CHEMICAL COMPOSITION OF ESSENTIAL OILS FROM *Origanum onites* L. AND *Cymbopogon citratus*, AND THEIR SYNERGISTIC EFFECTS WITH ACYCLOVIR AGAINST HSV-1

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Origanum onites L. and *Cymbopogon citratus* have a number of pharmacological activities such as antimicrobial, anti-inflammatory and antioxidant. In this study, we aimed to investigate the components and the synergistic effect of *Origanum onites* L. and *Cymbopogon citratus* and their combinations with acyclovir against HSV-1 on HEp-2 cell line. The main essential oil components of Origanum onites L. were Thymol (68,28%), γ-Terpinene (5,50%), p-Cymene (5,47%) and Linalool (4,40%) and for Cymbopogon citratus the main components were E-Citral (47,53%), Z-Citral (36,29%), β-Myrcene (9,31%). Initially, 100 $TCID_{50}$ (Tissue Culture Infective Dose) of HSV-1 on HEp-2 was calculated in the study. Antiviral activity studies were carried out at the concentration of 1, 10 and 100 $TCID_{50}$ of HSV-1. The antimicrobial efficacy of the *Origanum onites* L. and *Cymbopogon citratus* essential oils were assessed alone and in combination with acyclovir. In addition, the efficacy of acyclovir was determined on 100 $TCID_{50}$ of HSV-1. In conclusion, the essential oils of *Origanum onites* L. and *Cymbopogon citratus* against HVS-1 were found to have a significant inhibitory effect on viral replication. We think that the essential oils of *Origanum onites* L. and *Cymbopogon citratus* may be a very important agent against herpesviruses. In particular, the essential oils of *Cymbopogon citratus* can be a very promising plant.

Keywords: Acyclovir, HSV-1, Linalool, rosmarinic acid, HEp-2 cell line, antiviral

INTRODUCTION

Origanum species are plants that are commonly used as spices and marketed under the name of thyme. The genus Origanum is take place in the family Labiatae. Origanum genus has a large variation between species. This species is composed of many species and subspecies (Ozkan *et al*., 2010).

Cymbopogon citratus is a widely used in folk medicine. The essential oils of *Cymbopogon citratus* have various pharmacological activities. Terpenes, alcohols, ketones, aldehyde and esters are the important compounds. It has also been reported that Citral α, Citral β, Nerol Geraniol, Citronellal, are among the important phytoconstituents (Akhila, 2010; Oloyede, 2009).

Herpes viruses are broad-spectrum infectious agents that can range from superficial infections to deep tissue infections. It has been reported to cause serious morbidity and mortality, especially in immunosuppressed individuals. Medication is insufficient due to increased drug resistance in cases such as meningitis. For this reason, new active substance investigations continue against herpes viruses (Roizman, 1996).

In this study, we aimed to investigate the synergistic effect of *Origanum onites* L. and *Cymbopogon citratus* and their combinations with acyclovir against HSV-1 on HEp-2 cell line.

https://doi.org/10.24264/icams-2018.IV.1

MATERIALS AND METHODS

Plant Materials

Origanum onites L. and *Cymbopogon citratus* essential oils were supplied from market.

GC/MS Analysis

Analysis of essential oil was performed using the Thermo Scientific Focus gas chromatograph equipped with a DSQ II single quadrupole mass spectrometer, Triplus autosampler and fused-silica capillary column TR-5MS (5% phenyl-polysilphenylene-siloxane, 30 m×0.25 mm inner diameter, film thickness 0.25 µm). The injection volume was 2 µL. The samples were injected with a split ratio of 250:1 by using helium (99.99 %) as carrier gas, at a flow rate of 1 mL/min; ionization energy was 70 eV. The transfer line temperature of the mass spectrometer was 220 °C, while the temperature of orifice injection was of 220 °C. The temperature of oven was programmed in the range 50–220 °C at a rate of 3 °C/min. Data acquisition was made in the scanning mode. Identification was done on full scan mode in the m/z range of 50–650 a.m.u.

Determination of Minimum Bactericidal Concentration (MBC)

To dissolve the essential oils dimethyl sulfoxide [(DMSO); Sigma, USA] was used. In order to find the non-toxic concentration of DMSO, 1x 10^6 Hep-2 cells were inoculated into each well of micro plates containing RPMI medium (Gibco-BRL)], and cells were allowed to incubate for 48 hours in the presence of decreasing amounts of DMSO (25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39%). The non-toxic concentration was determined up to 3.12%. DMSO concentrations lower than 3.12%did not inhibit the growth of the HEp-2 cells. In the experiments, *Origanum onites* L. and *Cymbopogon citratus* essential oils were dissolved in 1 % DMSO.

Cytotoxicity Tests

To evaluate cytotoxicity of the essential oils, the HEp-2 cell line (human larynx epidermoid carcinoma cell line) was selected. The cells were cultured in RPMI medium with 10% (w/v) fetal bovine serum (FBS). The cells were incubated for 48 h at 37°C in an atmosphere with 5% CO_2.

To determine the effects of propolis samples on HEp-2 cells, they were infected with propolis. Then the infected and non-infected Hep-2 (uninfected with propolis) cells were observed under inverted microscopy. To evaluate the effects of the essential oils on HEp-2 cells, 1×10^5 cells were inoculated into flat bottomed microplates. Then, the cells were allowed to adhere for 6 h at 28 °C. And the cells were allowed to grow for an additional 72 hours. And then, the essential oils of *Origanum onites* L. and *Cymbopogon citratus* were diluted and decreasing amounts (2560, 1280, 640, 320, 160, 80, 40, 20, 10 and 5 µg/ml) were inoculated per well, and incubated for 96 hours. All experiments were performed in triplicate. The Cytotoxic concentrations of essential oils were determined by both cell morphological evaluation with invert microscopy and cell viability by trypan blue method. The highest non-cytototoxic (on HEp-2 cells) concentrations of the essential oils of *Origanum onites* L. and *Cymbopogon citratus*

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were determined to be 1280 and 640 µg/mL, respectively. Therefore, the essential oils concentrations were selected lower than 640µg/mL.

Virus Titrations

Firstly, 100 $TCID_{50}$ (Tissue Culture Infective Dose) of HSV-1 on HEp-2 was calculated in the study. Antiviral activity studies were carried out at the concentration of 1, 10 and 100 $TCID_{50}$ of HSV-1. The antimicrobial efficacy of the *Origanum* Onites L. and *Origanum* Onites L. essential oils were assessed alone and in combination with acyclovir. In addition, the efficacy of acyclovir was determined on 100 $TCID_{50}$ of HSV-1. In the study, *Origanum onites* and *Cymbopogon citratus* were studied at concentrations of 320, 160, 80, 40, 20, 10 and 5 µg/ml.

RESULTS

Origanum onites L. and *Cymbopogon citratus* essential oil components results were given Table 1, 2 and Figure 1, 2.

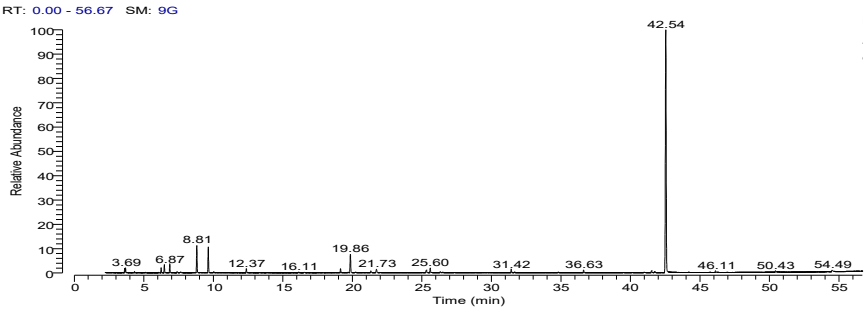

Figure 1. GC/MS chromatogram of *Origanum onites* L.

Table 1. The main essential oil components of *Origanum onites* L.

RT	Compound Name	SI	RSI	Cas #	%
6,48	β-Myrcene	969	974	123-35-3	1,47
6,87	α-Terpinene	974	983	99-86-5	1,64
8,81	γ-Terpinene	989	993	99-85-4	5,50
9,64	p-Cymene	975	975	99-87-6	5,47
19,86	Linalool	994	995	78-70-6	4,40
42,54	Thymol	969	969	89-83-8	68,28

https://doi.org/10.24264/icams-2018.IV.1

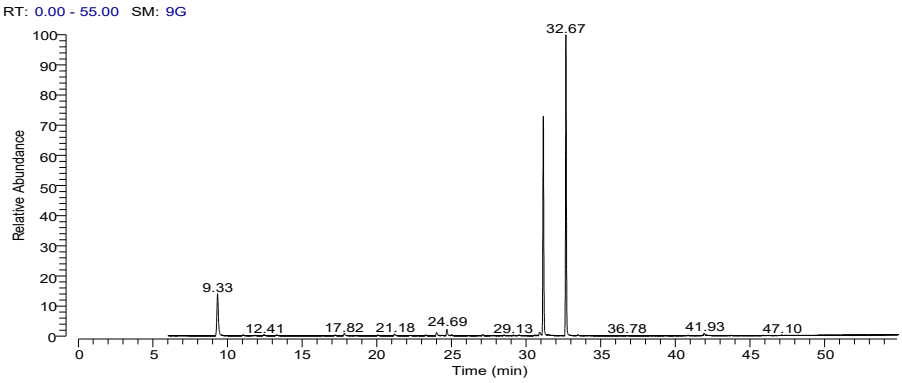

Figure 2. GC/MS chromatogram of *Cymbopogon citratus*

Table 2. The main essential oil components of *Cymbopogon citratus*

RT	Compound Name	SI	RSI	Cas #	%
32,67	E-Citral	977	977	141-27-5	47,53
31,15	Z-Citral	988	989	106-26-3	36,29
9,33	β-Myrcene	979	985	123-35-3	9,31
24,69	Verbenol	881	885	18881-04-4	1,19

The main essential oil components of *Origanum onites* L. were Thymol (68,28 %), γ-Terpinene (5,50 %), p-Cymene (5,47 %) and Linalool (4,40 %) and for Cymbopogon citratus the main components were E-Citral (47,53 %), Z-Citral (36,29 %), β-Myrcene (9,31 %).

In the experiments, it was determined that the concentration of 2.5 µg/ml of acyclovir completely inhibited viral replication at 100 $TCID_{50}$ of virus (Figure 3).

In the experiment, the essential oils of *Origanum onites* L. inhibited virus replication at the concentrations of 20, 40, and 80 µg/ml for 1, 10, 100 $TCID_{50}$ values, respectively, whereas *Cymbopogon citratus* inhibited these three different viral titers at concentrations of 10, 20, and 40 µg/ml (Figure 4). In addition, the *Origanum onites* L. and acyclovir combination inhibited virus replication at the concentrations of 1.5, 2.5, and 5.0 µg/ml for 1, 10, 100 $TCID_{50}$ of values, respectively. In addition, the *Cymbopogon citratus* and acyclovir combination inhibited virus replication at the concentrations of 1.0, 1.5 and 1.5 µg/ml for 1, 10, 100 $TCID_{50}$ of values, respectively (Figure 5).

https://doi.org/10.24264/icams-2018.IV.1

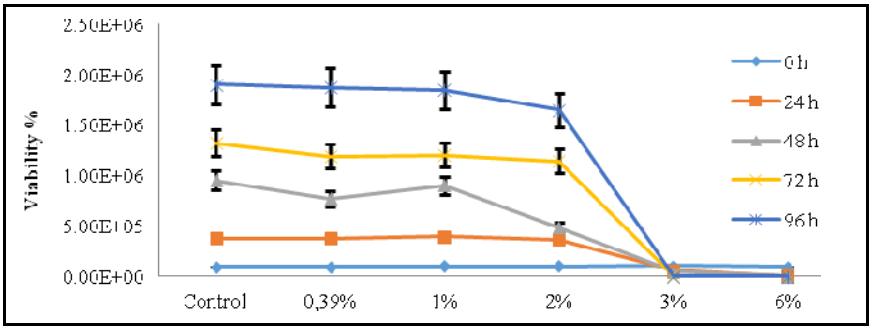

Figure 3. Effect of DMSO on cell viability in HEp-2 Cell Culture

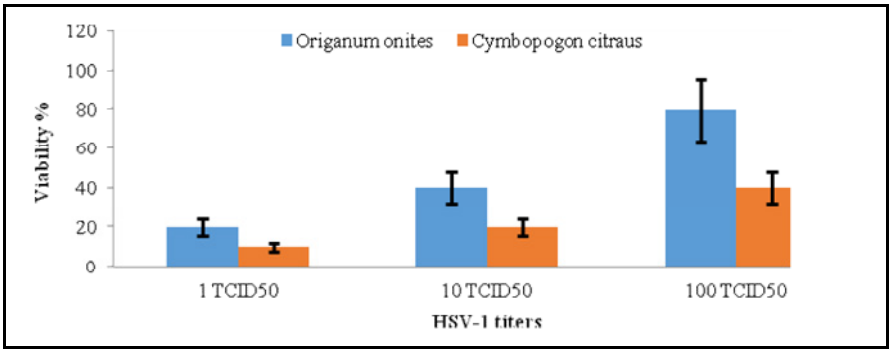

Figure 4. Antiviral activities of *Origanum onites* and *Cymbopogon citratus* essential oils against HSV-1

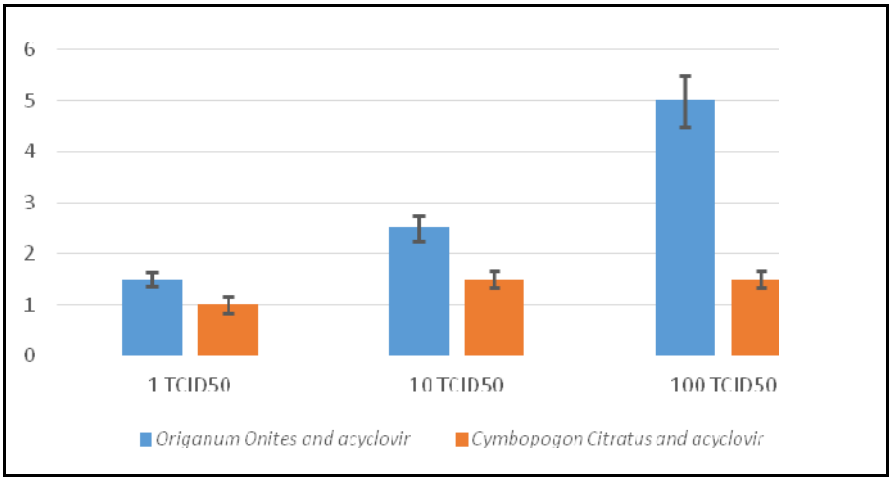

Figure 5. Antiviral activities of Acyclovir plus *Origanum onites* and *Cymbopogon citratus essential* oils against HSV-1

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DISCUSSION AND CONCLUSION

In conclusion, the essential oils of *Origanum onites* L. and *Cymbopogon citratus* against HVS-1 were found to have a significant inhibitory effect on viral replication. The essential oils of *Cymbopogon citratus* and acyclovir showed very strong synergism against HSV-1. The essential oils of *Origanum onites* L. acid with acyclovir significantly reduced viral replication at low concentrations of HSV-1 (1 and 10 $TCID_{50}$), but did not show a significant decreasing in viral replication against 100 $TCID_{50}$titres of virus. It is thought that these two substances may be useful for decreasing viral replication in HSV-1 infections.

Considering the increased resistance to antiviral drugs in recent years, we think that the essential oils of *Origanum onites* L. and *Cymbopogon citratus* may be a very important agent against herpesviruses. In particular, the essential oils of *Cymbopogon citratus* can be a very promising plant.

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