Investigation of the effects of six medicinal plants with antiviral effects against COVID-19

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Article

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Abstract

Today, the coronavirus epidemic, which caused the death of 79 million cases and 1743 thousand people in 218 countries around the world, continues to increase its impact all over the world. Researchers are still trying to develop an effective solution against covid-19, including vaccines and drugs. However, there are few studies that determine the effect of natural products obtained from plants on covid-19. Medicinal and aromatic plants have been used for therapeutic purposes since the existence of humanity. In this study, the effects of some important medicinal plants including Licorice (*Glycyrrhiza glabra*), Saffron (*Crocus sativus* L.), Nigella (*Nigella sativa* L.), Laurel (*Lauris nobilis*), Karabaş (*Lavandula stoechas*), and Zahter (*Thymbra spicata* L*. var. Spicata*) against Covid-19 were investigated in vitro conditions. The six plants were evaluated for cytotoxic effect on Vero cells and determining inhibition of viral replication in Vero-E6 cells at concentrations of broad-spectrum antiviral non-cytotoxic against Covid-19 in cell culture and an additional antiviral effect against Covid-19. According to the results, the five examined plants (Saffron, Nigella, Laurel, Karabaş, Zahter) were ineffective against Covid-19 in vitro conditions. Interisingly, the water extract obtained from the root of the licorice plant (*Glycyrrhiza glabra*) inhibited Covid-19 in vitro conditions in the 2nd dilution (1: 4) following the initial concentration in Vero-E6 cells.

Introduction

Covid-19 is a new strain of coronavirus, and this virus family is zoonotic and can infect humans from animals. Besides, the contamination is very high and spread rapidly over the world. It is seen that this virus can use human angiotensin-converting enzyme II (ACE2) effectively and can multiply in human respiratory tract cells. The virus (2019-nCoV) first appeared in the Hubei Province of China in late 2019. The agent was defined as a coronavirus that was not previously detected in humans in a group of patients presenting with pneumonia, and the name of the disease was accepted as Covid-19 (1–3). Today, the coronavirus epidemic, which has killed 79 million cases and 1743 thousand deaths in 218 countries around the world, continues to increase its impact all over the world (4).

Covid-19 treatment is primarily supportive, and the role of antiviral agents has not yet been determined. Promising approaches to Covid-19 focus on traditional medicine such as medicinal plant extracts. Traditional medicine of many countries of the world recommends some herbs for the prevention, treatment, and rehabilitation of diseases, including Covid-19 (5). Herbal remedies for various diseases have been prescribed by mankind for thousands of years. According to numerous articles, some herbs have antiviral activities and have shown positive effects in practically treating many viral infections. Herbs that act as antiviral agents to treat viral infections have been applied or prescribed as supportive therapy (6).

Herbal and traditional medicines have been used since the first days of the Covid-19 epidemic in China. These traditional medicines have been shown to result in 90% recovery of 214 treated patients. Besides, some traditional herbal medicines have been reported to prevent SARS-CoV-2 infection in healthy people and improve the health status of patients with mild, or severe symptoms (7,8).

Medicinal and aromatic plants are very important as they contain bioactive compounds that can be used in the development of official medicines against various diseases with little or no side effects.

The aim of this study is to investigate the effects of six medicinal and aromatic plants (*Glycyrrhiza glabra*, *Crocus sativus*, *Nigella sativa*, *Lauris nobilis*, *Lavandula stoechas* and *Thymbra spicata var. spicata*) known to have antiviral effects on Covid-19 in vitro.

Material And Methods

The first stage of the study was carried out Altınözü Vocational School of Agricultural Sciences Medicinal and Aromatic Plants Laboratory in Hatay Mustafa Kemal University. In the second stage of the study, the cytotoxic effect of the examined plant extracts and theirs effect on COVID-19 were determined in Ankara University Biotechnology Institute Laboratory and BSL3 laboratory.

Plant Materials

The plant materials were collected from Hatay/Turkey. The plants used in the study are in Table 1.

Table 1. Areas where plant material is collected in Hatay province

No	Plant name	Drog	Location of the plant
1	Licorice	root	Kumlu
2	Saffron	stigma	Kırıkhan
3	Nigella	seed	Antakya
4	Laurel	fruits	Defne
5	Laurel	leaf	Defne
6	Karabaş	flowers	Yayladağı
7	Zahter	herbaceous herb	Altınözü

Water Extraction

The water extraction method was applied to the root of the licorice plant and the stigma of the Saffron plant. Samples were prepared as 10 g of the root in 100 mL of distilled water, and 1 g of stigma in 100 mL of distilled water. Obtained mixtures were extracted in an ultrasonic bath (ALEX MACHİNE, İstanbul/ Turkey) at 28 ^{o}C for 15 mi (9, 10).

Cold Pressed Extraction

The seeds of the Nigella and the fruit of the Laurel plant were extracted by the cold press method (11). Oil extraction was carried out using a propeller (Kocmaksan, İzmir, Turkey). A screw expeller powered by a 10 kW electric motor was used to extract oil from the seeds of the plants tested. The expellers were cleaned after each extraction. Obtained oils were filtered and stored 4^{o}C (12-13).

Essential Oil Extraction

The plants collected from nature were dried at room temperature without washing. To obtain essential oil, distillation was carried out in a Clevenger-type hydrodistillation device in Hatay Mustafa Kemal University Altınözü Agricultural Sciences Laboratory for 3 hours. The essential oil obtained was measured in ml and % ratios (v/w) were determined. It was stored in the refrigerator by putting it in sealed storage bottles. (14-16).

Cell Culture

Vero cells (African green monkey kidney Vero E6 cell) was purchased from ATCC. Vero cells were maintained in DMEM medium (Sigma, USA) containing 10% FBS (Biological Industries, Israel), and 1% penicillin/streptomycin (Biowest, USA) at 37 °C in a humidified incubator in an atmosphere of 5 % CO_2. In vitro assay was carried to determine the cytotoxic effects of six plant samples. Vero cells were seeded into a 96-well plate at a density of 1x10^4 cell and incubated at 37°C. Vero cells were treated with plant samples at a concentration of ……mg/mL and six different concentrations for 24, and 48 h. The cell viability was evaluated by following the MTT (3- (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) reduction assay. MTT solution (5 mg/mL in PBS) was added into each well and then incubated for 4 h at 37 °C. The 100 µL DMSO was added into each well to extract the insoluble formazan crystals within the cells. The absorbance was measured at 540 nm using a microplate reader. The results represent the average values of six experiments.

Viral Infection

Vero E6 cells (ATCC: CRL-1586) were used to propagate SARS-CoV-2 and performed all cytotoxicity and antiviral tests. Infectivity tests related to antiviral assays were performed in BSL-3 facility of the Department of Virology, Faculty of Veterinary Medicine, Ankara University. To evaluate the effect of the extracts on Vero E6 cells viability, 10-fold dilutions of plant extracts were added on 90% confluent monolayers in 96-well culture plates. After 72 hours of incubation, the maximum noncytotoxic concentration (MNCC) for all extracts was determined by microscopic observation and CC50 (extract concentration that is toxic for at least half of the cells) was determined by the crystal violet uptake method. Briefly, cell monolayers were fixed and stained with a crystal violet 0.75% in 40% methanol solution and incubated for 15 minutes at 37°C.

A primary antiviral screening test was conducted by cytopathic effect (CPE) reduction assay, which involves the protection of SARS-CoV-2 caused lysis of Vero E6 cells by extracts. Briefly, in a 96-well culture plate, 0.1 moi of the virus was inoculated on Vero E6 cells. After 1 hour of adsorption at 37°C in a

5% CO2 humidified atmosphere, cells were washed with phosphate buffer saline (PBS) and the highest non-toxic dilution (in DMEM High glucose, Gibco, Germany) detected before were added. Cytotoxicity and cell controls were included. After 72 hours of incubation at 37°C in a 5% CO2 humidified atmosphere, cells were fixed and stained as described above. The SARS-CoV-2 activity of the extracts was evaluated by direct observation of CPE reduction (17-18).

Results And Discussion

The effect graph of the medicinal plants used in this study on COVID19 in various concentrations between 25 µl and 250 µl is shown as following figures; Licorice (Fig. 1), Saffron (Fig. 2), Negella seed (Fig. 3), Laurel seed (Fig. 4), Laurel leaf (Fig. 5), Karabaş (Fig. 6) and Zahter (Fig. 7) plants, respectively.

As shown in Fig. 1, licorice root aqueous extract showed antiviral activity at the second dilution after the first concentration and inhibited the replication of the SARS CoV-2 virus. It is thought that the aqueous extract of licorice root may be effective at concentrations lower than the concentration in the study. This shows that it is a phytotherapy drug candidate for the safe use of its phytotoxic effect in terms of effectiveness.

In literature studies and consuming medicinal herbs such as Allium sativum, Camellia sinensis, Zingiber officinale, Nigella sativa, Echinacea spp. They reported that immune-enhancing herbs such as Hypericum perforatum and Glycyrrhiza glabra could be effective against COVID 19 (19). However, these plants have not been studied in vitro [19]. Among these plants, the antiviral activity of the Nigella sativa plant included in our study against COVID 19 has not been found. Again, from these plants, Glycyrrhiza glabra emerged as an antiviral agent. Although there are many studies on the antiviral effectiveness of licorice and glycyrrhizic acid, the fact that there are no studies on the effectiveness of COVID 19 shows the importance of this study (20, 22).

There is much literature about the use of the saffron plant as an important antiviral agent (23, 24). In one of these studies, the antiviral activities for saffron extract and its main components affected, and Crocin and picrocrocin could be promising anti-HSV and anti-HIV agents for herbal treatment against viral infections (25). In this study, the saffron plant with proven antiviral activity did not show antiviral effects against COVID 19.

The cold press essential oil obtained from the seed of the Nigella plant (*Nigella sativa* L.) was ineffective in vitro conditions against COVID-19 infectivity at all concentrations following the initial concentration (Fig. 3). Antiviral activity of *Nigella sativa* alcoholic extracts against PPRV was investigated in vitro. It showed an antiviral effect in Vero cell line and at the prepared dose of 50 µg / ml (26). *Nigella sativa* did not show an antiviral effect against COVID 19 viral infection in this study.

The cold press essential oil obtained from the seed of the laurel plant (*Laurus nobilis*) was found to be ineffective in vitro conditions against COVID-19 infectivity at all concentrations following the initial concentration (Fig. 4).

The essential oil obtained from the leaf of the laurel plant (*Laurus nobilis*) was ineffective in vitro conditions against COVID-19 infectivity at all concentrations following the initial concentration (Fig. 5). Bay leaf essential oil has a wide range of bioactive properties due to its bioactive functions such as antimicrobial, antifungal, antioxidant, antiviral, pesticide and food applications (27). However, in this study, Laurus nobilis did not show an antiviral effect against COVID 19 viral infection.

The essential oil obtained from the flower of the Karabaş plant (*Lavandula stoechas*) was ineffective in vitro conditions against COVID-19 infectivity at all concentrations following the initial concentration (Fig. 6).

The essential oil obtained from the herb of the Zahter plant (*Thymbra spicata* L. *var. spicata*) was ineffective in vitro conditions against COVID-19 infectivity at all concentrations following the initial concentration (Fig. 7).

Conclusion

Previously, medicinal and aromatic plants have been successfully used to treat many viral diseases. Although many plants have been proposed that are effective against COVID19, no in vitro studies have been found on the effectiveness of these plants to date. According to the findings obtained in this study, Licorice plant (*Glycyrrhiza glabra*) was discovered to be effective against COVID-19 in vitro conditions. It is easy to use, rapid results can be obtained in combating the epidemic. We hope that speeding up studies on other medicinal and aromatic plants to achieve faster results after this study can be valuable in combating the epidemic.

Declarations

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Ethical Statement

The permission of the Animal Experiments Local Ethics Committee is not required for this study.

Conflict of Interest

The authors declared that there is no conflict of interest.

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Figures

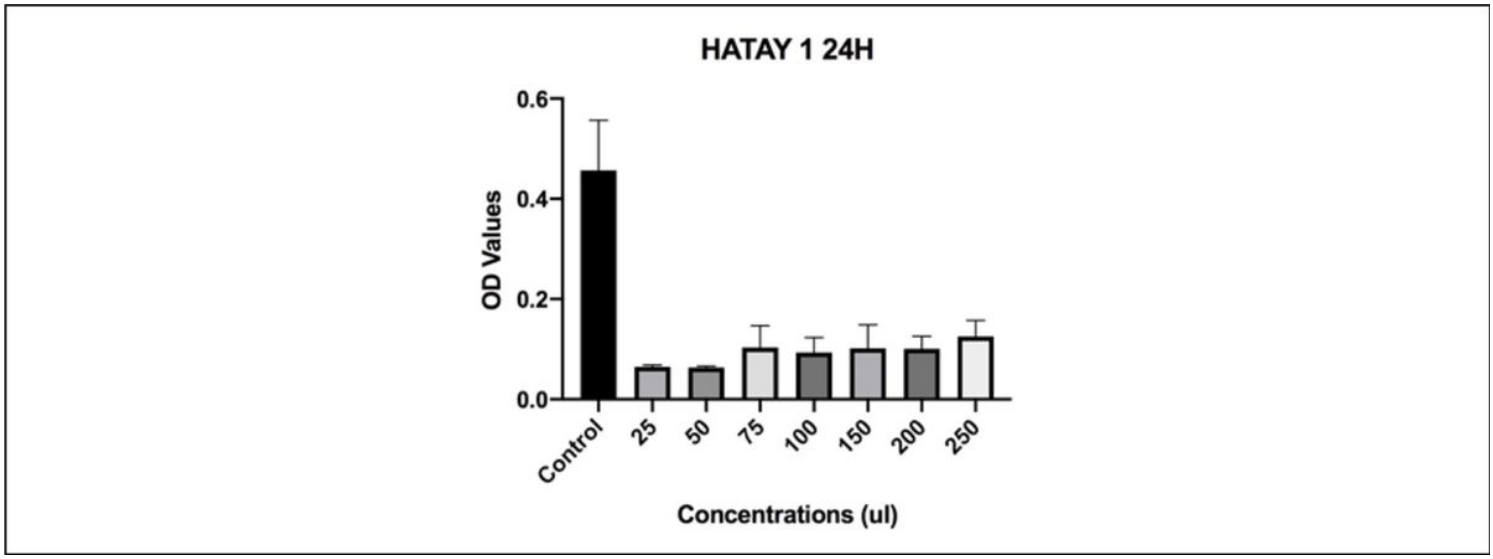

Figure 1

Concentrations and effect graph of Glycyrrhiza glabra water extract on COVID-19

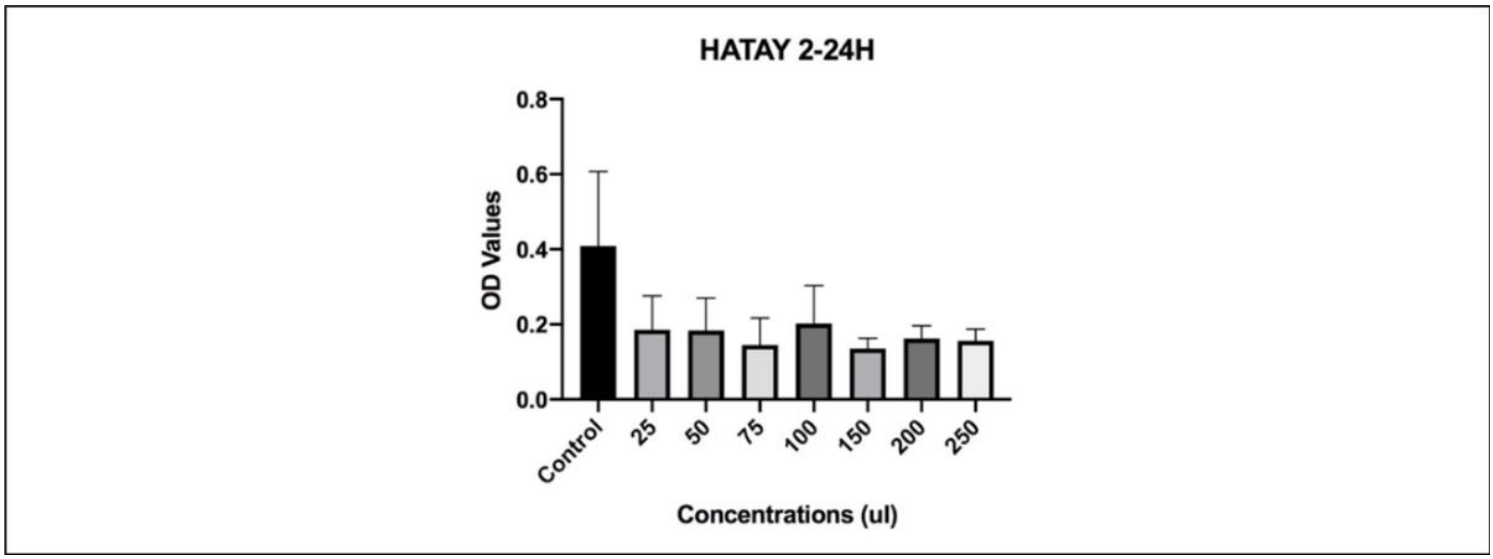

Figure 2

Concentrations and effect graph of Crocus sativus L. water extract on COVID -19

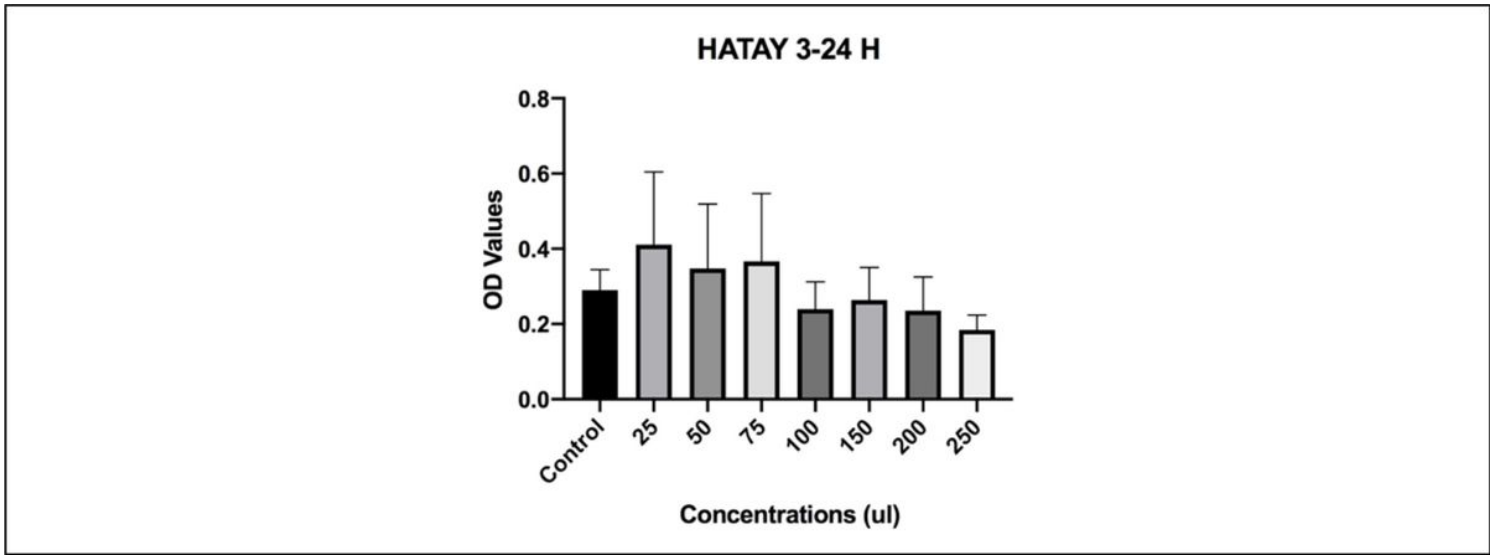

Figure 3

Concentrations and effect graph of Nigella sativa L. seed cold press essential oil on COVID-19

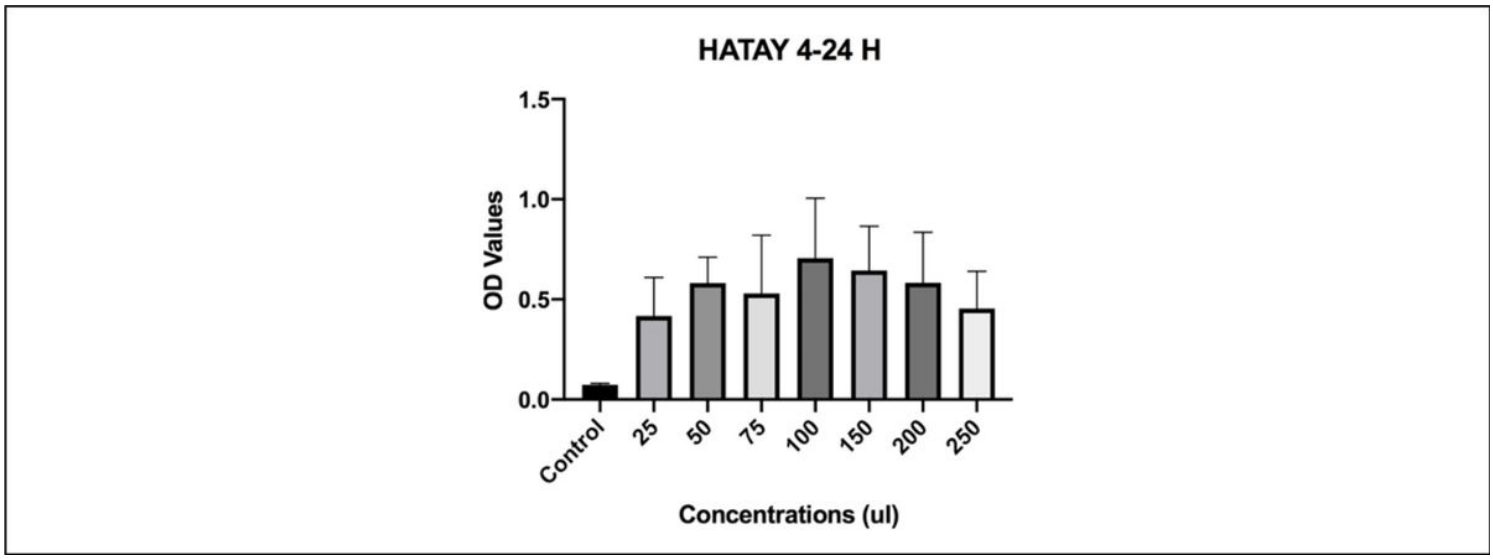

Figure 4

Concentrations and effect graph of Laurus nobilis seed cold press essential oil on COVID-19

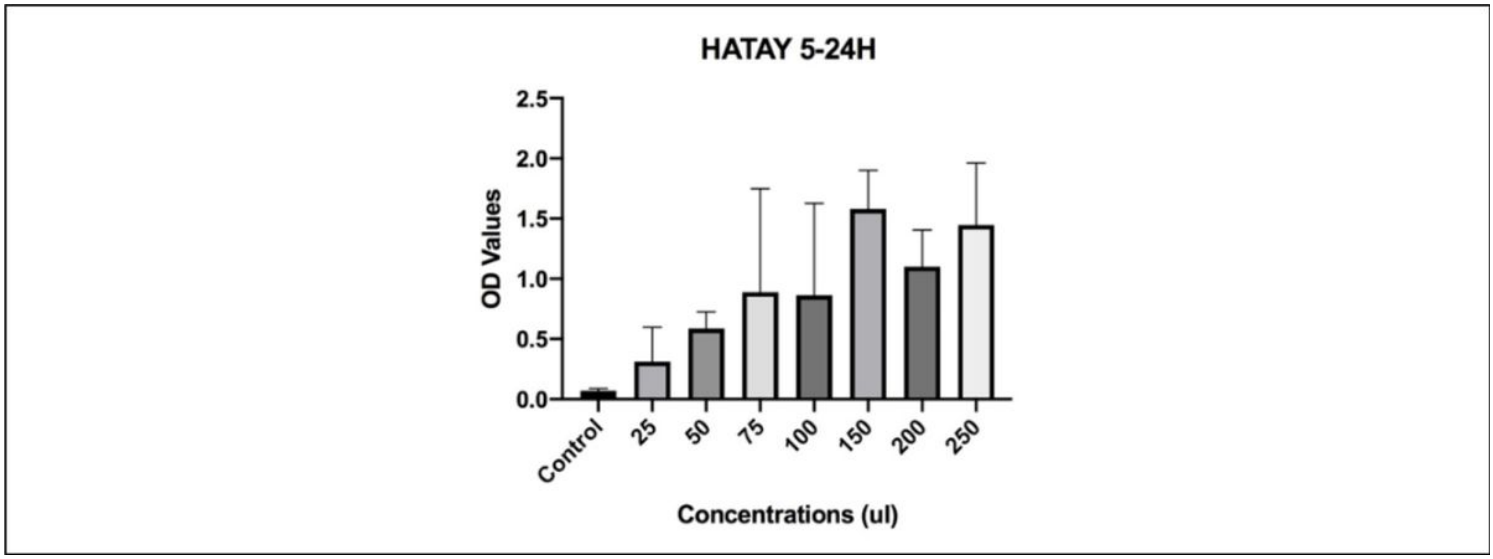

Figure 5

Concentrations and effect graph from Laurus nobilis leaf essential oil on COVID-19

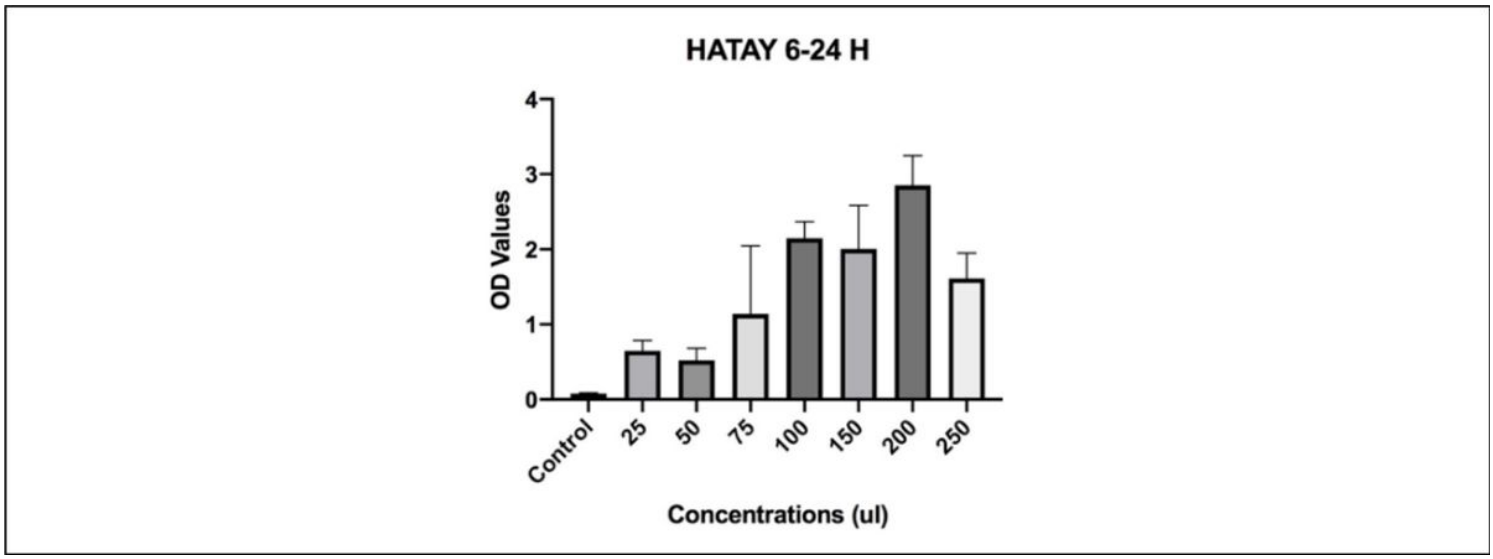

Figure 6

Concentrations and effect graph from Lavandula stoechas essential oil on COVID-19

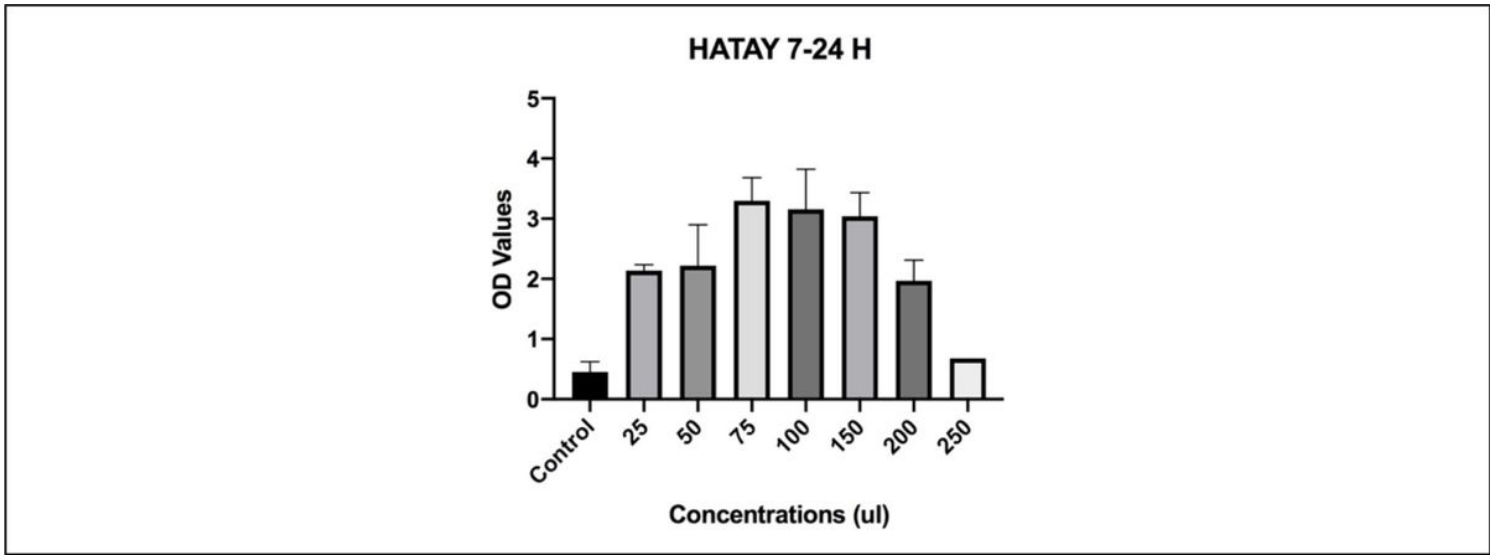

Figure 7

Effects of Thymbra spicata L. var. spicata essential oil on COVID-19 depending on its concentrations.