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ORIGINAL ARTICLE

Analysis of the Chemical Components Found in The Volatile Oils of *Thymus vulgaris* and *Lavandula angustifolia* and Their Antiviral Properties: A Case Study of Russia

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ABSTRACT: Herbal medicines are natural remedies derived from plants or plant extracts. They have been used for thousands of years in traditional medicine and are still widely used today as alternative treatments for various health conditions. In this study, the antiviral effects of the volatile oils of *Thymus vulgaris* and *Lavandula angustifolia* were examined separately after identifying the constituents of the volatile oils of these two plants. Two plants, *Thymus vulgaris* and *Lavandula angustifolia*, grown in the open air, were collected from the surrounding plains of Mirny and Ust-Ilimsk cities in Russia, respectively. Using a Clevenger apparatus, the oil was independently extracted from each plant by distillation with water. By using gas chromatography tandem mass spectrometry (GC-MS), the obtained volatile oil was examined. The antiviral effects of these two plants' essential oils were then studied independently on herpes simplex virus (HSV-1). These viruses can quickly multiply on HeLa or HEp-2 cancer cells and produce effects that specifically cause cytopathic effect. In the essential oil of the *Lavandula angustifolia*, 24 compounds including thymol, carvacrol, *p*-Cymene and caryophyllene were identified. In the essential oil of *Thymus vulgaris*, 27 substances including terpenin-4-L, *p*-cymene, γ-terpinene and sabinene were identified. Also, the antiviral effects of the volatile oil of these two plants were investigated separately with a concentration of 10^{-4} on HeLa cells infected with HSV-1, and the result was negative.

INTRODUCTION

Thymus vulgaris, also known as common thyme or garden thyme, is a small perennial herb native to the Mediterranean region but now found worldwide. It is commonly used as a culinary herb and for medicinal

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purposes [1,2]. Thyme contains a variety of chemical compounds that give it its characteristic flavor and aroma [3]. The main chemical constituents of thyme include thymol, carvacrol, p-cymene, linalool, and borneol. These compounds are responsible for thyme's antiseptic, antifungal, and antioxidant properties [4]. Thymol is one of the most abundant and biologically active compounds in thyme. It has been shown to have antibacterial, antifungal, and antiviral properties, and is often used as a natural preservative in food and cosmetic products [5,6]. Thymol also has antioxidant properties, which help protect cells from oxidative damage. Carvacrol is another important compound in thyme. It has been shown to have antimicrobial properties, and is used in the food industry as a natural preservative [7]. Carvacrol also has anti-inflammatory properties, which may make it useful in the treatment of conditions such as arthritis [8,9]. In addition to thymol and carvacrol, thyme also contains other compounds that have been shown to have medicinal properties. For example, linalool has been shown to have anxiolytic (anti-anxiety) properties, while p-cymene has been shown to have analgesic (pain-relieving) properties [10,11].

Lavandula angustifolia, commonly known as English lavender, is a small flowering plant native to the Mediterranean region but now cultivated worldwide for its fragrant flowers and essential oil. Lavender has been used for centuries for its medicinal and aromatic properties, and is commonly used in aromatherapy, perfumes, and cosmetics. The essential oil of *Lavandula angustifolia* is composed of numerous chemical compounds, including linalool, linalyl acetate, ocimene, terpinen-4-ol, and camphor [12]. These compounds are responsible for the distinct aroma and therapeutic properties of lavender. Linalool is the most abundant compound in lavender essential oil, and is responsible for its relaxing and calming effects. It has been shown to have anxiolytic (anti-anxiety) and sedative properties, and is often used to promote relaxation and improve sleep quality [13–15]. Linalyl acetate is another important compound in lavender essential oil, and has been shown to have anti-inflammatory and analgesic properties [16]. It is often used topically to help reduce pain and inflammation, and is also thought to have antispasmodic properties, which may make it useful for conditions such as asthma and irritable bowel syndrome [17]. Ocimene is a terpene found in lavender essential oil that has been shown to have antifungal and antimicrobial properties. It may also have potential as an insecticide [18,19]. Terpinen-4-ol is a terpene found in lavender essential oil that has been shown to have antimicrobial properties, and may be effective against a range of bacteria, viruses, and fungi [20,21]. It is also thought to have antioxidant properties, which may help protect cells from oxidative damage [22]. Camphor is a terpene found in small amounts in lavender essential oil. It has been shown to have anti-inflammatory and analgesic properties, and is often used topically to help reduce pain and inflammation [23].

The aim of this study is to analyze the composition of the essential oil obtained from *Thymus vulgaris* and *Lavandula angustifolia*, which were grown in the open air and collected from the surrounding plains of Mirny and Ust-Ilimsk cities in Russia, respectively. Consequently, as a logical continuation of this research, we have investigated the potential antiviral effects of these two volatile oils on the herpes simplex virus (HSV-1), which was cultured in HeLa cells.

MATERIAL AND METHODS

In this study, *Thymus vulgaris* and *Lavandula angustifolia* collected from Mirny and Ust-Ilimsk cities in Russia, respectively were employed. In order to separate the volatile oil from the mentioned plants, 120 grams of each species was extracted by distillation with water and using a Clevenger [24]. After dehydrating with sodium sulfate without using any water, the weight of the volatile oil recovered was measured, and the yield was estimated. The obtained volatile oil was maintained in the refrigerator until usage after being sealed in dark glass containers with nitrogen gas.

A gas chromatograph (GC) device (Agilent Technologies model 6890 plus) was used to examine volatile oil that was collected from both plants. In order to identify the compounds, present in the volatile oil of *Lavandula angustifolia* and *Thymus vulgaris* plants, an Agilent Technologies model 6890 plus GC connected to a model 1200 triple quadrupole mass spectrometer (MS) detector was used. The ionization energy was reported as 75 eV (Figure 1).

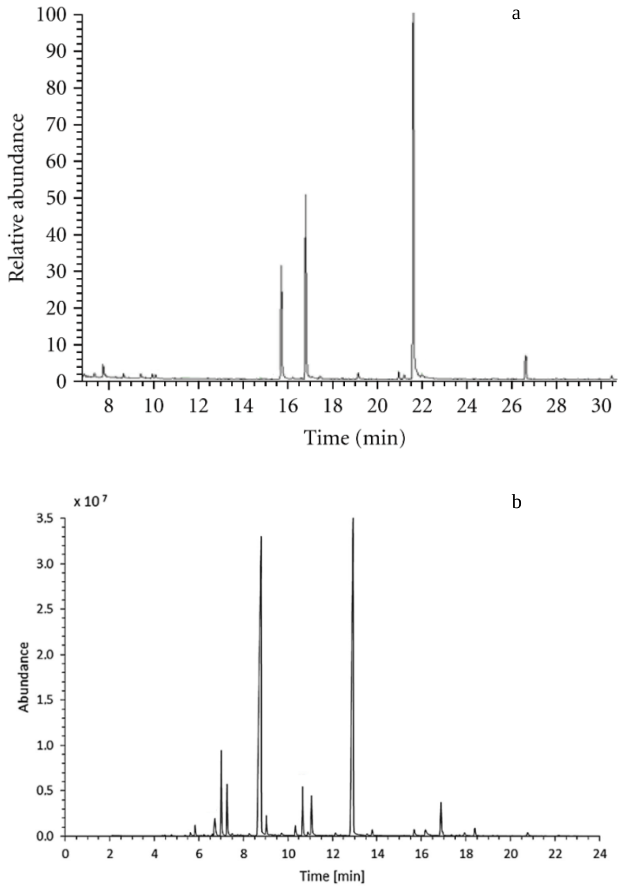

Figure 1. Exemplary chromatogram of (a) *Thymus vulgaris* and (b) *Lavandula angustifolia* essential oil.

Using information from studies on the herpes simplex family, the HSV-1 was chosen to study the antiviral properties of the essential oils of the plants described. These viruses can quickly multiply on cancerous HeLa or HEp-2 cells and cause their own cytopathic effect.

To explore the potential cytotoxic effects of *Lavandula angustifolia* and *Thymus vulgaris* essential oils, dilutions of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} were prepared in Minimum Essential Medium (MEM) and applied to HeLa cells in four cell culture plates. The cells were incubated at 38°C for a week and monitored daily to assess any lethal impact. This study aimed to investigate the antiproliferative activity of these oils on HeLa cells. The findings of this investigation demonstrated that a dilution of 10^{-4} for each essential oil was devoid of any cytotoxic impact on HeLa cells, thereby suggesting its suitability for assessing the antiviral activity of essential oils in a cell culture medium. Following the aforementioned assay, the 0.0001 dilution of volatile oils extracted from *Lavandula angustifolia* and *Thymus vulgaris* plants was identified and subsequently evaluated for their antiviral potential in cell culture medium using three distinct approaches mentioned in Ref. [23]:

1. The virus was prepared to a precision of 100 $TCID_{50}$ (i.e., a dose of virus capable of infecting 50% of the inoculated cultures) and subsequently inoculated into two distinct cell culture tubes. Subsequently, a 10^{-4} dilution of each volatile oil was separately added to the tubes containing both the virus and the cell culture, without any delay.

2. The virus was diluted to 100 $TCID_{50}$ and then inoculated into two cell culture tubes. Following an incubation period of one hour, a 10^{-4} dilution of each volatile oil was separately added to the tubes containing the virus and cell culture.

3. Using a dilution of 100 $TCID_{50}$ for the virus, the aforementioned volatile oils were added at a dilution of 10^{-4}, and after an incubation period of one hour, the resulting mixture was inoculated into two distinct cell culture tubes.

Subsequently, all the tubes were placed in an incubator set at 35°C, and were observed daily under an inverted microscope until three days after the viral controls tested positive. The samples were then stored in a freezer at a temperature of -18°C. The frozen samples were then thawed for the second viral passage, and the contents were then reinoculated into fresh cell culture tubes. In each of the aforementioned scenarios, two control tubes for the virus and two control tubes for the desired plant's volatile oil are added, each with a dilution of 10^{-4}, as per the experimental protocol. Furthermore, the experiments were replicated thrice to validate the intended outcomes' accuracy. This was done to ensure the reliability and reproducibility of the results. Generally, approaches 1 and 2 are used to study any potential antiviral characteristics of substances intended for use as pharmaceuticals, while method 3 is used to examine any prospective antiviral properties of substances that are categorized as disinfectants [25]. Using the Excel spreadsheet program, the average percentage of identified volatile oil components was computed.

RESULTS

Analysis of the volatile oil extracted from *Lavandula angustifolia* yielded the identification of 24 constituents, which constitute 98.52% of the total volatile oil content of the plant. The results, obtained through both qualitative and quantitative analyses, provide valuable insight into the composition of *Lavandula angustifolia*'s volatile oil (Table 1).

Typically, monoterpenoids make up 91.72% of the constituents present in volatile oils, with 8.94% being monoterpene hydrocarbons and 82.78% being oxygenated monoterpenoids. The essential oil in question exhibits a significant presence of terpenoid sesquiterpenes, comprising 6.84% of the total constituents. Of this, 5.02% are hydrocarbon terpenoid sesquiterpenes, and 1.82% are oxygenated terpenoid sesquiterpenes. Moreover, the oil is typically abundant in oxygenated compounds.

Table 1. The volatile oil composition the plants *Lavandula angustifolia* and *Thymus vulgaris*.

#	Compound name	Retention Index	*Lavandula angustifolia*, %	*Thymus vulgaris*, %
1	Eucalyptol	1022	0.52	--
2	Ascaridole	1230	--	0.47
3	Borneol	1160	0.31	--
4	Carvacrol	1291	34.41	0.47
5	Carvacrol acetate	1367	1.61	--
6	Carvacrol methyl ether	1238	1.77	--
7	Caryophyllene oxide	1575	1.39	1.88
8	Cis-Pinene hydrate	1135	--	1.39
9	Cis piperitol	1186	--	0.77
10	Cis-Sabinene hydrate	1062	--	0.39
11	Cis-β-Terpineol	1137	--	1.46
12	Di hydro linalyl acetate	1268	--	0.37
13	E-Anethole	1276	--	0.39
14	Geranyl acetate	1376	--	0.59
15	Isobornyl acetate	1279	0.39	--
16	Linalool	1090	1.79	3.86
17	Linalyl acetate	1248	--	1.85
18	Meta-Cymene	1076	0.11	--
19	Myrcene	980	0.48	1.62
20	Neryl acetate	1354	--	0.97
21	Para-Cymene	1016	7.67	14.44
22	Sabinene	963	--	6.07
23	Spathulenol	1570	0.46	1.64

24	Terpinen-4-ol	1169	1.02	34.73
25	Terpinolene	1078	--	2.49
26	Thymol	1281	36.66	--
27	Thymol acetate	1348	1.74	--
28	Thymol methyl ether	1228	1.07	--
29	Trans-Dihydro carvone	1195	--	0.36
30	Trans-Piperitol	1199	--	0.70
31	Valencene	1489	0.64	--
32	α-Humulene	1447	0.18	--
33	α-Pinene	930	0.26	0.67
34	α-Terpinene	1009	--	2.35
35	α-Terpineol	1180	1.18	5.74
36	α-Thujene	920	0.24	0.33
37	α-Caryophyllene	1410	2.90	2.62
38	β-Humulene	1429	1.31	--
39	β-Pinene	984	0.08	0.04
40	γ-Terpinene	1049	0.35	7.82
	Total		**98.52**	**96.49**

Through this study, 27 constituents, comprising 96.49% of the volatile oil compounds of the *Thymus vulgaris*, were successfully identified (Table 1). Monoterpenoids make up 88.80% of the constituents present in volatile oils, with 32.63% being monoterpene hydrocarbons and 56.17% being oxygenated monoterpenoids. This essential oil has 6.07% terpenoid sesquiterpenoids, of which 2.01% are hydrocarbon sesquiterpenoids and 4.06% oxygen and is rich in oxygen compounds. According to the findings, oxygenated terpenoids make up 84.06% of the volatile oil constituents in *Lavandula angustifolia* and 60.23% of the volatile oil in *Thymus vulgaris*. Table 2 presents the findings of the investigation into the effects of the aforementioned essential oils on the HSV-1 virus-contaminated environment.

Table 2. The antiviral effect of the volatile oil of the plants against HSV-1.

Method	*Lavandula angustifolia*	*Thymus vulgaris*
1	+	+
2	+	+
3	+	+
Virus control	+	+
Cell control	-	-

Note: (+) Virus growth, (-) No virus growth

DISCUSSION

The experiments carried out in this study and other researches demonstrate the impact of the growing environment, including perhaps the growth season, on the primary volatile oil constituents [26]. An study revealed that the volatile oil of *Lavandula angustifolia* primarily consisted of carvacrol (59.17%) as the main compound and thymol (23.74%) as the second compound, in the species found in Northern Iraq [27]. In contrast, the current study reports the percentage of carvacrol and thymol present in the volatile oil are 34.41 and 36.66, respectively. Linalool (57.39%) and linalyl acetate (13.80%) were found to be the primary volatile oil constituents of *Lavandula angustifolia* in a different investigation [12]. The location and timing of the plant's collection are likely what produced such a big difference. Carvacrol is more poisonous than thymol [28,29], therefore it would seem that the volatile oil from the *Lavandula angustifolia* would be better suited for usage in food and medicine. According to study conducted in 2016-2017, the two main volatile oil constituents of *Thymus vulgaris* growing in Dołuje (northwestern Poland) are terpinen-4-ol (10–17%) and cis-sabinene hydrate (34–51%) [4]. In the present research, the main compounds of the essential oil of the plant were terpinen-4-ol (34.73%), para-cymene (14.44%), γ-terpinene (7.82%) and cis-sabinene hydrate (0.39%) as one of sub-compositions of volatile oil have been reported. Another study which investigated the volatile oil of *Thymus* species indicates the presence of terpinen-4-ol, cis-sabinene hydrate, and *p*-cymene as the main compounds of the volatile oil [30]. The variations in the composition of the volatile oil could be attributed to the differences in the growing climate and the time of collection of the plants.

In this study, the antiviral properties of the essential oils extracted from *Lavandula angustifolia* and *Thymus vulgaris* plants were investigated against HSV-1 virus using HeLa cell culture medium. The oils were tested at a concentration of 10^{-4} to determine their effectiveness. These cells have the capability to replicate and propagate, and their ease of handling allows for the study of viruses that specifically thrive in this environment. It is important to note that these cells are highly sensitive to fluctuations in pH and chemicals, and even the slightest changes can lead to their destruction. Therefore, it is crucial to determine the appropriate concentration of the substance being investigated that is not toxic to the cells before continuing with the research. It is worth mentioning that the volatile oil of the plants under investigation was tested separately on HeLa cells at a concentration of 10^{-4}, and at this concentration, it did not show any significant effect on the HSV-1 virus. Therefore, it cannot be completely ruled out that the essential oils of these two plants possess antiviral properties. By maintaining adequate cell culture conditions on the virus, it is first important to separate all of this essential oil's constituents in order to study the mechanism underlying its antiviral effects. Also, the processing of this volatile oil allowed for the creation of a cell culture that can contain higher amounts of it without harming the cells [31,32].

CONCLUSIONS

In this research, the volatile oils of *Thymus vulgaris* and *Lavandula angustifolia* were analyzed to identify their constituent compounds. Our analysis revealed that 96.49% and 98.52% of the compounds in *Thymus vulgaris* and *Lavandula angustifolia*'s volatile oils, respectively, were identified. The major components of the essential oils were found to be oxygenated terpenoids, accounting for 60.23% of the essential oil of *Thymus vulgaris* and 84.06% of the essential oil of *Lavandula angustifolia*. Our investigation also revealed that the volatile oils of these two plants exhibit toxicity to HeLa cells at concentrations higher than 10^{-4}. Despite this toxicity, the antiviral properties of the volatile oils cannot be entirely dismissed. However, our findings underscore the need for more precise techniques to assess the potential therapeutic effects of these plants.

Conflict of interests

The authors have disclosed that there are no conflicts of interest that could potentially influence the publication of this paper.

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