, the retention indices (Kovats indices) of the EOs' components were calculated[22].

2.4. Antiviral activity

2.4.1. Viruses, strains and cell culture conditions

From the Laboratory of Virology (Science Way for Scientific Research and Consultations, Al-Azhar University, Egypt) HAV and HSV-I were collected. Normal VERO cell line (adherent kidney epithelial cells from Cercopithecus aethiops, CCL-81) was cultured in RPMI 1640 medium (Gibco, Tunisia) supplemented with streptomycin (100 mg/mL), penicillin (100 U/mL), L-glutamine (2 mM), and fetal bovine serum (10% v/v). The cells followed by incubation at 37 °C in a humidified environment with 5% CO_2[23].

2.4.2. Determination of cytotoxicity on VERO cells

The maximum non-toxic concentration (MNTC) of the four essential oils on VERO cells was determined by the MTT colorimetric assay. Different concentrations of the investigated oil samples were prepared. After forming a confluent sheet of VERO cells, the culture medium was poured out of 96-well microtiter plates. The cell monolayer was removed twice using rinse media. The investigated oil samples were double-diluted in minimum needed media, and 0.1 ml of each dilution was tested in separate wells, leaving three wells as standards. The plate was then incubated at 37°C and monitored periodically for up to two days. Physical manifestations of toxicity, such as shrinkage, rounding, granulation, or partial or whole disappearance of the monolayer, were checked in the cells. 5 mg/ml MTT solution in PBS was made (BIO BASIC CANADA INC). After adding 20 µl of MTT solution to each well, the media was mixed with the MTT by shaking the well for five minutes at 150 rpm. MTT was allowed to metabolize for up to five hours at 37°C with 5% CO_2. After that, the media was discarded (dry plate on tissue paper to remove residue if desired). To thoroughly mix the formazan and solvent, the formazan is re-suspended in 200 µl of DMSO and shaken for five minutes at 150 rpm. The optical density is measured at 560 nm, and the background is eliminated at 620 nm. There should be a strong correlation between optical density and cell count[24].

2.4.3. MTT assay protocol

The antiviral activity was measured using an MTT test with 10,000 cells covered with 200 µl of medium per well in a 96-well plate. After an hour of incubation at equal volumes (1:1 v/v) with a nonlethal dilution of the examined samples and the virus suspension, 100 µl of the viral/sample suspension was added, and the combination was agitated for five minutes at 150 rpm. For blank controls, three wells were left empty. After that, the cells were left to adhere to the wells for a whole night at (37°C, 5% CO_2). To allow the virus adequate time to reproduce itself, the viral/sample solution was incubated for one day at 37°C with 5% CO_2. For every 96-well plate, two milliliters or more of MTT solution at a concentration of 5 mg/ml were made in phosphate buffered saline (PBS). After adding 20 µl of MTT solution to each well, the plates were shaken at 150 rpm for five minutes to thoroughly mix the MTT into the media. The plate was then incubated at (37°C, 5% CO_2) for 1-5 hours to permit the MTT to be digested before the media was removed (dry plate on paper towels to eliminate rest if necessary). A shaking table was used to properly mix the formazan (MTT metabolic product) into the solvent for five minutes while it was re-suspended in 200 µl DMSO. The optical density (OD) was measured at 560 nm. The number of cells should be proportionate to the

optical density[24]. The following equation was used to calculate the antiviral activity of four oil samples;

$$\text{Antiviral activity \%} = \frac{(\text{Absorbance of cells without treatment} - \text{Absorbance of cells with treatment})}{(\text{Absorbance of cells without treatment})} \times 100$$

2.4.4. Calculation of Selectivity Index (SI)

Determination of SI towards the supporting host cell is an essential step of antiviral testing. Calculation of selectivity index according to the following equation: SI= CC_{50} /IC_{50} where; Cytotoxicity (CC_{50}) was expressed as mean ± standard deviation (SD) to Amantadine and Acyclovir; IC_{50}: Concentration that inhibit 50% of virus cells [25].

2.4.5. Statistical analysis

The means ± standard errors of the means were displayed for our data. The results were assessed using a one-way ANOVA and then the Tukey multiple comparison test. The significance of the data was determined using the *p*-value, with a value of $p < 0.05$ being deemed significant. Statistical analysis was performed using GraphPad Prism (Version 8.0.2 (2019) Inc., C.A., U.S.A.)[26].

3. RESULTS

3.1. Essential oil yield

The essential oils from MTL and MTF yielded 1.3% and 1.8% V/W, respectively, while the volatile oils from MVL and MVF yielded 1.1% and 1.6% V/W, respectively, when they were hydro-distilled.

3.2. Physical characteristics of the oil samples

There were discernible differences in the physical properties of the four oil samples. The volatile oils extracted from the leaves and the ripe fruits of Merav had an aromatic mandarin scent, were miscible with 70% ethanol, and had a light orange color. The specific gravities were 0.75 and 0.77 at 25°C, while the refractive indices were 1.351 and 1.407 at 20°C, respectively. The leaves and ripe fruit rind of the Murcott plant produced essential oils that were miscible with 70% ethanol, had a good aromatic scent, and were yellow in color. The specific gravities were 0.79 and 0.82 at 25°C, while the refractive indices were 1.392 and 1.422 at 20°C, respectively.

3.3. Chemical Composition of the hydro-distilled oils

The results of GC/MS analyses of the examined oils were discussed in Tables (1 and 2) and Fig. (1). The results revealed both qualitative and quantitative variation in the composition of the EOs., the total identified components under specified operating parameters were 42 and 7 compounds in MVL and MVF respectively; while that in MTL and MTF were 31, 14 compounds respectively. Fig. (1) represents the comparison between *C. reticulata* cv Merav and Murcott regarding their phytochemical constituents obtained from their leaves and fruit rind essential oil.

The hydrocarbon constituents in the essential oil of MVL and MVF were 67.01% and 97.68% respectively. Table (1) showed that sabinene (21.19%) and caryophyllene (11.98%) were identified in MVL. While MVF was dominated by D-limonene (96.02%) as a major active constituent. On the other hand, the oils of MTF and MTL appeared to be dominated by hydrocarbons (97.23% in the fruits rind and 55.49 % in the leaves). D-limonene (96.39%) was the major constituent of hydrocarbons in the fruit rind EOs. While Sabinene (23.40%) and β-trans ocimene (11.47%) were observed to be the most significant components of the leaves EOs. Considering the total percentage of oxygenated compounds of MVL was 33.99% were represented by linalool alcohol being the major oxygenated constituent (10.89%) followed by terpinen-4-ol (6.30%) and caryophyllene oxide (4.15%). While in MTL and MTF, alcohols were the major constituent (43.51% and 1.92%) respectively, being represented by linalool (31.63%) in MTL oil samples and (1.02%) in MTF oil samples.

3.4. Antiviral properties of studied oil samples

The MTT antiviral assay was used to examine the examined essential oils' antiviral efficacy against the HAV and HSV-1 viruses. According to Fig. (2), all tested oils showed dose-dependent antiviral potential against HAV and HSV-1. From Table (3) the essential oils extracted from MTF had the highest antiviral efficacy against HAV and HSV-1, with SI (selectivity index) of 10.86 and 12.79 respectively. Amoros, *et al.,*(1992)[25] proposed that a selectivity index of not less than 4 can be used when required. Statistical analysis data of antiviral activity represents a significant difference between the four tested samples when compared to each other and to amantadine and acyclovir ($p < 0.05$). Similarly, the volatile oil of MTL exhibited the lowest antiviral effect against HAV and HSV-1 with SI 3.517 and 4.43 respectively. All the tested volatile oils displayed marked antiviral activity against HSV-1 according to Amoros, *et al*.,(1992)[26].

Table 1. The identified chemical components in the hydro-distilled essential oil of the leaves and fruits of *C. reticulata* cv. Merav and Murcott

				Percentage (%) of the essential oils of:			
Peak	**KI***	**Identified Compound**	**Formula**	**MVL**	**MVF**	**MTL**	**MTF**
1	860	n-Hexanol	$C_6H_{14}O$	**-**	**-**	0.05	**-**
2	868	cis-3-Hexene-1-ol	$C_6H_{12}O$	**-**	**-**	0.10	**-**
3	897	Sabinene	$C_{10}H_{16}$	**21.19**	**-**	**23.40**	0.08
4	902	3-Thujene	$C_{10}H_{16}$	0.43	0.55	0.68	-
5	948	α-Pinene	$C_{10}H_{16}$	2.20	0.27	1.61	0.21
6	948	Δ-Carene	$C_{10}H_{16}$	0.50	-	-	-
7	958	β- Myrcene	$C_{10}H_{16}$	2.63	0.84	2.85	0.53
8	976	β- trans-Ocimene	$C_{10}H_{16}$	0.21	-	**11.47**	-
9	976	β- cis-Ocimene	$C_{10}H_{16}$	2.68	-	-	-
10	990	*p*-Mentha-2,8-diene	$C_{10}H_{16}$	0.16	-	-	-
11	998	α-Terpinene	$C_{10}H_{16}$	0.45	-	1.61	-
12	998	γ-Terpinene	$C_{10}H_{16}$	2.41	-	3.23	-
13	1005	n- Octanal	$C_8H_{16}O$	-	-	-	0.49
14	1018	D-Limonene	$C_{10}H_{16}$	2.96	**96.02**	2.03	**96.39**
15	1041	4-Thujanol	$C_{10}H_{18}O$	0.67	-	-	-
16	1042	o-Cymene	$C_{10}H_{14}$	0.80	-	0.83	-
17	1052	Terpinolene	$C_{10}H_{16}$	-	-	0.84	-
18	1059	1-Octanol	$C_8H_{18}O$	**-**	-	-	0.19
19	1062	α-Thujone	$C_{10}H_{16}O$	0.10	-	-	-
20	1082	Linalool	$C_{10}H_{18}O$	**10.89**	1.03	**31.63**	1.02
21	1109	2-*p*-Menthen-1-ol	$C_{10}H_{18}O$	0.57	-	0.74	-
22	1125	Citronellal	$C_{10}H_{18}O$	-	0.32	0.11	0.11
23	1137	Terpinen-4-ol	$C_{10}H_{18}O$	**6.30**	**-**	7.82	**-**
24	1143	α-Terpinol	$C_{10}H_{18}O$	2.85	0.97	0.96	0.09
25	1164	trans- Linalool oxide	$C_{10}H_{18}O_2$	0.25	-	-	-
26	1171	p-Anisaldehyde	$C_8H_8O_2$	-	-	0.68	-
27	1175	1-*p*-Menthen-3-ol	$C_{10}H_{18}O$	0.42	-	0.68	0.51
28	1179	Citronellol	$C_{10}H_{20}O$	-	-	-	0.11
29	1221	α-Copaene	$C_{15}H_{24}$	1.15	-	-	-
30	1339	β-Bourbonene	$C_{15}H_{24}$	2.33	-	-	-
31	1402	Dodecanal	$C_{12}H_{24}O$	-	-	-	0.13
32	1431	γ-Elemene	$C_{15}H_{24}$	0.11	-	-	-
33	1435	γ-Cadinene	$C_{15}H_{24}$	0.39	-	0.05	0.01
34	1440	Cubenene	$C_{15}H_{24}$	0.10	-	-	-
35	1458	α-Farnesene	$C_{15}H_{24}$	2.64	-	1.16	-

Table 1 cont. The identified chemical components in the hydro-distilled essential oil of the leaves and fruits of *C. reticulata* cv. Merav and Murcott

				Percentage (%) of the essential oils of:			
Peak	**KI***	**Identified Compound**	**Formula**	**MVL**	**MVF**	**MTL**	**MTF**
36	1469	Δ-Cadinene	$C_{15}H_{24}$	1.72	-	0.08	-
37	1484	Cubebol	$C_{15}H_{26}O$	0.49	-	-	-
38	1490	α-Guaiene	$C_{15}H_{24}$	-	-	0.27	-
39	1494	Caryophyllene	$C_{15}H_{24}$	**11.98**	-	4.29	-
40	1494	Alloaromadendrene	$C_{15}H_{24}$	0.21	-	-	-
41	1500	β-Bisabolene	$C_{15}H_{24}$	-	-	1.09	-
42	1507	Caryophyllene oxide	$C_{15}H_{24}O$	4.15	-	-	-
43	1515	Germacrene	$C_{15}H_{24}$	5.84	-	-	-
44	1530	Epiglobulol	$C_{15}H_{26}O$	0.22	-	-	-
45	1536	Spathulenol	$C_{15}H_{24}O$	2.12	-	-	-
46	1564	trans-Nerolidol	$C_{15}H_{26}O$	1.13	-	1.08	-
47	1579	Humulene	$C_{15}H_{24}$	3.77	-	-	-
48	1580	τ-Cadinol	$C_{15}H_{26}O$	1.34	-	-	-
49	1597	Sesquirosefuran	$C_{15}H_{22}O$	0.24	-	-	-
50	1598	Agarospirol	$C_{15}H_{26}O$	-	-	0.10	-
51	1607	Dendrolasin	$C_{15}H_{22}O$	0.17	-	-	-
52	1613	Neointermedeol	$C_{15}H_{26}O$	-	-	0.19	-
53	1646	Caryophyllene oxide	$C_{15}H_{24}O$	-	-	0.20	-
54	1646	α-Sinensal	$C_{15}H_{22}O$	0.84	-	-	-
55	1673	α-Bergamotol	$C_{15}H_{24}O$	0.49	-	-	-
56	1762	Humulenol II	$C_{15}H_{24}O$	-	-	0.05	-
57	2045	Phytol	$C_{20}H_{40}O$	0.17	-	0.11	-
58	4085	1,1- Bis	$C_{40}H_{82}O_2$	-	-	-	0.13
		Total identified percentage :		100	100	99.99	100

*KI:Kovats index; The major components are highlighted in bold; MVL: Merav leaves; MVF: Merav fruit rind; MTL: Murcott leaves; MTF: Murcott fruit rind

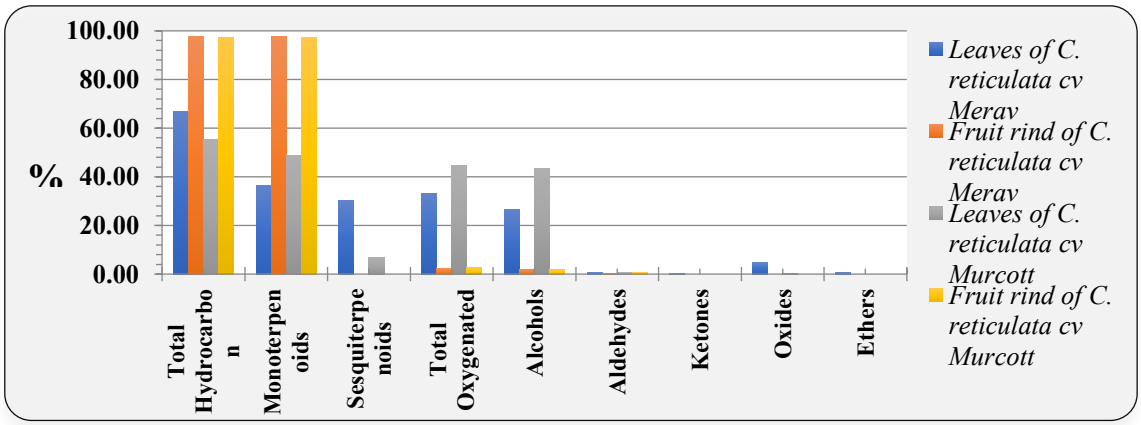

Figure. 1. Percentage of phytochemical constituents identified in the essential oils of MVL, MVF, MTL and MTF

Table 2. Comparison between the percentage of the chemical composition of the hydro-distilled essential oils of *C. reticulata* cv. Merav and cv. Murcot

Constituents	% of the essential oils:			
	MVL	**MVF**	**MTL**	**MTF**
Total Hydrocarbons:	**67.01**	**97.68**	**55.49**	**97.23**
Monoterpenes	**36.62**	**97.68**	**48.55**	**97.22**
Sesquiterpenes	**30.39**	**(-)**	**6.94**	**0.01**
Total Oxygenated:	**33.99**	**2.32**	**44.5**	**2.78**
Alcohols	**27.62**	**2.00**	**43.51**	**1.92**
Aldehydes	**0.84**	0.32	**0.79**	**0.73**
Ketones	**0.10**	(-)	(-)	(-)
Oxides	**4.7**	(-)	0.20	(-)
Ethers	**0.73**	(-)	(-)	**0.13**

(-): absence, **MVL**: Merav leaves; **MVF**: Merav fruit rind; **MTL**: Murcott leaves; **MTF**: Murcott fruit rind

Table 3. MNTC (µg/ml), Mean value of CC_{50}, IC_{50}, SI and antiviral activity (%) of MVL, MVF, MTL and MTF essential oils against HAV & HSV-1

Samples	Vero cells		HAV			HSV-1		
	MNTC ((µg/ml)	CC_{50} (Mean ± SD)	IC_{50} (µg/ml)	SI	Antiviral activity (%)	IC_{50} (µg/ml)	SI	Antiviral activity (%)
MTF	62.5 ug/ml	144.43 ± 1.18	13.29	10.86	97.11 %	11.29	12.79	99.48 %
MVF	125 ug/ml	226.16 ± 1.73	34.38	6.57	94.37 %	26.17	8.64	99.04 %
MTL	1.95 ug/ml	4.77 ± 0.08	1.356	3.517	71.88 %	1.076	4.43	90.47 %
MVL	7.81 ug/ml	19.01 ± 0.32	2.804	6.779	85.22 %	3.523	5.39	93.73 %
Amantadine	250 ug/ml	601.19 ± 2.81	196.77	3.05	70.60 %	-	-	-
Acyclovir	62.5 ug/ml	118.46 ± 1.9	-	-	-	20.44	5.7	96.68 %

MNTC: maximum non-toxic concentration; **Cytotoxicity (CC_{50})** was expressed as mean ± standard deviation (SD) with respect to Amantadine and Acyclovir; **IC_{50}**: Concentration that inhibit 50% of virus cells; Selectivity index (SI= CC_{50}/IC_{50}); **MTF**: Murcott fruit rind; **MVF**: Merav fruit rind; **MTL**: Murcott leaves; **MVL**: Merav leaves

4. DISCUSSION

Every year, more individuals are being diagnosed with viral infections, which remain a major cause of morbidity and mortality worldwide. Moreover, the negative side effects and inadequate reactions to resistant genotypes of synthetic antiviral drugs have encouraged us to seek effective and alternative remedies, such as antiviral compounds produced from plants[27]. Essential oils are one of these alternatives because they have the ability to ward off a variety of harmful viruses[28]. Viral infections are eradicated by powerful virucidal drugs via different mechanisms, including denaturation, protein aggregation, and cell structural alteration[29]. Extracellular factors and the mechanical properties of the viral capsid, such as its flexibility and stress tolerance during DNA wrapping and cell entry, have a major influence on survivability. Unlike synthetic antiviral components, which work in a way limited to a particular virus type, the action mechanisms of EOs are more broadly varied[30]. By rupturing the structure of the viral envelope or obstructing viral proteins, the

activity of EOs can hinder the virus's ability to enter host cells extracellularly. Moreover, EOs may have antiviral properties against intracellular infections[31].

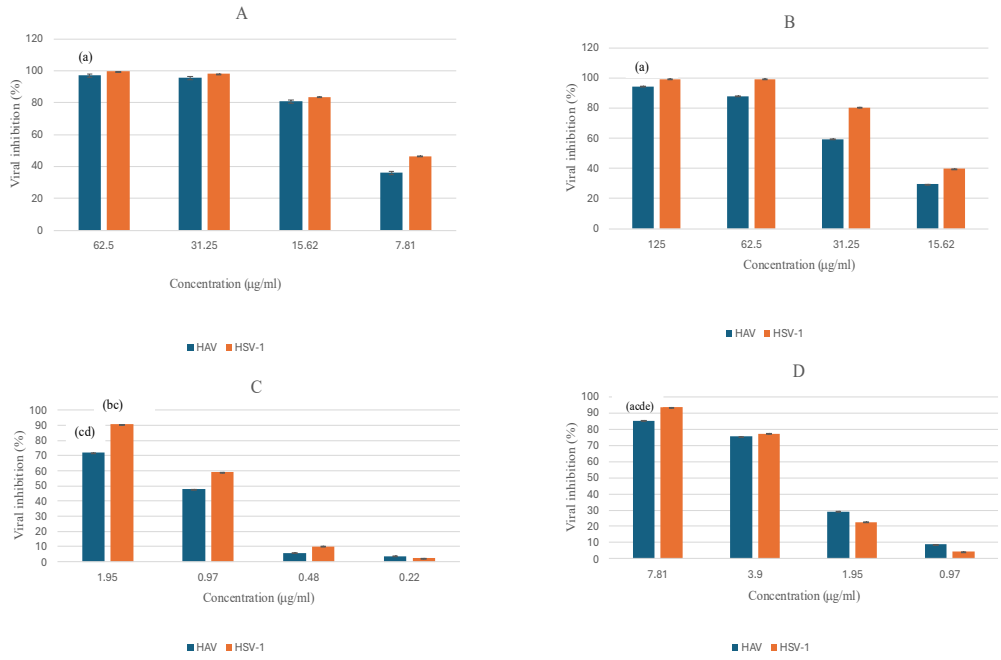

Figure. 2. Antiviral activity of MVL, MVF, MTL and MTF oils against HAV and HSV-1 expressed as viral inhibition (%) against concentration (µg/ml)
*A: MTF; B: MVF; C; MTL; D; MVL *Results are expressed as mean ± SE a: Significantly different from Amantadine b: Significantly different from Acyclovir c: Significantly different from the oil of MTF d: Significantly different from the oil of MVF e: Significantly different from the oil of MTL e: Significantly different from the oil of MTL

Vero and RC-37, two cell lines from monkey kidneys, are among the cell cultures used to test essential oils for their antiviral properties. Determining the cytotoxicity of essential oils is crucial to verify that an observed diminution in virus infection is caused by the direct action of the essential oil on the virus and not by a harmful effect on the host cells. Many essential oils and chemicals interact with HSV particles to impede cell adsorption, while some show direct virucidal activity or limit intracellular proliferation. Essential oils can also affect HSV strains resistant to acyclovir since they work differently than synthetic drugs[32]. Fadilah *et al*., (2022) is the first publication on the virucidal potential of both forms (Dextro and Levo) of limonene[33]. The accessible publication provides confirmed data on the use of different EOs of Citrus against HAV for example, *C. indicum* oil, and *C. morifolium* oil[34].

Against enclosed viruses, such as the herpes virus, influenza virus, and SARS CoV-2, the EOs are more effective. Due to changes in the envelope glycoproteins, which are required for virus adsorption and entrance into host cells, a virucidal impact is one possible mechanism of action for EOs against these viruses[35].

The highest antiviral activity of EOs of MTF and MVF can be ascribed to their elevated amount of limonene which was 96.39 and 96.02 % respectively. The cyclohexenyl moiety of limonene, which has been shown to have an antiviral effect in recent years, may be the reason for its relatively powerful binding and inhibition of the active site of the M^{pro} protein, a crucial homodimeric cysteine protease enzyme that splits polyproteins into separate proteins required for virus reproduction and translation[36].

The oil of MVL shows moderate antiviral activity against HAV and HSV-1 which besides the presence of limonene can be attributed to the presence of terpinen-4-ol which exerts antiviral activity through direct deactivation, which occurs when substances attach to the viral proteins that facilitate the virus's adhesion and invasion into the host cell[36]. Meanwhile, MTL oil is particularly effective against HSV-1 due to the presence of linalool. In fact, it acts on the protease enz

responsible for the transcription of viral proteins during the viral replication phase[37].

5. CONCLUSION

Our research concluded that the volatile oils obtained from the studied varieties had comparable both qualitative and quantitative differences in their chemical composition, with limonene being the main constituent of the oils extracted from the Murcott and Merav fruits. EOs of Murcott fruit and Merav fruit rind exhibited excellent antiviral activity against both HAV and HSV-1 when compared with amantadine and acyclovir respectively, while Merav leaves EO displayed good activity against HAV. In contrast, Murcott leaves and Merav leaves EO showed moderate antiviral activity against HSV-1 compared to acyclovir.

These findings led us to recommend Murcott and Merav fruit oils and Merav leaves oil as potentially useful antiviral drugs that can be used against HAV and HSV-1 infection after *in-vivo* and clinical studies.

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Author Contribution: Shaimaa A. El Zanaty performed the hydrodistillation to obtain essential oils, participated in GC/MS analysis, and wrote the paper. Salwa A. Abu El Wafa revised and finalized the paper. Heba A. El Gizawy participated in the interpretation of GC/MS charts and revised the paper. Abeer Temraz revised, finalized the paper and conceived the project.

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