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CYTOTOXIC AND ANTIVIRAL ACTIVITY OF *RIBES UVA CRISPA* LINN. AND *RIBES MULTIFLORUM* KIT. EX ROMER AND SCHULTES EXTRACTS

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ABSTRACT: To find new antiviral agents from natural sources, methanol and aqueous extracts of leaves and fruits of *Ribes uva - crispa* and *Ribes multiflorum* naturally grown in Turkey were investigated as *in vitro* to reveal their antiviral activities against Herpes simplex virus type 1. All experiments were performed in 96-well plates and results were measured with XTT-based colorimetric assay. Results demonstrated that all extracts have no cytotoxic effect on vero cells at 10000 µg/mL, while they also exhibited anti-HSV-1 activity with different percentages of protection (varying between 2.65% - 50.40%) in 10000 µg/mL which was at the highest concentration in vero cells. 50% effective concentrations (EC_{50}) of the extracts which were determined having percentage of protection against HSV-1 at concentrations lower than 10000 µg/mL were calculated using GraphPad Prism Version 5.03 statistics program with non-linear regression analysis. These extracts were determined to have EC_{50} values ranging between 9710 - 70600 µg/mL and selectivity index (SI) are ranging between 0.14 - 1.03. On the basis of these results we believe that it would be worthwhile expanding these studies to include additional species of Turkish plants.

INTRODUCTION: Viral diseases have always been a major health problem in the world, and therefore people have tried to find new antiviral drugs [1]. Herpes labialis is one of the most common viral diseases, and its main etiological agent is Herpes simplex virus type 1 (HSV-1) belonging an enveloped DNA virus of Herpes viridae family [2]. The occurrence of latent infections due to HSV (Herpes simplex virus) infection in sensory ganglia is a major obstacle to its treatment [3].

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In view of the prevalence and importance of HSV-1 complications throughout the world, most of the antiviral agents were developed against Herpes viruses, but most of the antiviral agents developed specifically against Herpes viruses, especially nucleoside analogs, have serious side effects and cannot completely cure HSV-1 infections [4].

Following long-term use of nucleoside analogues, some resistant virus mutants have emerged. For this reason, it seems particularly important to include natural anti-HSV-1 agents, especially in natural resources [5]. Many of taxa that are used as berries are naturally grown in Turkey, these fruits are rich in Vitamins and minerals, also important in terms of human health, and their use in the food sector is also increasing [6].

One of the families with grape berries is Grossulariaceae, plants of this family especially spread in the northern temperate zone, and they spread on the Andes Mountains and south of Tierra Del Fuegoin the southern hemisphere. Grossulariaceae is restricted to the genus Ribes and contains more than 200 species [7]. Eight species of Ribes genus (*R. biebersteinii* Berl. ex DC., *R. nigrum* L., *R. uva-crispa* L., *R. alpinum* L., *R. orientale* Desf. and Schultes and *R. anatolica* Behçet) are naturally grown in Turkey, and one of them (*R. rubrum* L.) is a culture plant in the flora [8]. The biological activities of Ribes species (antimicrobial, antioxidant, antitumour, anti-hypertensive, anti-inflammatory activity studies) have been particularly studied in recent years [9 - 13]. Studies on the antiviral activities of Ribes species are rather inadequate and more focused on *Ribes nigrum*. This species, naturally growing in our country, has been also shown to have antiviral activity against Herpes simplex virus type 1 - 2, Varicella zoster virus and Influenza viruses [14 - 16].

In this study; with the increasing usage of *R. nigrum* species in the world, we aimed to evaluate antiherpetic activities from new *Ribes* species (*R. uva-crispa* and *R. multiflorum*) naturally grown in Turkey and contribute to the efforts of developing antiviral drugs.

MATERIALS AND METHODS:

Plant Material and Reagents: Fresh leaves and fruits of Ribes species used in this study were collected in the given localities;

***Ribes uva-crispa*: A4 Ankara:** Kızılcahamam, Sarayköy, on the way of Çukurören, 3th km, stony area, near the old stream, 1137 m, 15.05.2015-14.07.2015.

***Ribes multiflorum*: B3 Afyon:** Şuhut, Başören village south, on the way of Büyük Toklu Tepe (Kumalarmount) the village exit, the pathway around the road, 1400 m, 28.05.2015 - 12.08.2015.

They have been taxonomically identified by Prof. Dr. Muhittin DİNÇ and voucher samples were deposited in Kon Herbarium at Selcuk University, Faculty of Science, and Department of Biology.

Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), Dulbecco's Phosphate Buffered Saline (DPBS), Trypsin-EDTA solution and XTT Cell Proliferation Assay Kit were purchased from Biological Industries Israel Beit Haemek Ltd., (Kibbutz Beit Haemek 25115, Israel). Acyclovir (ACV), Trypan Blue and Antibiotic Antimycotic Solution were purchased from Sigma Chemical Co (USA). Vero cells (African gren monkey kidney cell, ATCC-CCL81), *Herpes* simplex virus type 1 (HSV-1) strain HF (ATCC VR-260) were purchased from American Type Culture Collection (ATCC), and they were reproduced and stored in Virology Laboratory in Selcuk University, Science Faculty, Biology Department.

Preparation of Plant Test Samples: For the preparation of methanol (ME) and aqueous (AE) extract of leaves and fruits of *Ribes* species, both fresh materials were pulled in a blender. Ten grams for each of respective samples (leaves and fruits for both species) were extracted in 250 mL methanol and bi distile sterile water, respectively by ultrasonication (Bandelin GM2070, Germany) at 100% power, 37°C, for 60 min. In order to protect heat-labile substances in the plant leaves and fruits, ultrasonication has been chosen as an extraction method as it as done at relatively low temperatures.

The remaining plant materials were separated from the extracts by filtration (Wattman No. 1). The solvent of each extract was evaporated by rotary evaporator (IKA RW10BT99, Germany), at room temperature for 40 to 45 min. The extracts were collected by distilled water to the vials and were frozen overnight at -80 °C. Finally they were freeze dried in lyophilizer (Labconco, USA), at -85 °C.

Each lyophilized extract (1000 mg) was dissolved in 10 mL of DMEM (Dulbecco's Modified Eagle Medium) (without serum), and 100 mg/mL stock solution were prepared. 15 mg ACV was dissolved in 10 mL of DMEM (without serum) and 1.5 mg/mL (1500 µg/mL) stock ACV were prepared. Stock solutions of the extracts and ACV were sterilized through 0.22 Millipore filter, each 1 mL of the extracts and ACV were aliquoted into 2 ml tubes, separately, and stored at +4 °C until use. Extract dilutions and ACV used in cytotoxicity and antiviral activity assay were prepared from this stock [17].

Cell and Virus: Vero cells were cultured with Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 units/mL penicillin G, 100 µg/mL streptomycin and 0.25 µg/mL amphotericin B. In the antiviral assay, the medium was supplemented with 2% FBS and the above-mentioned antibiotics. The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2. Vero cell was subcultured twice a week. The stock of Herpes simplex virus type 1 (HSV-1) was prepared from culture supernatant of HSV-1 infected vero cells. The virus titer of the supernatant was determined using the 50% tissue culture infective dose ($TCID_{50}$) method as described previously by Kaerber [18]. The virus stock was stored as aliquots at -80 °C until used.

Cellular Toxicity: The cellular toxicity of the extractson Vero cells were evaluated by means of the Cell Proliferation Kit (Biological Industries, Beit Haemek, Israel) in 96 well flat bottomed microtiter plates [19].

Briefly, each 8 well of first column of plates was used as medium control (MC) and filled with 150 µLDMEM (without serum). Each 8 well of second column of microplate was used as cell control (CC) and 100 µL DMEM (without serum) was put in to wells. All the wells (2, 4, 5, 6, 7, 8, 9, 10, 11 and 12) except the third column were filled with 100 µLDMEM (without serum). Extract solutions were prepared at a concentration of 15 mg/mL from the stock solutions of the extracts (100 mg/mL) (total 2 ml). 200 µL of the extract solution (15 mg/mL) was added to each of the 8 wells in the third column of the microplates. Then, 100 µL of the working solution in the wells from the 3rd column were taken and transferred to 4th column. 100 µL of the wells in the 4th well were transferred. Transportations (100 µL) were made to 12th column and two fold dilutions were obtained according to the log2 base (15, 7.5, 3.75, 1.875, 0.938, 0.469, 0.234, 0.117, 0.059, 0.029 mg/mL). Finally, 50 µL cells were seeded in to 96-well microtiter plates (5×10^3 cells/well) and plates were incubated for 72 h at 37 °C in a humidified incubator with 5% CO_2. Then, XTT reagent was applied to form a soluble dye. The mixture of 0.1 mL PMS (N-methyl dibenzopyrazine methyl sulfate) and 5 mg/5 mL XTT (sodium 3′-[1-(phenylaminocarbonyl)-3, 4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate) were added as 50 µL to each well, seperately. The plates were re-incubated for an additional 2 hours to allow the production of formazan. The optical densities were determined with the ELISA reader (Multiskan EX, Lab systems) at a test wavelength of 490 nm and a reference wavelength of 630 nm. The percentage of cytotoxicity was calculated as [(A - B) / A × 100], where A and B are the absorbance of cell control and treated cells, respectively.

The 50% cytotoxic concentration (CC_{50}) corresponded to the concentration required to kill 50% of the Vero cells for each extract was calculated by nonlinear regression analysis using GraphPad Prism software. Additionally the maximum non-cytotoxic concentrations (MNCCs) were determined as the maximal concentration of the extracts or ACV that did not exert a toxic effect in comparison with cell controls. Later, these values of the maximum non-toxic concentration determined were used in antiherpetic activity determination of the extracts using XTT assay [20-21, 19] (with minor modifications).

Acyclovir Toxicity: Each 8 well of first column of plates was used as medium control (MC) and filled with 150 µL DMEM (without serum). Each 8 well of second column of microplate was used as Cell control (CC) and 100 µL DMEM (without serum) was put in to wells. All the wells (2, 4, 5, 6, 7, 8, 9, 10, 11 and 12) except the third column were filled with 100 µL DMEM (without serum). 200 µL ACV from stock solution (1500 µg/mL) was added to each of the 8 wells in the third column. Then, 100 µL of the stock solution in the wells from the 3rd column were taken and transferred to 4th column. 100 µL of the wells in the 4th well were transferred. Transportations (100 µL) were made to 12th column and two fold dilutions were obtained according to the log2 base (1500, 750, 375, 187.50, 93.75, 46.88, 23.44, 11.72, 5.86, 2.93 µg/mL). 50 µL of the Vero cell suspension containing 1×10^5 cells per milliliter were put in to each wells of the columns up to 12, including second column (as cell control, CC). Microplates were incubated for 3 days at 37 °C in a humidified incubator with 5%

CO_2. Optical densities were measured by the XTT method as described in cell toxicity.

Antiviral Assay Using XTT Method: The antiviral activity of the extracts and ACV was evaluated by the XTT method as described previously [20, 22, 23] (with minor modifications). Vero cells, treated by trypsin-edta were seeded in to 96-well culture plates at a volume of 70 µL/well and a concentration of 1.43×10^5 cells/mL. After 24-hour incubation, 20 µL of virus solution, diluted with DMEM supplemented with 2% FBS, which was equivalent to 50% tissue culture inhibitory dose ($TCID_{50}$) was added to each well and the infected cells were incubated for another 2 hours. Dilutions at 10 × MNTC were prepared from the stock solutions of the extracts and ACV containing 2% FBS. Subsequently, serial two-fold dilutions were prepared using maintenance medium (DMEM with 2% FBS) from extracts and ACV solutions at 10 × MNTC. Two fold dilutions for 10 µL of these extracts or ACV from first to 8th column were done, and then added to culture wells in triplicate. After further incubation at 37 °C with 5% CO_2 for 72 hours, the mixture of 0.1 mL PMS (N-methyl dibenzopyrazine methyl sulfate) and 5 mg/5 mL XTT (sodium 3′-[1-(phenylaminocarbonyl)-3, 4-tetrazolium]- bis (4- methoxy- 6- nitro) benzene sulfonic acid hydrate) were added as 50 µL to each well, seperately. The plates were re-incubated for an additional 2 hours to allow the production of formazan. Optical densities were determined with the ELISA reader (Multiskan EX, Lab systems) at a test wavelength of 490 nm and a reference wavelength of 630 nm. The percentages of protection of the extracts and ACV were calculated spectrophotometrically as [(AB) / (C-B) × 100], where A, B and C indicate the absorbances of extracts (or ACV), virus and cell controls, respectively. The antiviral concentration of 50% effectiveness (EC_{50}) of the extracts which were determined having cytoprotection level against HSV-1 infection at 10000 µg/mL and lower concentrations were calculated using Graph Pad Prism statistics program with non-linear regression analysis.

RESULTS AND DISCUSSION: Methanol and aqueous extracts prepared from fresh leaves and fruits of *R. uva-crispa* and *R. multiflorum* were found to have no toxic effect on Vero cells at the highest tested concentrations (10000 µg / mL) **Table 1**, and this concentration was accepted as the CC_{50} value for the extracts [24]. In the study, in order to determine MNTC and CC_{50} values of ACV used as a positive control against HSV-1, MNCC were found as 250 µg/mL and CC_{50} were determined as 2839 µg/mL **Table 1**.

TABLE 1: THE CYTOTOXICITY AND ANTI-HSV-1 ACTIVITY RESULTS OF THE EXTRACTS OF *R. UVA-CRISPA* AND *R. MULTIFLORUM*

Plants	Extracts	Toxicity		Anti-HSV-1 activity	
		MNCC (µg/ml)	CC_{50} (µg/ml)	EC_{50} (µg/ml)	Selectivity Index (SI)
R. uva-crispa	LME	10000	10000	12740	0.78
	LAE	10000	10000	NE	NE
	FME	10000	10000	21650	0.46
	FAE	10000	10000	NE	NE
R. multiflorum	LME	10000	10000	9710	1.03
	LAE	10000	10000	NE	NE
	FME	10000	10000	17560	0.57
	FAE	10000	10000	70600	0.14
ACV		250	2839	1.564	1815.22

LME: Leaf methanol extract; LAE: Leaf aqueous extract; FME: Fruit methanol extract;
FWE: Fruit aqueous extract; NE: Not Evaluated; MNCC: Maximum non-cytotoxic concentration;
CC_{50}: Concentration that showed 50% cell cytotoxic effect against Vero cells; EC_{50}:
Concentration that inhibited 50% virus infection; SI: the ratio of CC_{50} to EC_{50}.

The titer of HSV-1 used in the experiments were determined by the method of 50% tissue culture infective dose ($TCID_{50}$) as $TCID_{50} = 10^{-4.5}/0.1$ mL (Kaerber, 1964). % protection values obtained in triplicates to determine the EC_{50} (concentration providing protection in 50% of infected cells) value of ACV used as a positive control for HSV-1 inhibition is given in **Table 2** and EC_{50} value in **Table 1**. The EC_{50} value of ACV was determined to be 1.564 µg/mL. The SI, defined as the ratio of CC_{50} to EC_{50}, was determined as 1815.22 **Table 1**.

As a result of the antiviral activity tests, it was determined that all of the extracts at the highest concentration (10000 µg/mL) have lower % protection values against HSV-1 **Table 2**.

TABLE 2: % PROTECTION RATES OF METHANOL AND AQUEOUS EXTRACTS PREPARED FROM LEAVES AND FRUITS OF *RIBES UVA-CRISPA* AND *RIBESMULTIFLORUM* WERE DETERMINED BY XTT TEST AGAINST HSV-1

	% protection of *R. uva-crispa*				% protection of *R. multiflorum*			
Concentration (µg/Ml)	**LME**	**LAE**	**FME**	**FAE**	**LME**	**LAE**	**FME**	**FAE**
10000.0	34.56	12.83	10.40	7.26	50.40	7.26	8.71	14.81
5000.0	8.46	0.00	1.73	0.00	25.60	0.00	0.53	11.38
2500.0	0.00	0.00	0.00	0.00	6.93	0.00	0.00	6.08
1250.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.65
625.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
312.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
156.3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
78.1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
39.1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

For the calculation of the EC_{50} values of the extracts which were found to have antiviral activity at concentrations of 10000 µg/mL and lower, % protection rates were utilized against extract concentrations. On the other hand, EC_{50} values were not determined for the exctract which only had antiviral activity at a concentration of 10000 µg/mL. EC_{50} values of leaf methanol extract and fruit methanol extract of *R. uva-crispa* were determined to be 12740 µg/mL and 21650 µg/mL, respectively **Table 1**, **Fig. 1** and **2**, while EC_{50} values of the leaf methanol extract, fruit methanol extract and fruit aqueous extract of *R. multiflorum* were determined to be 9710 µg/mL, 17560 µg/mL and 70600 µg/mL, respectively **Table 1**, **Fig. 3**, **4** and **5**.

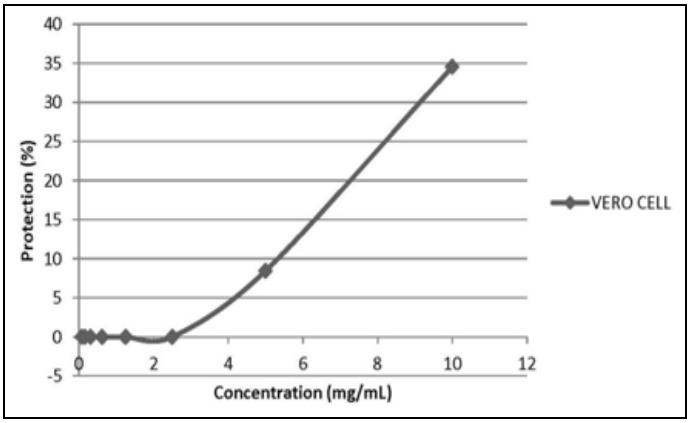

FIG. 1: THE ANTI-HSV-1 ACTIVITY OF LEAF METHANOL EXTRACT OF *R. UVA-CRISPA* (EC_{50}: 12.74 mg/ml, SI: 0.78)

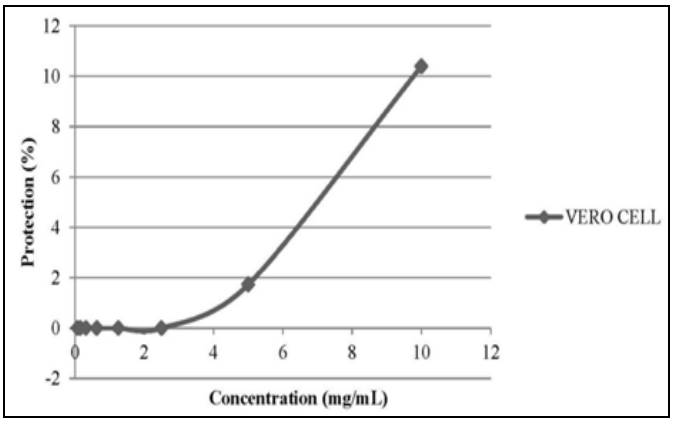

FIG. 2: THE ANTI-HSV-1 ACTIVITY OF FRUIT METHANOL EXTRACT OF *R. UVA-CRISPA* (EC_{50}: 21.65 mg/ml, SI: 0.46)

The SI (CC_{50} / EC_{50}) values of the extracts are; *R. uva-crispa* leaf methanol extract and fruit methanol extract were determined to be 0.78 and 0.46, respectively **Table 1**, **Fig. 1** and **2**, while leaf methanol extract, fruit methanol extract and fruit aqueous extract of *R. multiflorum* were determined as 1.03, 0.57 and 0.14, respectively **Table 1**, **Fig. 3**, **4** and **5**. As seen in **Table 2**, all of the plant species extracts were found to have % protection ranging from 2.58 - 50.40 against HSV-1 at 10000 µg/mL (the highest concentration in the test). Extracts with % protection being 10000 µg/mL and lower concentrations were found to have EC_{50} values ranging from 9710 to 70600 µg/mL and low SI values (ranging from 0.14 - 1.03) **Table 1**.

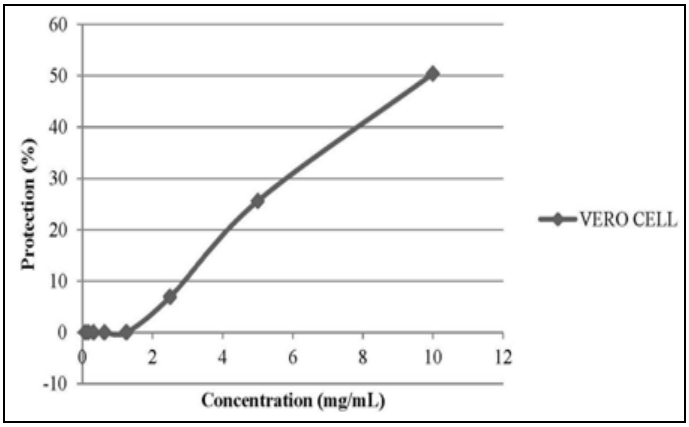

FIG. 3: ANTI-HSV-1 ACTIVITY OF LEAF METHANOL EXTRACT OF *R. MULTIFLORUM* (EC_{50}: 9.71 mg/ml, SI: 1.03)

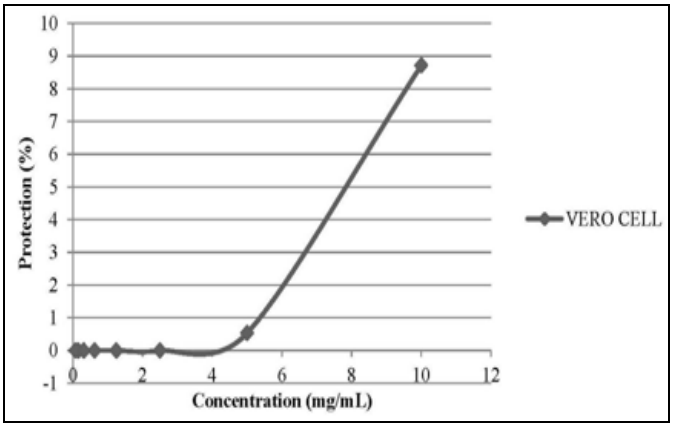

FIG. 4: ANTI-HSV-1 ACTIVITY OF FRUIT METHANOL EXTRACT OF *R. MULTIFLORUM* (EC_{50}: 17.56 mg/ml, SI: 0.57)

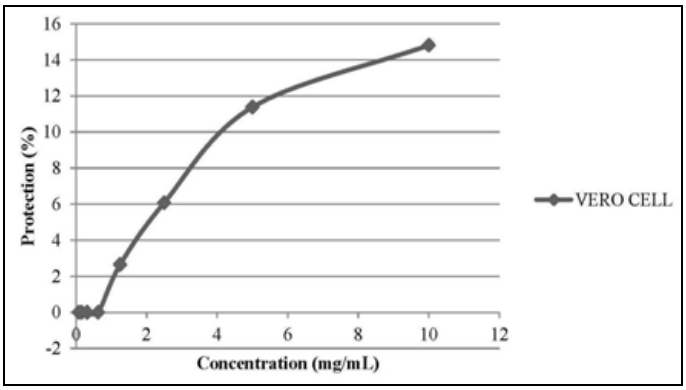

FIG. 5: ANTI-HSV-1 ACTIVITY OF FRUIT AQUEOUS EXTRACT OF *R. MULTIFLORUM* (EC_{50}: 70.60 mg/ml, SI: 0.14)

Chattopadhyay *et al.*, [25] reported that SI values, which is bigger than 3, should be regarded as indicative of potentially reliable antiviral activity for test extracts; therefore, it can be said that extracts may have weak antiviral activity. Studies on the antiviral activities of *Ribes* species have been rather inadequate and focused more on *Ribes nigrum* [14 - 16, 26, 28,]. The LADANIA067 application, obtained from the leaves of wild blackcurrant (*Ribes nigrum folium*), caused a reduction on the progeny virus titers in the cell cultures infected by prototype Avian influenza virus titers and Human influenza virus strains occurred from different subspecies. The extract at the effective dose of 100µg/mL did not show any significant deleterious effects on cell viability, metabolism or proliferation. In addition, viruses did not tend to develop resistance to LADANIA067 when compared to amantadine, which resulted in the formation of resistant variants only after a few passages. The application of LADANIA067 on the mouse infection model reduced the virus titres in the lung by intranasal application [16].

Inhibitory effects of an extract obtained from blackcurrant (*Ribes nigrum*), were investigated for pathogens that are Respiratory syncytial virus (RSV), Influenza virus A and B (IFV-A and IFV-B), Adeno virus (AdV), HSV-1, Haemophilus influenzae Type B, *Streptococcus pneumoniae* and *Streptococcus mutans* associated caused oral, nasopharyngeal, and upper respiratory tract infectious diseases. Concentrations lower than 1% of the black currant extract inhibited the replication of RSV, IFV-A and B, HSV-1 by more than 50%, and an extract of 10% inhibited the adsorption of these viruses to the cell surface by more than 95% [26]. Inhibitory effects of the fractions of anthocyanins, which are compounds having antiviral activity, from *Ribes nigrum* fruit extract "called kurocarin" were investigated against Influenza virus tip A and Influenza virus tip B, and the mechanism of this antiviral effect was examined.

The anthocyanin fraction was divided into 7 fractions A'-G '. D'-G 'fractions showed strong antiviral activity against both viruses. Fraction E'

were identified as 3-*O*-α-L- ramnopyranosyl- β- D-glucopyranosyl-cyanide and 3-*O*-β-D-glucopyrano syl-cyanide, and Fraction F' were identified as 3-*O*-α-L-rhamnopyranosyl - β - D- glucopyranosyl-delfidine and 3- *O*- β- D-glucopyranosyl-delfidine by HPLC and high resolution mass spectrometry. It was determined that F' fraction did not directly inactivate viruses but can inhibit adsorption of viruses to the cells and virus propogation from infected cells [27].

Antiviral activity of *Ribes nigrum* raw fruit extract against Influenza virus type A and Influenza virus type B viruses was investigated. It has been reported that 3.2 g/mL extract is required to inhibit 50% plaque formation. It was determined that 10 g/mL extract directly inactivated both types of virus 99% at pH 2.8 and 95 - 98% at pH 7.2. It was observed that after 6 hours of treatment with 10 and 100 g/mL extracts, the infection caused by Influenza virus type A was completely stopped. After 1 hour of the treatment with the 100 g/mL Kurocarin extract, the infection of the virus titers in the culture media of the cells was stopped in 8 to 9 hours, and extract was presented to inhibit virus propogation in infected cells [14].

The antiviral activity of the methanol extract of 100 medicinal plants used in the province of British Columbia, Canada in the light of ethnobotanical and pharmacological activity information, was examined against seven different viruses (Bovinecorona virus, Bovineherpes virus type 1, Bovinepara influenza virus type 3, Bovinerota virus, Bovine respiratory syncytial virus, Vaccinia virus, Vesicular stomatitis virus). Any antiviral activity against Bovineherpes virus type 1 was found in the methanol extract of *Ribes sanguineum* twigs, one of the plant species used in the research [28].

The antiherpes virus activity of *Ribes nigrum* fruit extract known as Kurocarin in Japan has been investigated as *in vitro*. The extract completely inhibited Herpes simplex virus type 1 (HSV-1) bound to the cell membrane at 100-fold dilution. In addition, it inhibited at the the rate of 50% or at a lower concentration to HSV-1 and *Herpes simplex* virus 2 (HSV-2) viruses in the plaque form, Varicellazoster virus at 400 fold dilution. Finally, it has been observed that it inhibits virus replication in cells as it slows down protein synthesis in infected cells in the early stages of infection [15].

In this study, the results of methanol and aqueous extracts obtained from leaves and fruits of *R. uva-crispa* and *R. multiflorum* were different weak anti-HSV-1 activity than those of Herpesvirus studies [15, 26]. Suzutani *et al*., [15] noted that phenolic compounds (such as anthocyanins) found in the extracts of *Ribes* species may be a key factor in the antiherpesvirus activity of these extracts. The total phenolic contents of methanol and aqueous extracts prepared from the leaves of *R. uva-crispa* were 273.13, 341.25 mg/g, respectively, while total phenolic contents of the methanol and aqueous extracts prepared from the leaves of *R. multiflorum* were 368.44, 490.63 mg/g, respectively from a study [29].

Although the species in our research have quantities that are not underestimated in terms of their total phenolic contents by Kendir and Köroğlu [29], comparing to the results of Suzutani *et al*., [15] they have poor anti-HSV-1 activity, therefore other chemical components found in these species may be responsible for the low anti-HSV-1 activity of our studied *Ribes* species. Because; *Ribes* species include many active components which may be responsible for anti-herpes virus activity, such as flavonoids [30 - 32], tannins [33], biphenyls [34], nitryl-containing compounds [31], polyunsaturated fatty acids [35] and aromatic compounds (including terpenes, esters and alcohols) [36].

CONCLUSION: Methanol and aqueous extracts prepared from fresh leaves and fruits of *R. uva-crispa* and *R. multiflorum* were found to have anti HSV-1activity. These results are very important for natural sources and they may be used for further antiviral studies. When people consume *Ribes* species in their daily diet, they can get natural antiviral immunity. We also believe that future in vivo studies may be useful in the investigation of a new generation of drugs.

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CONFLICT OF INTEREST: There is no conflict of interest.

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