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Research Article

Assessment of *In-vitro* Cytotoxicity and *In-ovo* Virucidal Antiviral Efficacy of Various Plant Extracts and Bioactive Molecules

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Abstract

The viral diseases that occurred in recent years have increased the interest in non-toxic to healthy cells and naturally isolated agents to struggle with these diseases. The key intention of this research is to examine both antiviral potentials against the Infectious Bronchitis model virus (IBV) and cytotoxic activities on determined cell lines of different active ingredients and medical herbs extracts for developing new antiviral agents or drugs towards SARS-CoV-2. The antiviral potency of the samples against IBV was determined as *in ovo* virucidal antiviral activity in specific pathogen-free (SPF) embryonated chicken eggs (ECEs). To detect antiviral activity, the haemagglutination test was performed after 48 h of incubation for all samples. The cytotoxic activity of the samples was identified on HepG2, Caco-2, HeLa, HEK293, PANC-1, PC-3, A549, MDA-MB-231, and CCD-34Lu cell lines by the MTT protocol. *Hypericum perforatum* extract was found to have a dominant role in cytotoxicity and antiviral activity. In addition, while nobiletin and *Sambucus nigra* do not exhibit cytotoxic activity on cells, they play a significant role in antiviral activity. As a consequence of our investigation, the cytotoxic and antiviral properties of *Laurus nobilis, H. perforatum*, and *S. nigra* extracts were found remarkable and the potential of these extracts was demonstrated.

Keywords: Antiviral, Cytotoxicity, Extract, *In ovo*, Infectious bronchitis virus

Introduction

Population in different regions can be negatively impacted by various viruses, which can induce epidemics and pandemics, deaths, and severe economic losses. Studies conducted by the World Health Organization (WHO) have stated that the population affected by viral pathogens reaches 80%. Therefore, developing efficient treatments against viruses is crucial to prevent these losses. In addition to the current strategies against viruses, the search for alternative approaches has gained momentum. For this purpose, *in vitro* and *in vivo* studies have been used to specify the potential of various natural plant sources as therapeutic agents against different viruses [1]. Antiviral effects for plant-derived components have been broadly evaluated on lots of viruses such as influenza, herpes simplex, and respiratory syncytial virus in the last two decades [2]. One of the critical virus families among these viruses is the *Coronaviridae* which has created major socioeconomic problems and caused serious outbreaks or pandemics worldwide [3]. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) which belongs to this family, has swiftly spread into the population and has caused the COVID-19 pandemic [4]. This novel coronavirus, which causes numerous deaths and diseases globally, has once again revealed the necessity of vaccines and therapeutics studies. Despite significantly increasing investment in the pharmaceutical field, prophylactic treatments or existent antiviral approaches for coronaviruses are currently limited. Herbal medicines and naturally-purified molecules may guide new antiviral drugs [3]. Infectious Bronchitis virus (IBV), taking part

in the same virus family as SARS-CoV-2, is known for its high contagion rate, especially among chickens. It is a Gamma coronavirus in the *Coronaviridae* family [5] and causes dangerous respiratory diseases by infecting poultry through the respiratory system [6]. Owing to weak cross-protection of the vaccines among virus serotypes, alternative approaches for effective antiviral agents have become imperative to preventing IBV infection. The research on new antiviral drugs gravitates toward natural products because of resistance against current antiviral agents [7].

In recent years, scientific curiosity has increasingly concentrated on active compounds of plants responsible for biological properties and therapeutic effects [8]. According to research, plant-derived compounds have been used in medicine at a rate of 80% because of active molecules such as alkaloids, glycosides, steroids and tannins. They can exhibit potent bioactivities as antimicrobial, antioxidant, antibacterial, antifungal, and anti-inflammatory in a variety of living systems [9,10]. Furthermore, the cytotoxic and antiviral properties of these plants provide opportunities for studies to discover new therapeutic agents [8,9]. Many studies indicate that phenol content of different herbs plays active role in antiviral activity and affects different RNA viruses generally by inhibition of nucleic acids, gene expression or entry protein [11]. The cytotoxic effect and antiviral activity studies performed by extracts obtained from *Camellia sinensis*, *Hypericum perforatum*, *Sambucus nigra* and molecules such as tangeretin, catechins, nobiletin have also importance in the challenging against viruses.

The focus of this research is to investigate the selected extracts and active ingredients for antiviral activity against IBV. Thus, compounds having anti-IBV activity against model virus system are planned to be pioneers in developing new antiviral agents for SARS-CoV-2.

Material and Methods

Ethical Statement

This study was approved by the Ege University Animal Experiments Local Ethics Committee (Approval no: 2020/051).

Plant Extracts

The plant extracts performing in this study were supplied by NPRO Natural Products Company and prepared by dissolving in Dimethyl sulfoxide (DMSO) to use in all tests.

Cell Lines

Human prostate adenocarcinoma (PC-3), human colorectal adenocarcinoma (Caco-2), human embryonic kidney (HEK293), human cervix adenocarcinoma (HeLa), human alveolar adenocarcinoma (A549), human pancreatic carcinoma (PANC-1), human breast adenocarcinoma (MDA-MB-231), human hepatocarcinoma (HepG2), and a healthy human lung fibroblasts (CCD-34Lu) cells were purchased from ATCC (Manassas, VA, USA) and used for evaluating the cytotoxic activity of samples. The cell lines were cultured at 37°C in a humidified atmosphere containing 5% CO_2 in Dulbecco's Modified Eagle Medium/ Nutrient Mixture F-12 (DMEM/F12) supplemented with 10% fetal bovine serum (FBS), 400 µg/mL penicillin/ streptomycin mixture, and 1% L-glutamine (Gibco, NY, USA).

Virus and Specific Pathogen-Free Embryonated Chicken Eggs (SPF-ECEs)

The IBV D274 strain, present in our laboratory stock was used for antiviral activity assay. Specific pathogen-free embryonated chicken eggs (SPF-ECEs) that were procured from İzmir Bornova Veterinary Control Institute, Türkiye were used for the determination of antiviral potential.

In vitro Cytotoxicity Assay

Cytotoxicity of samples was identified by performing MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] test, a common assay for evaluating cytotoxicity of agents depending on the viability of cells [12]. For the MTT assay, the cell lines having initially $1x10^4$ cells/well concentration were cultivated in 96-well microplates and overnight incubation was performed at 37°C in a humidified atmosphere. Then, the samples at three determined concentrations (0.5, 5, and 50 µg/mL) were treated with the cells and incubated for a further 48 h. While all samples except *S. nigra* were incubated in all selected cell lines, *S. nigra* has been treated with 2 cells (CCD-34Lu and HEK293). Following the incubation for 48 h, MTT solution (diluted from 2.5 mg/mL stock) was added to the cells at 20 µL volume and incubated for 4 h. After the incubation period, formazan crystals were dissolved by using DMSO and the spectrophotometer (Thermo Fisher Scientific) was used to measure the absorbance values at 570 nm. Percent viability of treated cells was assessed by comparing control cells. The half maximal inhibitory concentration (IC_{50}) values were determined by calculation on Prism 5 software (GraphPad5, San Diego, CA, USA).

Preparation of Virus and Inoculation of SPF-ECEs *in ovo*.

Antiviral activity of some plant extracts and molecules were evaluated as virucidal effects against IBV *in ovo* model [13]. Reed and Muench method [14] was used to calculate the Embryo Infective Dose 50% (EID_{50}) by performing the hemagglutination test specified in the WOAH protocol. Based on Reed-Muench calculation, $100EID_{50}$/0.1 mL

virus stock was used in antiviral experiments [15]. The desired final concentration of molecules and extracts diluting by phosphate buffer saline (PBS) was prepared and mixed with the virus 1:1 ratio for 1 h incubation at 25°C. Randomly grouped (n=4) 9-11-day-old SPF-ECEs were inoculated into the chorioallantoic membrane with the 0.1 mL sample-virus mixture. Favipiravir used for clinically antiviral agent was tested in control groups as positive control at 10 mg/g amd 25 mg/g concentrations, respectively. SPF-ECEs were incubated for 48 h and their viability was checked daily. After the incubation period, the SPF-ECEs were stored overnight at +4°C. Following overnight storage, chorioallantoic fluid (CAF) of them were collected to detect HA titer [16].

Hemagglutination (HA) Assay

For the hemagglutination (HA) test, V-bottom 96-well microplates were used according to the WOAH protocol [15]. In the HA assay, 25 mL of PBS was put into all wells. Then, 25 mL of collected CAF was placed in the first column of the microplate for each sample and diluted 2-fold to the last column of the microplate. As a final step, 25 mL chicken red blood cells (1% v/v)(RBC) were added all wells to incubate them for 45 min at 25°C. After incubation, button shape formation for wells was evaluated as HA negative and agglutination was considered as HA positive. The highest HA dilutions and percent viability of the eggs were recorded [15,16].

Statistical Analysis

Statistical analysis of the results was calculated by one-way ANOVA using SPSS (version 23, SPSS Inc., Chicago, IL, USA). Mean and standard deviation (SD) values are expressed and statistical significance (P<0.05) was determined.

Results

In vitro Cytotoxicity Assay

The cytotoxicity assay of samples on different cancerous and noncancerous cell lines was realized. The effect of samples on treated cells was first examined under the microscope and statistically analyzed. According to the MTT assay results the obtained IC_{50} values of samples was given *(Table 1)*. Among the samples, *Abies* spp. essential oil, along with *Laurus nobilis*, *H. perforatum*, and gallic acid had cytotoxic effects on the cells to which they were applied (P<0.05). *H. perforatum* was performed to evaluate the cytotoxic effect for different cancerous and noncancerous cell lines and a remarkable cytotoxic effect has been seen on PANC-1 (IC_{50}=6.69±1.32 mg/mL) (P<0.001) and HeLa (IC_{50}=10.86±0.13 mg/mL) (P<0.001), while both cell lines have approximate values with doxorubicin (IC_{50}=5.52 mg/mL, IC_{50}=9.04 mg/mL, respectively). Also, the results demonstrated that *L. nobilis* extract shows a significant impact on HeLa, PANC-1 and HepG2 cell lines (IC_{50}=11.71±0.12, 7.54±1.20 and 7.33±0.82 mg/mL respectively)(P<0.001). Moreover, the PANC-1 cell line has been observed as more active than doxorubicin (IC_{50}=9.04 mg/mL) which chemotherapeutic agent. *Abies* spp. essential oil against Caco-2 and HeLa cells cytotoxic effect has been not demonstrated (P>0.05). The highest dose of *Abies* spp. essential oil (0.5% v/v) was found to have a percent viability of 50% in PC3, MDA-MB-231 and

Table 1. *The IC_{50} values of C. sinensis, S. nigra, H. perforatum, E. purpurea, M. officinalis, oleuropein, L. nobilis, neohesperidin, nobiletin, gallic acid, tangeretin, catechin hydrate, and doxorubicin µg/mL*

Sample	MDA-MB-231	A549	PANC-1	PC-3	CCD-34Lu	HEK293	HepG2	HeLa	Caco-2
Camellia sinensis	>50	>50	ND	>50	21.65±6.18	>50	>50	>50	ND
Sambucus nigra	-	-	-	-	ND	>50	-	-	-
Echinacea purpurea	>50	ND	ND	ND	ND	>50	>50	>50	ND
Melissa officinalis	>50	ND	ND	>50	ND	>50	ND	>50	ND
Hypericum perforatum	19.53±0.73	18.78±1.75	10.86±0.13	14.27±1.50	13.58±1.17	28.32±1.08	45.38±5.50	6.69±1.32	14.76±1.98
Oleuropein	ND	ND	ND	ND	ND	ND	ND	ND	ND
Laurus nobilis	40.09±0.40	16.14±2.14	7.54±1.20	31.56±6.85	19.82±2.28	27.95±3.23	7.33±0.82	11.71±0.12	46.08±5.21
Neohesperidin	ND	ND	ND	ND	ND	ND	ND	ND	ND
Nobiletin	ND	>50	ND	ND	ND	>50	ND	ND	ND
Gallic acid	42.21±2.6	36.91±5.38	42.06±6.93	38.8±1.65	25.70±1.2	25.70±1.2	40.19±2.6	20.40±0.34	24.14±2.8
Tangeretin	ND	ND	ND	ND	ND	>50	ND	ND	47.36±6.1
Catechin hydrate	ND	ND	ND	ND	ND	ND	ND	ND	>50
Doxorubicin	14.77	2.36	9.04	9.88	4.42	2.76	6.79	5.52	4.45

ND: non detected, -: not analyzed

HEK293 cells among other cells (P<0.01). Besides, it has been found that high cytotoxicity was performed on CCD 34-Lu, PANC-1, A549, HepG2 cells (P<0.001). Although *C. sinensis* and tangeretin demonstrated comparable results, *C. sinensis* showed statistically more significant results than tangeretin in HEK293 and HeLa cell lines (P<0.01). In contrast, tangeretin was more effective than *C. sinensis* in Caco-2 cell line (P<0.01). The other extracts were not active at the highest dose (50 mg/mL) tested.

In ovo Antiviral Activity

The *in ovo* experiment was used to assess the antiviral activity of the plant extracts against IBV [16]. After 48 h incubation of SPF-ECEs, the numbers of embryo deaths, and percentage viability of eggs were recorded and mean HA titers were calculated *(Table 2)*. In addition, embryos after 48 h incubation with sample-virus mixture were evaluated. Deaths in SPF-ECEs were observed between 24-48 h in virus control and vehicle control groups. In

Table 2. *Mortality and HA titers of SPF-ECE's after 48 h incubation*

Sample Groups (n=4)	Concentration	Egg Mortality			Mortality%	Mean HA Titer	Mean HA Titer (log_2-based)
		24 h	48 h	Total			
Untreated control		-	-	0/4	0%	0	0
Virus control		-	1	1/4	25%	2048	11
Vehicle control (5% DMSO)		-	1	1/4	25%	2048	11
Favipiravir (antiviral agent)	10 mg/g	-	-	0/4	0%	512	9
Camellia sinensis	5 mg/g	-	-	0/4	0%	512	9
	10 mg/g	-	-	0/4	0%	512	9
Sambucus nigra	0.1 mg/g			0/4	0%	256	8
	1 mg/g			1/4	25%	32	5
Echinacea purpurea	5 mg/g	-	-	0/4	0%	512	9
	10 mg/g	-	-	0/4	0%	128	7
Melissa officinalis	5 mg/g	-	1	1/4	25%	1024	10
	10 mg/g	-	-	0/4	0%	512	9
Hypericum perforatum	0.1 mg/g	-	-	0/4	0%	64	6
	1 mg/g	-	1	1/4	25%	0	0
	5 mg/g	-	-	0/4	0%	0	0
	10 mg/g	-	-	0/4	0%	0	0
Abies spp. volatile oil	0.5% (v/v)	-	-	0/4	0%	512	9
Oleuropein	5 mg/g	-	-	0/4	0%	512	9
	10 mg/g	-	-	0/4	0%	64	6
Laurus nobilis	5 mg/g	-	1	1/4	25%	256	8
	10 mg/g	-	-	0/4	0%	128	7
Tangeretin	5 mg/g	-	1	1/4	25%	512	9
	10 mg/g	-	1	1/4	25%	128	7
Cateshin hydrade	5 mg/g	-	-	0/4	0%	512	9
	10 mg/g	-	-	0/4	0%	128	7
Neohesperidin	5 mg/g	-	-	0/4	0%	512	9
	10 mg/g	-	-	0/4	0%	256	7
Gallic acid	5 mg/g	-	-	0/4	0%	512	9
	10 mg/g	-	-	0/4	0%	128	8
Nobiletin	5 mg/g	-	-	0/4	0%	512	9
	10 mg/g	-	-	0/4	0%	64	6

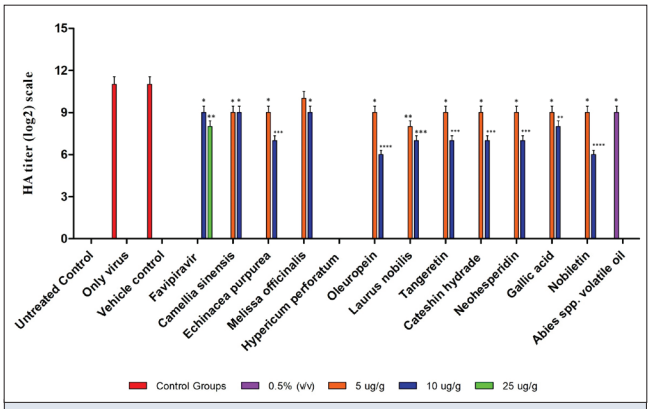

Fig 1. HA titers of IBV-extract/molecule samples on SPF-ECEs (P<0.05, P<0.01, P<0.001, P<0.0001)

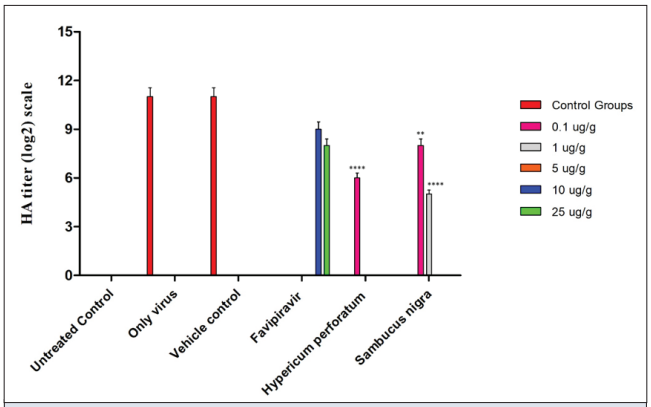

Fig 2. HA titers of IBV-*H. perforatum*, IBV-*S. nigra* samples on SPF-ECEs (P<0.05, P<0.01, P<0.001, P<0.0001)

both groups, the HA titer was calculated as 2048. It was determined that DMSO vehicle control did not affect deaths and HA titer. In the untreated control group, no deaths in SPF-ECEs were detected and HA titers were negative. As a positive antiviral control, favipiravir which can be used commercial antiviral agent, was examined at 10 and 25 mg/g concentrations. It was found that favipiravir could decrease the HA titer compared to virus control. Depending on the results obtained, $\log_2$-based HA titers were determined 3-fold at 10 mg/g and 4-fold at 25 mg/g lower than control. Between 24-48 h observation, 25% embryo deaths were determined for the following groups; *S. nigra* at 1 mg/g, *H. perforatum* at 1 mg/g, *L. nobilis* at 5 mg/g, *Melissa officinalis* at 5 mg/g, tangeretin at 5 mg/g and 10 mg/g. These deaths may be due to some manipulation during inoculation or weak embryo development [17]. All samples except *S. nigra* were tested for antiviral activity at concentrations of 5 mg/g and 10 mg/g. *H. perforatum* was also tested at a concentration of 0.1 and 1 mg/g besides 5 mg/g and 10 mg/g. *In ovo* antiviral activity of *S. nigra* with known antiviral activity was measured at 0.1 and 1 mg/g concentrations. The concentration of *Abies* spp. essential oil was prepared as 0.5% by volume (v/v). The average HA titers of all samples which were analyzed for 5 mg/g, and 10 mg/g were shown *(Fig. 1)*. When all samples were compared, the most potent antiviral activity results were observed in *S. nigra* and *H. perforatum*. Therefore the lower concentrations for the two extracts were attempted *(Fig. 2)*. The efficacy of all groups was evaluated statistically compared to the virus control group. All extracts were statistically significant (P<0.05) except *M. officinalis* (at 5 mg/g). Compared to control groups, it showed that samples at concentrations of 5 mg/g and 10 mg/g had the potential to significantly lower the HA titer by 1 to 5 $\log_2$ HA. Depending on the dose, the extracts showed more effective antiviral activity at a concentration of 10 mg/g. Oleuropein and nobiletin at 10 mg/g had also the highest statistical significance (P<0.0001). Also, *S. nigra* (P<0.01 at 0.1 mg/g) and *H. perforatum* (P<0.0001 at 0.1 mg/g) gave statistically significant results. Antiviral activity was negative in *H. perforatum* at 1, 5 and 10 mg/g concentrations. At 0.1 mg/g, it significantly reduced HA activity with 5 $\log_2$ HA. In the *S. nigra* sample, the antiviral activity value was found between 0.1 mg/g and 1 mg/g. HA activity decreased by 6 $\log_2$ HA at 1 mg/g, while at 0.1 mg/g, HA activity decreased by 3 $\log_2$ HA. This allowed the focus on the potential antiviral activity of both samples.

Discussion

In our study, it has been demonstrated the cytotoxic effects and antiviral activity of medicinal plants and active ingredients. *L. nobilis* was one of these samples and its fruit and leaves have been used in traditional Turkish medicine as antimicrobial, antiseptic, antivenom, diuretic agent and stomach-ached curative [18]. It was reported in studies that aqueous extract obtained from leaves of *L. nobilis* has cytotoxic effects without genotoxicity [19]. According to Oliveira et al.[20], although there is no relationship between cytotoxicity and genotoxic effect, changes in genetic material may occur indirectly. Research findings indicate that costunolide, zaluzanin D, and sesquiterpenes obtained from *L. nobilis*, exhibit potentially inhibitory effects by preventing the growth of human promyelocytic leukemia (HL-60) cells [21]. Compared with other results, the higher cytotoxicity results on PANC-1, HeLa, and HepG2 cells were obtained for *L. nobilis* in our study. Gallic acid which is one of the plant phenols, exhibits selective cytotoxicity to tumor cells compared with a normal cell. In a study performed on HL-60RG cells, it was indicated that gallic acid has the potency to induce apoptosis by way of reactive oxygen forms [22]. This study also evaluated that gallic acid reduced cell viability by 20-50% on different cancerous and non-cancerous cell lines. In parallel with the studies in the literature, obtained results in this experiment emphasize that a certain dose has a high cytotoxic effect on the cell. Spectrometric indications have proven that the activity of *H. perforatum* extracts results from phloroglucinols which

include hyperforin, and zaphthodianthrones which involve hypericin [23]. Hypericin as a potent natural photosensitizer and hyperforin as an inhibitor of the drug-metabolizing enzyme in liver, activate the biological system strongly [24]. *H. perforatum* also involves various active components such as avicularin, rutin, quercetin and derivatives to act as ROS scavengers [25]. It has been shown in another study that hyperoside which is another flavanol of *H. perforatum*, not only prevents of PC12 cells propagation, but also promotes the healthy cell growth [26].

Herbal medicines have been historically preferred for therapy against various viral diseases. These phyto-chemicals are considered to be crucial antiviral compounds for treatment opposed many important viruses. The potential usage of many extracts and bioactive molecules as antiviral agents against the SARS-CoV-2 pandemic is being investigated [27]. As viral infections have become increasingly important, the trend towards antiviral drugs has also accelerated. Assessing the antiviral activity of different plant species or bioactive compounds isolated from them suggests potential therapies [1]. In addition to studies evaluating the *in vitro* and *in vivo* antiviral potential of different plant species, *in ovo* antiviral activity experiments are an effcient method that offers three-dimensional study and provides advantages in ethical issues [13]. In our study, the anti-IBV virucidal activities of the samples were determined as *in ovo*. Compared with previous studies, the antiviral activity of *L. nobilis*, oleuropein and nobiletin compounds exhibit similar results. Serkedjieva et al.[28] evaluated both *S. nigra* and *H. perforatum* extracts *in vitro* and *in vivo*, and reported that the strongest antiviral activity was observed against herpes simplex and influenza A viruses. They stated that the antiviral activity is due to the flavonoid, triterpene, tannin and polysaccharide content, although the mechanism of action is not precise. Chen et al.[29], showed in their study that the *S. nigra* extracts inhibit viral replication and demonstrated the effect of these extracts on avian IBV replication. Treatment of *S. nigra* extracts with the virus before infection to a great extent inhibited the virus at early stage in the infection. In another study, Chen et al.[30] confirmed that ethyl acetate extraction (HPE) of *H. perforatum* exhibited an antiviral effect on IBV. In chicken embryo kidney (CEK) cells, this extract was significantly reduced virus titer by inhibiting mRNA expression.

In our study, *S. nigra* reduced the HA titer by 3-5 $\log_2$ HA between concentrations of 0.1-1 mg/g in parallel with the literature, and for the chloroform phase of ethyl alcohol extraction of *H. perforatum*, the antiviral activity was found to decrease by 5 $\log_2$ HA at 0.1 mg/g. It is thought that the significant antiviral activity of both extracts was due to flavonoid, triterpene, saponin or phenolic acid compounds and exhibits activity by inhibiting viral replication. Phenolic compounds and oleic acids from olives play an effective role in biological activities. Furthermore, oleuropein in olives may serve as an antiviral agent against various viruses through inhibition of replication and entry protein [31,32]. In this study, when the results of cytotoxicity and antiviral activity are compared together, it is suggested that oleuropein can be used as a potential antiviral agent against coronaviruses by promoting cell proliferation in healthy cell lines (CCD-34Lu). It has been stated that nobiletin, the most abundant compound in *Citrus* spp., has potential effects against influenza, hepatitis B and C, human respiratory syncytial, vesicular stomatitis viruses. Antiviral effect against SARS-CoV-2 with protein inhibition is also predicted [27]. This study showed that the nobiletin molecule, which gives promising effects *in ovo*, can be assessed as a potential antiviral agent against IBV. Rosmarinic acid (RA) and its derivatives such as sulfated or hexoside form of rosmarinic acid were identified as the primary bioactive phenolic compound in *M. officinalis* [33]. Lelesius et al.[8] observed *in vitro* virucidal antiviral potential in *M. officinalis* in their experiment. Otherwise, in this study, *M. officinalis* showed low antiviral potency by decreasing HA titer to 2 $\log_2$ HA. This indicated that the samples could exhibit different potential antiviral activity between *in ovo* and *in vitro*. This result is thought to be possible due to the inhibition mechanism targeting the entry and envelope proteins [8]. A computational analysis conducted in a study has revealed the potential of both hesperidin, a flavonoid, and rosmarinic acid, derived from plant extracts, to inhibit SARS-CoV-2 [34]. Moreover, Al-Hatamleh et al. have demonstrated that hesperidin is the only natural compound capable of attaching to the receptor binding domain of the S protein, thereby neutralizing the binding between ACE2 receptor and spike-RBD [35].

In conclusion, studies have proven that traditional medicinal plant extracts and molecules display antiviral activities against numerous viruses. The antiviral activity against IBV and the cytotoxic effect on cancerous cell lines were analyzed for plant extracts and active ingredients in this study. Comparing cytotoxicity and antiviral results, it is shown that *L. nobilis* and *H. perforatum* have a dominant role in both analyses. Also, it is noteworthy that nobiletin and *S. nigra* do not exhibit cytotoxic activity on any cell but play a role in antiviral activity.

Declarations

Availability of Data and Materials: Materials and datasets provided from the study can be obtained from the corresponding author (F. O. Çöven) upon request.

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Author Contributions: All authors participaed to design the methodology, FOÇ, SG, KAHMA: writing the original draft, SG, EU and KAHMA: software and visualization, FK, FÇ and İÇ: data curation, FK, İÇ, AN: investigation and validation, FOÇ, FÇ, AN: reviewing and editing.

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