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Effective Antiviral Activity of Tea Tree and Lemon Essential Oils against Influenza A virus (H1N1), Herpes Simplex virus Type 2 and Adenovirus Type 40

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Abstract

Since drug-resistant mutations continue to arise, there are currently few effective therapeutic medicines for the control of the influenza virus, herpes simplex type 2, and adenovirus. In this study, we assessed the potential antiviral effects of the lemon and tea oils. For this purpose, Gas Chromatography-Mass Spectrometry (GC-MS) was used to determine the composition of the essential oils (EOs) for lemon and tea tree. The oils were put through a virucidal test to check for cytotoxicity and in vitro inhibitory action against the H1N1, HSV-2, and Adenovirus (ADV) type 40.The obtained results showed that the dominant compounds in tea tree EO were terpinen-4-ol (62.51%), and in lemon EO, citral (51.9%) and neral (39.43%). Among the analyzed EOs, lemon essential oil is more powerfully antiviral than tea essential oil against the viruses under study compared with the recommended therapies. The lemon essential oil has a high activity (IC_{50} values of 2.501, 9.677, and 10.488) against H1N1, HSV-2, and ADV, respectively. On the other hand, the tea tree essential oil showed a moderate activity against H1N1 and HSV-2 with IC_{50} values (28.76, 39.462, respectively) and a high activity against (ADV) with IC_{50} (39.462). Both essential oils have a selectivity index ($SI=IC_{50}/MIC$) higher than 4, which is considered effective and safe for the host cell. Therefore, lemon and tea essential oils could be a new natural source of antivirals with potential therapeutic benefits for people suffering from influenza, herpes simplex type 2, and adenovirus, and possibly other membrane-containing viruses.

Keywords: Tea tree; Lemon; Antiviral; Cytotoxicity; Terpenes; Eos; H1N1; Influenza virus; Herpes (HSV) and Adenovirus (ADV).

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1. Introduction

Viral infections are the root cause of a wide range of human diseases and health issues, including self-limiting and over 20% of all recorded fatalities worldwide (Sofy et al., 2020; Pronin et al.,2021). Although scientists have always been interested in viral diseases (Jasim et al., 2021), their focus has lately dramatically grown as a result of the present worldwide coronavirus disease epidemic (Al-Tayyar et al., 2020; Boban, 2021; Behzadi et al., 2023). H1N1 is one of these viral diseases. According to (Kshatriya et al., 2018) Influenza A contains multiple types, such as the one that infects pigs, which is known as swine influenza, a transmissible viral infection that can infect the respiratory system of its host. The World Health Organization (WHO) estimates that each year, seasonal influenza epidemics result in between 250,000 and 500,000 fatalities and three to five million instances of serious disease (Organization, 2021). Adenoviruses (ADV) are widespread viruses that can cause a variety of diseases in humans, including bronchitis, pneumonia, gastroenteritis, hepatitis, and myocarditis (Sofy et al.,2018; Shieh, 2022). They are immune to numerous viruses and disinfectants since they are not enclosed viruses, a major pathogen of gastroenteritis in children is enteric (ADV), which plays an important role in causing inflammation of the upper respiratory system. It accounts for 5–10% of respiratory tract infections and causes a wide range of clinical diseases (Ghasemi et al., 2014; Kim et al., 2023; Liu et al., 2023;). Many people around the world have been infected with herpes simplex virus types 1 and 2 (HSV-I and HSV- II), and because herpes is a chronic condition. Globally, it is estimated that 3.7 billion people have HSV-1 infection, which is the main cause of oral herpes (Silva et al., 2022), whereas 491 million people have HSV-2 infection, which is the main cause of genital herpes (Devine et al., 2022).

Many people die due to the presence of microbes capable of antimicrobial resistance (AMR), and this number is expected to rise to 20 million by 2050 (Sofy et al.,2020; Uddin et al., 2021). As a result, several potent drugs have been created during the past ten years to treat viral infections (Tompa et al., 2021). Plant phytochemicals are effective antiviral and antibacterial defense agents (Sofy et al., 2017; Moustafa *et al.*, 2023). Therefore, during the past ten years, One of the most powerful potential sources of antiviral treatment derived from plant extracts is essential oils (Wani, et al., 2021; Moustafa et al., 2022; El-Gebaly *et al.*, 2024). One of these essential oils, Tea tree oil (TTO), is made up of roughly 100 distinct molecules with varying quantities of volatile aromatic terpene hydrocarbons (Khan et al., 2023). Its effectiveness against enveloped viruses has been studied previously. TTO, in particular, can decrease the development of plaques caused by (HSV-I and HSV-II), especially when react with the virus only before it infects cells (Sharma et al., 2023; El-Gebaly *et al.*, 2024). It can also reduce the replication of

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adenovirus (Yin et al., 2022) influenza virus (Garozzo et al., 2009) and other viruses in vitro. Additionally, Lemon essential oil also has several health anlages as an aromatherapy. It has been demonstrated that adding lemon essential oil to fruit and berry packaging can lower viral loads of the non-enveloped viral pathogen hepatitis A virus and the feline calicivirus (Battistini et al., 2019; Saada *et al* ., 2020; Pellegrini et al., 2023; El-Sayed et al., 2024).

The current study sought to describe the essential oil compositions of lemon and tea and their antiviral efficacy against H1N1, HSV-2, and Adenovirus in light of the current widespread interest in natural antivirals.

2. Material and methods

2.1 Materials

Lemongrass and tea tree leaves and twigs were collected from faculty of Agriculture, Ain Shams University. Plant materials were stored in cool and dry place. The collected leaves were washed with distilled water before crushing for extraction.

2.1.1. Cell culture

(MDCK), Vero, and Hep-2 cells that were bought from Nawah-Scientific, Egypt, were submerged in DMEM media supplemented with 0.1% bovine serum and 0.1% antibacterial/antifungal solution. Gibco BRL provided the fetal bovine serum, trypsin-EDTA, antibacterial/antifungal solution, and DMEM medium. The cell cultures were maintained at 37ºC in 5% CO2 humidified air.

2.1.2. Viruses

From Nawah-Scientific, Egypt, stockpiles of influenza virus (H1N1), herpes simplex virus type 2 (HSV-2), and adenovirus (AD) were collected. MDCK cells were used to replicate the viruses, and the supernatant fluids from the infected cells were collected, titrated, and kept at -80 °C until use.

2.2. Methods

2.2.1. Extraction of essential oils

` At the Department of Chemistry, National Research Centre, Dokii, Egypt, essential oil was extracted using the hydrodistillation procedure. The apparatus was made up of a thermometer, condenser, and distillation flask or tank. Twelve litters of water were added to a flask containing eight to ten kilograms of plant material. The process was then initiated at a temperature of 250 °C after the setup was sealed firmly. For 4-5 hours, the process persisted. After extracting the oil in a separating funnel, the EO was put in amber glass containers and refrigerated at 4°C until the antimicrobial screening and phytochemical analysis were carried ou**t.**

2.2.2. Cytotoxicity Assays

One day before to infection, MDCK cells were planted into a culture plate at a density of 2 x 104 cells per well for viral titration. After being introduced to the cells, the cells are washed with a saline solution mixed with phosphate after removing the culture medium, which contains the gradually diluted samples. By tracking the cytopathic (CPE) inhibitory effect and calculating the percentage of cell viability, the crystal violet technique was used to assess the infectivity of viruses (Donalisio et al., 2013). Mammalian cells received 0.1 mL of the essential oils solution that was diluted and contained CCID50 (1.0 × 106) of virus stock. Forty-eight hours after the injury, this dosage was chosen to result in the required CPEs. Utilizing the Graph Pad PRISM program (San Diego, USA), both cytotoxic concentrations (CC_{50}) and inhibitory concentrations (IC_{50}) were calculated.

2.2.3. Antiviral Assay

In order to administer a medication to the cells, 100 µl of medium containing the necessary amount of the molecule was added. All sample's antiviral effectiveness was evaluated at levels that were diluted two times, starting at 1000 µg/ml. The viral control (virus-infected, untreated cells) as well as the cell controls (untreated, non-infected cells). Culture plates were kept in an environment at room temperature and 5% CO_2 for 72 hours. By using an optical microscope, the cytopathic effect's development was tracked. The antiviral activity had been determined according to (Pauwels et al., 1988). The 50% CPE inhibitory dose (IC_{50}) was determined as a result of these studies.

2.2.4. Characterization of essential oils by (GC-MS)

An Agilent Technologies gas chromatograph (model 7890B) and mass spectrometer detector (model 5977A) were installed on the GC-MS system in the main laboratory of Cairo's National Research center. In dichloromethane, the sample was dissolved. The DB-624 pillar, which has a width of 30 m by 320 m and a film width of 1.8 m, was installed in the GC. The analysis utilized the following temperature program: 40°C for 1 minute, followed by a 5-minute hold at 250 °C after increasing 7 °C/min, with hydrogen serving as the carrier gas at a flow rate of 3.0 ml/min at a split of 1:20 and an injection volume of 1 µl. The injector and indicator were kept at 250 °C. Utilizing electron ionization (EI) at 70 eV, mass spectra were created utilizing a spectral range of m/z 30-440 and a solvent delay of 360 seconds. It was possible to identify between several components by contrasting the pattern of spectrum fragmentation observed in the Wiley and NIST collective spectral library data.

2.3. Statistical analysis

(SPSS software version 25, Chicago, Illinois) programs for statistical analysis, Results were analyzed and summarized using the means ± standard deviation. Significant P-values were defined as those less than 0.05.

3. Results and discussion

3.1 Activity of essential oil on the cell

The survival of MDCK and Hep-2 cells were assessed following medium incubation with oil or without oil to ascertain if essential oil directly decreased viral infectivity and progeny formation through cellular cytotoxic effects. The cause of variability was also investigated using a similar technique when all systems, cell lines, and vesicles were taken into account. The morphological profiles of the all infected cells with adenovirus, herpes virus type 2, and H1N1 and treated with lemon and tea oil as shown in (**Figures 1-3**).

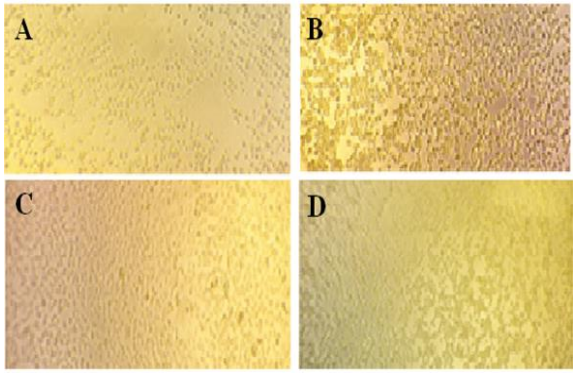

Figure 1: A) Cytotoxicity of 1000 µg/ml Tea, **B)** Inhibitory effect of 1000 µg/ml Tea **C)** Cytotoxicity of 1000 µg/ml Lemon and **D)** Inhibitory effect of 1000 µg/ml Lemon Essential oils on Adenovirus

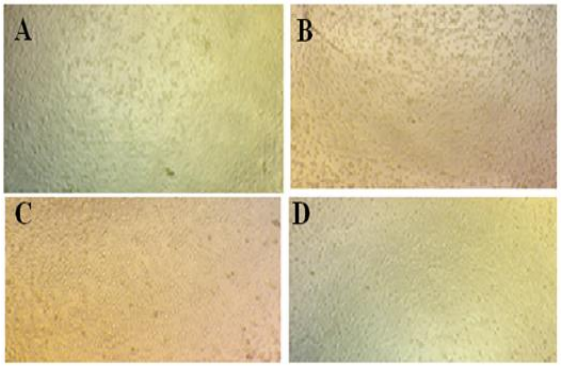

Figure 2: A) Cytotoxicity of 1000 µg/ml tea , **B)** Inhibitory effect of 1000 µg/ml Tea, **C)** Cytotoxicity of 1000 µg/ml Lemon and **D)** Inhibitory effect of 1000 µg/ml Lemon Essential oils on herpes virus type 2**.**

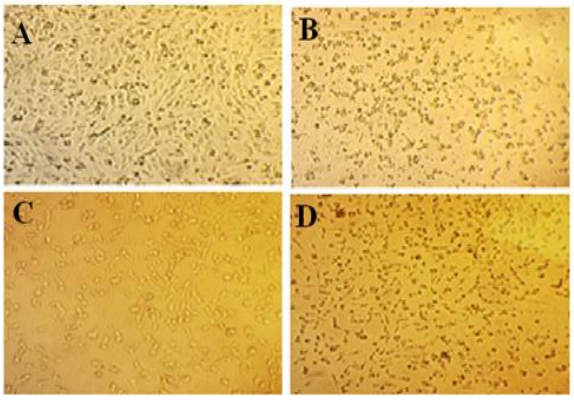

Figure 3: A) Cytotoxicity of 1000 µg/ml tea , **B)** Inhibitory effect of 1000 µg/ml Tea **C)** Cytotoxicity of 1000 µg/ml Lemon and **D)** Inhibitory effect of 1000 µg/ml Lemon essential oils on H1N1

5.2 Antiviral activity of Lemon and Tea Essential Oils

The Lemon and Tea essential oils had similar antiviral effects on the H1N1, herpes simplex, and Adenovirus type 40 viruses. The IC_{50} as shown in (**Figure 4**) and CC_{50} in (**Figure 5)** the values at different concentrations and selectivity index ($SI=IC_{50}/MIC$) of these essential oils treatment was summarized in **(Table 1)**. The IC_{50} positivity correlated with concentration, while CC_{50} negatively correlated. These results showed the powerful antiviral effect of lemon and tea essential oil against the viruses under study comparing with oseltamivir, acyclovir, and Gallic acid the recommended therapies for H1N1, herpes simplex, and adenovirus, respectively. The results for both oils similarly showed a significant difference at a P value less than 0.5 when compared to the values of the positive control.

For instance, the antiviral activity of tee tree oil is mainly attributed to preventing the virus from attaching to the cell and merging with its contents, which leads to disruption in the formation and weakening of large molecules located on the outside of the virus (Romeo et al., 2022; El-Gebaly et al., 2024). As an example, molecular docking and MD simulations proposed TTO's prevention of influenza A (H1N1) virus entry into host cells could be due to the fusion and interference of terpinen-4-ol with the viral hem agglutinin protein, which directly prevents its action (Romeo et al., 2022).

Table1. The cytotoxicity effect of lemon and tea essential oils against studied viruses.

Treatment	Viruses	CC50	IC50	SI
Lemon Oil	H1N1	23.42	2.501	9.36
	HSV-2	67.214	9.677	6.95
	(ADV)	49.707	10.488	4.73
Tea Oil	H1N1	244.1	28.76	8.48
	HSV-2	702.181	39.462	17.8
	(ADV)	358.274	11.07	32.4

This compound is supposed to interfere with the virus's peptide, slowing down protein formation, which in turn impedes rearrangement processes and prevents virus entry (El-Wakil et al., 2022). In this study the EOs of Tea tree demonstrated an activity against Influenza virus at an IC_{50} value of 28.76 µg/mL, CC_{50} 244.1µg/ml and SI of 8.48. According to this study's findings, the TTO has a modest level of activity against the H1N1 virus and causes less cytotoxicity in the host cell. Those that target distinct hosts and those that hinder and prevent the development of crucial viral components, including proteins or genes components for reproduction and dissemination are the two main categories of lead anti-influenza medications' molecular mechanisms of action (Chakraborty & Chauhan, 2023). These pathways can be utilized to more precisely categorize anti-influenza drugs (virus inhibitors). Entrance and attachment (fusion) inhibitors make up the first group and are often used as adjuvants in the development of influenza vaccines (Su et al., 2023). According to an in silico simulation study, tea tree oil (TTO) prevents the influenza virus from entering cells and joining forces with other viruses (Madia et al., 2022).

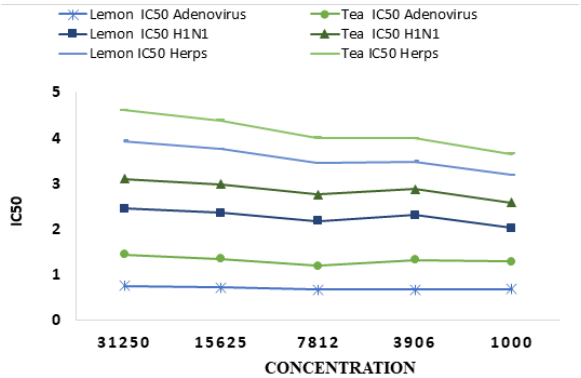

Figure 4: The IC_{50} values of lemon and tea oil at different concentrations against viruses (Adenovirus, Herpes and H1N1).

In this study the TTO demonstrated an activity against HSV-2 at an IC_{50} value of 39.46 µg/mL, CC_{50} 702.18µg/ml and SI of 17.8. With an excellent and safer SI, this finding showed that the TTO has a moderately active impact against HSV-2. This antiviral activity might be the result of TTO's inhibiting effect on the development of two types of herpes (HSV-I and HSV-II) plaques, especially when administered to a virus that is free before it infects cells, but it did not exhibit any antiviral activity against the replication cycle of HSV-1 and HSV-2 (Jamshidinia et al., 2023). Due to the Terpinen-4-ol and -terpineol components of TTO (Romeo et al., (2022) found a significant reduction in HSV-2-related symptoms following application of 6% gel containing tea tree oil.

The TTO demonstrated an activity against (ADV) at an IC_{50} value of 11.07µg/mL, CC_{50} 358.274 µg/ml and SI of 32.4. This outcome demonstrated that the TTO has an excellent and safer SI and a highly active impact against (ADV) (Sofy et al., 2021; El-Sayed& Youssef, 2019). This is the first report on the virucidal action of TTO against (ADV), to the best of our knowledge. TTO's true anti-adenovirus mechanism is unclear as it has different mechanisms of action. For example, some medications aim to render a virus fully inactive, while others inactivate virus particles or disrupt the steps necessary for the replication of specific viruses, like the herpes simplex virus (El-Wakil et al., 2022). These stages involve the virus attaching itself, entering the cells, proliferating inside of them, and then exiting the infected cells (Kausar et al., 2021). Essential oil vapor was examined by (Boone et al., 2023) for its potential to kill virus virions. After being exposed to the oil vapor for 60–120 minutes, adenovirus was fully rendered inactive.

In the lemon essential oil Monoterpenoids, sesquiterpenoids, and phenylpropanoids are some of the special main ingredients of essential oils that are responsible for their pharmacological qualities. Furthermore, a growing body of research from controlled trials and in vitro investigations indicates that essential oils may be used as antiviral medicines to treat human viral illnesses, such as SARS coronaviruses (Nadjib, 2020; Sofy et al., 2020). The majority of these antiviral essential oils' tests have been performed Against viruses with free nucleic acid, such as adenovirus type 3 (Youssef et al., 2021; José-Rita et al., 2022), Together with RNA or DNA viruses like influenza virus, dengue virus, and herpes virus (Elsebai & Albalawi, 2022). The majority of these antiviral drugs are that clinically effective work by targeting various stages of the viral biosynthesis and specifically preventing viral reproduction. Conversely, virucidal drugs reduce or eliminate the ability of virus particles to infect others by denaturing the structure or glycoproteins of the virus (Freitas et al., 2022).

The lemon essential oil demonstrated an activity against H1N1 at 50% inhibitory concentration value inhibitory concenteration of 2.501µg/mL, CC_{50} 23.42µg/ml and SI of 9.36. This finding demonstrated the extremely active activity of lemon oil against H1N1 with an excellent and safer SI. By directly interacting with the viral particles, the lemon balm essential oil prevents the influenza virus H1N1 from replicating at different phases of the reproduction series (Swamy et al., 2016; Meeran et al., 2021).

The lemon essential oil demonstrated an activity against HSV-2 at an IC_{50} value of 9.677 µg/mL, CC_{50} 67.214 µg/ml and SI of 6.95. With an excellent and safety SI, this study showed that lemon oil has a highly active impact against HSV-2. Citral and citronellal in lemon essential oil may also prevent HSV-2 from replicating (Rani et al., 2023). The results of the viral binding experiment point to the possibility that the antiviral activity of lemon essential oil occurs after the virus has already entered the host cell, rather than before to it as suggested by the mechanism of the antiviral action (Ponticelli et al., 2023). On the other hand, lemon extract can prevent the virus from attaching and penetrating the host cell. The main mechanism for inhibiting the attachment and penetration of these virus mucous cells is the direct interaction between the viral glycoproteins of the herpes simplex virus (such as glycoproteins GB and GD) and lemon balm extract. Among the other chemicals that showed some antiherpes simplex virus properties was the lone phenolic compound found in rosmarinic acid in lemon balm extract (Gabrani, Ishaque, & Jain, 2023; Youssef et al., 2023).

The lemon essential oil demonstrated an activity against (ADV) with 50% inhibitory concentration value 10.48 µg/mL, CC_{50} 49.707 µg/ml and SI of 4.73. This finding was shown that lemon oil has a potent action against (ADV) with a good and secure SI. To our knowledge, this is the first account of lemon oil's virucidal action against (ADV) (Sofy et al., 2018). It is uncertain how lemon oil works to prevent adenoviruses. Numerous investigations have shown that citrus oils, like lemon, and some of their constituents effectively inactivate viruses with lipid envelopes, such as the herpes and influenza viruses, by interfering with the virion cover structures or by concealing viral structures required for entrance into host cells (Astani et al., 2010; Arena et al., 2021).

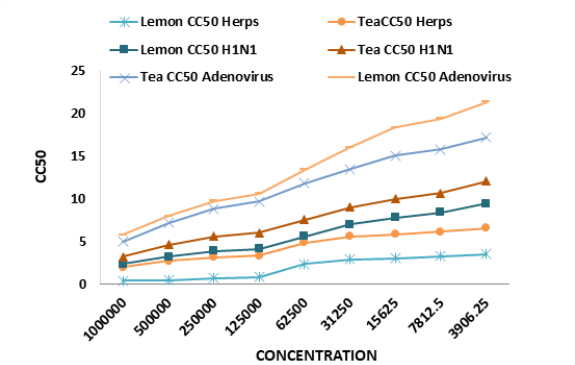

Figure 5: The CC_{50} values of lemon and tea oil at different concentrations against viruses (Adenovirus, Herpes and H1N1).

5.3 GC-MS of the essential oils

The Essential oils of Lemon and Tea were investigated by GC/MS (**Figure 6**). The fragmentation pattern was compared to the mass spectral library data to identify the 32 chemical substances found in tee oil. These identified compounds were mainly Terpinen-4-ol (62.51%) and gamma-Terpinene (16.24%) beside other components Cyclohexene, 4-methyl-3-(1-methylethylidene) (4.53%), p-Cymene (2.99%), alpha-Terpineol (1.89%), Cyclohexene, 1-methyl-4-(1-methylethylidene) (1.59%), Eucalyptol (1.38%), Aromandendrene (1.35%) and alpha-Pinene (1.23%). In addition to, minor compounds of concentration less than 1% are listed in (**Table 2**).

GC-MS analyses of lemon essential oil identified 20 constituents, representing that the major constituents were 2,6-Octadienal, 3,7-dimethyl- (E) (51.9%), Neral (39.43%), and other compounds beta-Myrcene (3.94) and cis-Verbenol (1.04%). In addition to the minor (< 1 %) compounds were listed in (**Table 3**).

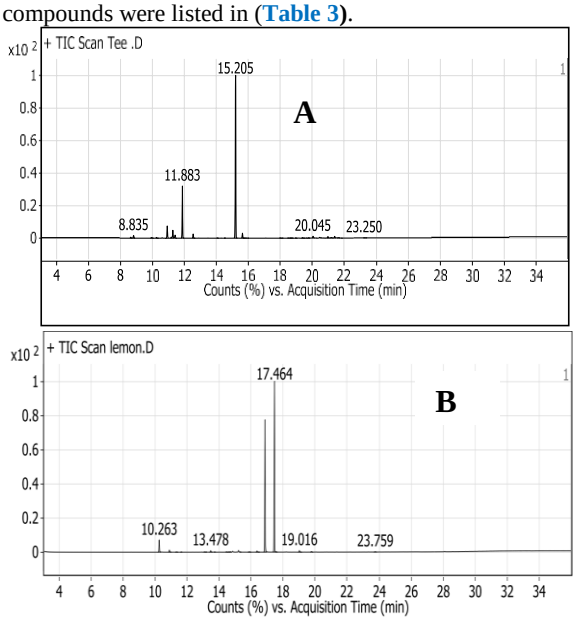

Figure 6.GC-MS spectrum of **A**: Tea tree; **B**: Lemon essential oils.

In **Table (2)** terpinen-4-ol (62.1%) and numerous monoterpenes and sesquiterpenes, including gamma-terpinene (16.24%), P-Cymene (2.99%), -Terpineol (1.89%), Eucalyptol (1.38%), and -Pinene (1.23%), are responsible for a portion of TTO's antiviral efficacy. This result is consistent with those of (Panagiotopoulos et al., 2021) who reported that P-cymene has the potential to be a successful agent against influenza viral infection,as well as(Bravo, 2020; Bravo, Vila, & Bonté, 2021)reported that eucalyptol and α-pinene are the most significant antiviral active principles of the mixture of terpenes that constitutes the essential oil.

In **Table (3)** as presented the main component of lemon oil according to this study was Citral (3, 7-dimethyl-2, 6-octadienal) (51.9%), and Neral (39.43%). Citral is a compound made up of the isomers neral and geranial, both of which have been deemed GRAS (Generally Recognized as Safe) to date. This substance has been shown to have a range of biological actions (spasmolytic, analgesic, anti-inflammatory, antioxidant, diuretic) (Oladeji et al., 2019; Wojtunik-Kulesza, 2022). Additionally, beta-Myrcene was (3.94%) and cis-Verbenol (1.04%) in the lemon. essential oil. (Masyita et al., 2022) reported that the *p*-cymene and citral isomer, in addition to suppressing the growth of pathogens.

Table 2: Identification of the Compounds in tea tree essential oil by GC-MS.

Peak	RT	Name	Formula	Area	Area Sum %
1	8.659	alpha.-Phellandrene	C10H16	1552217.3	0.6
2	8.835	alpha.-Pinene	C10H16	3202668.6	**1.23**
3	9.968	beta.-Pinene	C10H16	1015860	0.39
4	10.264	beta.-Myrcene	C10H16	811070.3	0.31
5	10.94	Cyclohexene, 4-methyl-3-(1-methylethylidene)-	$C_{10}H_{16}$	11783190	**4.53**
6	11.183	Limonene	$C_{10}H_{16}$	1080481.1	0.42
7	11.287	p-Cymene	$C_{10}H_{14}$	7776168.5	**2.99**
8	11.427	Eucalyptol	$C_{10}H1_{8O}$	3591681.7	**1.38**
9	11.883	gamma.-Terpinene	$C_{10}H_{16}$	42210743	**16.24**
10	12.554	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	$C_{10}H_{16}$	4121875.5	**1.59**
11	14.059	1,3,7-Octatriene, 3,7-dimethyl-	$C_{10}H_{16}$	296276.07	0.11
12	14.54	3-Decen-1-yne, (E)-	$C_{10}H_{16}$	210041.13	0.08
13	15.205	Terpinen-4-ol	$C_{10}H_{18}O$	162513309	**62.51**
14	15.635	alpha.-Terpineol	$C_{10}H_{18}O$	4915974.1	**1.89**
15	15.73	Benzene, 1-cyclopropyl-2-nitro-	$C_9H_9NO_2$	119092.89	0.05
16	15.849	3-Nonynoic acid	$C_9H_{14}O_2$	153621.87	0.06
17	15.992	4-Vinylcyclohexene diepoxide (isomer 2)	$C_8H_{12}O_2$	119280.32	0.05
18	17.988	trans-2-Caren-4-ol	$C_{10}H_{16}O$	318503.33	0.12
19	18.14	"1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS*)]-"	$C_{15}H_{24}$	795882.4	0.31
20	18.497	"trans-2-Caren-4-ol"	$C_{10}H_{16}O$	261799.19	0.1
21	18.626	"1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)]-"	$C_{15}H_{24}$	155577.42	0.06
22	18.711	"Bicyclo[4.1.0]-3-heptene, 2-isopropenyl-5-isopropyl-7,7-dimethyl-"	$C_{15}H_{24}$	326553.05	0.13
23	18.964	"1-Decen-4-yne, 2-nitro-"	$C_{10}H_{15}NO_2$	271454.27	0.1

24	19.388	"1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]-"	$C_{15}H_{24}$	1358050	0.53
25	19.769	"BICYCLO[7.2.0]UNDEC-4-ENE, 4,11,11-TRIMETHYL-8-METHYLENE-, [1R-(1R*,4E,9S*)]-"	$C_{15}H_{24}$	661731.21	0.25
26	20.045	Aromandendrene	$C_{15}H_{24}$	3523349.4	**1.35**
27	20.978	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-	$C_{15}H_{24}$	2139590.6	0.82
28	21.188	.gamma.-Elemene	$C_{15}H_{24}$	769406.04	0.3
29	21.402	δ-Cadinene (CAS)	$C_{15}H_{24}$	2039483.9	0.78
30	21.621	cis-Calamenene	$C_{15}H_{22}$	651259.35	0.25
31	23.25	trans-Caryophyllene	$C_{15}H_{24}$	554370.87	0.21
32	23.34	Tetracyclo[6.1.0.0(2,4).0(5,7)]nonane, 3,3,6,6,9,9-hexamethyl-, cis,cis,trans--	$C_{15}H_{24}$	678362.36	0.26

Table3: Identification of the Compounds in lemon by GC-MS.

Peak	RT	Name	Formula	Area	Area Sum %
1	10.263	.beta.-Myrcene	$C_{10}H_{16}$	9805665.8	**3.94**
2	10.902	6-METHYL-5-HEPTEN-2-ONE	$C_8H_{14}O$	1804750.2	0.73
3	11.335	"(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene"	$C_{10}H_{16}$	158676.14	0.06
4	11.64	5-methyl-3-(1-methylvinyl)-1,4-hexadiene	$C_{10}H_{16}$	126159.76	0.05
5	13.087	Furan, 3-(4-methyl-3-pentenyl)-	$C_{10}H_{14}O$	219929.11	0.09
6	13.183	Butyraldehyde, 4-(methylenecyclopropyl)-	$C_8H_{12}O$	130092.94	0.05
7	13.478	Linalool	$C_{10}H_{18}O$	1303004.9	0.52
8	13.725	1,6-Octadiene, 3,7-dimethyl-	$C_{10}H_{18}$	130553.6	0.05
9	14.473	cis-Verbenol	$C_{10}H_{16}O$	2608957	**1.04**
10	14.635	Citronellal	$C_{10}H_{18}O$	357139.21	0.14
11	14.721	trans-Chrysanthemal	$C_{10}H_{16}O$	280985.85	0.11
12	15.302	(E)-3(10)-Caren-4-ol	$C_{10}H_{16}O$	207801.52	0.08
13	15.916	"1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6- one"	$C_{13}H_{20}O_2$	604085.06	0.24
14	16.368	Citronellol	$C_{10}H_{20}O$	1208925.4	0.49
15	16.868	Neral	$C_{10}H_{16}O$	98094887	**39.43**
16	17.464	2,6-Octadienal, 3,7-dimethyl-, (E)-	$C_{10}H_{16}O$	129126391	**51.9**
17	17.54	2-β-PINENE	$C_{10}H_{16}$	351576.75	0.14
18	19.016	Geranyl acetate	$C_{12}H_{20}O_2$	1439300.7	0.58
19	19.764	trans-Caryophyllene	$C_{15}H_{24}$	521399.95	0.21
20	23.759	1,3a-Ethano-3aH-indene, 1,2,3,6,7,7a-hexahydro-2,2,4,7a-tetramethyl-, [1R-(1.alpha.,3a.alpha.,7a.alpha.)]-	$C_{15}H_{24}$	329201.29	0.13

A major global concern for healthcare providers today is viral infection because of the uncontrolled incidence of morbidity and mortality (Sofy et al., 2020). Human health has been impacted for many years by a number of viruses that can cause death, such as the herpes simplex virus (HSV), influenza virus, adenovirus (ADV), and others (Gisondi et al., 2020; Youssef et al., 2023). Unfortunately, the issue has gotten worse due to a shortage of both safe and effective antiviral medications for these infections (Sofy et al., 2018). Numerous plant extracts have had their antiviral potential investigated in an effort to create new treatments. Numerous essential oils reduce viral infectivity, according to laboratory research conducted during the past ten years (Wani et al., 2021;Cozzi et al., 2023; Youssef et al., 2023). According to the American National Cancer Institute's (NCI) methodology, plant-based crude extracts should be regarded as relevant if their IC_{50} values ≤ 30 µg/ml and their CC_{50} values between 100 µg/ml to1000 µg/ml. Another measure to assess their safety is the therapeutic index, often known as the selectivity index (SI). In vitro, any SI value below 4 is regarded as cytotoxic to the host cells (Reichling, 2022). This assessment is also consistent with the given data that categorized the IC50 values as follows: IC_{50} ≤ 20 µg/ml = highly active, IC_{50} 21-200 µg/ml =moderately active, IC_{50} 201-500 µg/ml = weakly active and IC_{50}> 501 µg/ml is inactive (Srisawat et al., 2013;Ibrahim et al., 2021).

Conclusions

Essential oils of lemon and tea tree contain active components like citral and Terpinen-4-ol that have antiviral potential. The lemon essential oil is more potent antiviral than tea essential oil, as it showed high activity vs moderate activity of tea tree essential oil. The findings of this study contribute to our understanding of the antiviral activity of these EOs and may be used as a starting point for the development of drugs that are effective against H1N1, HSV-2, and adenovirus.

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