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# ANTIVIRAL ACTIVITY OF *SPARTIUM JUNCEUM* AGAINST HERPES SIMPLEX VIRUS TYPE 1: AN *IN-VITRO* STUDY

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**Keywords:**

Anti HSV-1 activity, Cytotoxic concentration, Cytopathic effect, *Spartium junceum*

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**ABSTRACT:** This study was carried out to evaluate the antiviral activities of methanol and aqueous extracts prepared from the flowers and stems of *Spartium junceum* L., used as herbal medicine in the treatment of gastric ulcer in Turkish folk medicine, against herpes simplex virus type 1 (HSV-1). The maximum non-toxic concentration of the extracts (MNTC) was determined against Vero cells by using the serial two-fold dilution of MNTC (specific for each extract). The cytotoxic activity of the extracts and their ability to inhibit the cytopathic effect (CPE) caused by the virus in tissue culture was evaluated by colorimetric XTT assay after three days of inoculation and incubation. 50% cytotoxic concentration ($CC_{50}$) and 50% effective concentration ($EC_{50}$) were determined using graph pad prism, and the selectivity index (SI) was calculated from the $EC_{50}$ ratio of $CC_{50}$. As a result of the research, the strongest antiviral activity against HSV-1 was determined from flower aqueous extract with 1290.88 µg/mL $EC_{50}$ and 24.09 SI values, and this was followed by stem aqueous extract ($EC_{50}$ = 1217.52 µg/mL, SI = 12.22), flower methanol ($EC_{50}$ = 1673.41 µg/mL, SI = 6.74) and stem methanol extracts ($EC_{50}$ = 795.31 µg/mL, SI = 4.86). As a result, we can say that *S. junceum* extracts are worthy of further study to develop as an alternative to the drugs used clinically against HSV-1. This is the first report on *S. junceum* for anti-HSV-1 activity.

**INTRODUCTION:** Herpes simplex virus type 1 (HSV-1) in the herpesviridae family is an enveloped double-stranded DNA virus [1]. HSV-1 infections are common and contagious. Approximately 60% to 90% of the adult world population is seropositive for HSV-1 [2].

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Serious complications such as facial ulceration, herpes keratitis, meningoencephalitis, and encephalitis constitute the clinical manifestations due to HSV-1 infection [3-4]. The treatment of HSV-1 infections requires the use of viral DNA replication inhibitors, such as acyclic guanosine analogs, acyclic nucleotide analogs, and pyrophosphate analogs.

For over 30 years, acyclovir and its derivatives have been the important material in the treatment of HSV infections. However, long-term treatment with these antivirals leads to the emergence of resistance [5-6].

Due to the increased incidence of resistance commonly seen among immunocompromised patients, new antiherpetic drugs with different mechanisms of action and low toxicity need to be developed against HSV resistant to drugs [6]. *Spartium junceum* L., in the family of Leguminosae (Fabaceae), is a shrub species native to the Mediterranean region [7]. This plant, which is a typical Mediterranean element, is distributed throughout the world in Southern Europe and North Africa. In Turkey, it grows abundantly in the shrubbery which is close to the sea and roadside, mainly in North, West and Southern Anatolia [8]. It is known as "Katırtırnağı" or "AdiKatırtırnağı" among the people [9]. It has been reported that *S. junceum* flowers exhibit moderate sedative and diuretic activities [10], antiulcer [11-12], anti-inflammatory, analgesic [13] and antitumor [14] activities. The antimicrobial, antioxidant and cytotoxic activities of aromatic water obtained from *S. junceum* flowers were investigated, and it was determined that aromatic water had a significant cytotoxic and antioxidant activity [15]. Researchers have shown that this plant contains high amounts of quinolizidine alkaloids [16-18], flavonoids (flavonols, isoflavones, flavones) [19], triterpenoid saponin [20] and proanthocyanidin [21], including sparteine showing interesting biological activities.

Proanthocyanidins [22-23] and triterpenoid saponins [24-25] have been reported to have anti-herpes virus activity. Flavonoid glucoside (luteolin 4'-beta-glucoside) was also isolated from *S. junceum*[20–21]. Some studies have shown that luteolin, luteolin glycosides or luteolin-containing plants have antiviral activity [26-28].

In this study, taking into consideration the various biological activities of the compounds responsible for the antiherpetic activity of *S. junceum* used in the treatment of gastric ulcers in Turkish traditional medicine [29], crude extracts of the plant prepared from flowers and stems were tested against HSV-1 to find new and reliable antiviral agents.

## MATERIALS AND METHODS:

**Plant Material and Reagents:** The samples of *S. junceum* were collected from the 4$^{th}$ km of Soğukpınar-Uludağ road, Bursa, Turkey in June-August 2018, especially in the months of the flowering of plant species. Prof. Dr. Hüseyin DURAL identified them at Selcuk University, Science Faculty, Biology Department, Konya, Turkey. A voucher sample was deposited in the Kon Herbarium. The flowers and body parts of *S. junceum* were dried in the shade, fine powdered in a mill and stored in sterile black glass bottles at room temperature.

Eagle's Minimum Essential Medium (EMEM), 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).

**Preparation of Plant Test Samples:** For the preparation of methanol (ME) and aqueous (AE) extract of flowers and stems of *S. junceum*, twenty grams for each of respective samples (flowers and stems) were used. They were extracted in 250 mL methanol and sterile bi-distilled water, respectively by ultrasonication (Bandelin GM2070, Germany) at 100% power, 37 °C, for 60 min. To protect heat-labile substances in the plant flowers and stems, 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 (Whatman no. 1). The solvent of each extract was evaporated by a 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 EMEM (without serum), and 100 mg/mL stock solution was prepared. 3 mg ACV was dissolved in 3 mL of EMEM (without serum) and 1 mg/mL (1000 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 these stocks [30].

**Cell Line and Virus:** Vero cell line (African green monkey kidney cell line, ATCC-CCL81) was used as a suitable cell line for HSV-1 HF strain (ATCC VR-260) proliferation and antiviral experiments. Cells were produced and reproduced in the presence of 5% $CO_2$ at 37 ºC using Eagle's Minimum Essential Medium (EMEM) with 10% fetal bovine serum (FBS). Serum concentration was reduced to 2% during virus replication and antiviral tests. The titer of the propagated viral stock was determined using the 50% tissue culture infective dose ($TCID_{50}$) method as described previously by Kaerber [31]. The virus stock was stored as aliquots at –80 °C until used.

**Cytotoxicity Assay:** The cytotoxic effects on Vero cells for ACV (acyclovir), used as positive controls for HSV-1, as well as methanol and aqueous extracts from the flowers and stems of *S. junceum* was investigated on the XTT based cell proliferation kit according to the manufacturer's instructions (Biological Industries, Israel).In summary, the first column of a 96-well microplate was used as medium control (MC) and the second column as cell control (CC). 150 µL EMEM (serum-free) were seeded to each of the 8 wells in the 1st column used as MC. 100 µL EMEM (serum-free) was added to each of the 10 columns (*i.e.*, 2, 4, 5, 6, 7, 8, 9, 10, 11 and 12) except for the third column. A working solution of 75 mg/mL (75000 µg/mL) was prepared from stock solution of extracts (100 mg/mL) using serum-free EMEM.

200 µL of the working solution of the extracts (75000 µg/mL) were seeded into each of the 5 wells in the 3rd column. 100 µL working solution from each 5 well of the 3rd column was transferred to a 4th column and serial dilution based $log_2$ were done from 3rd to 12th column (75000.000, 37500.000, 18750.000, 9375.000, 4687.500, 2343.750, 1171.875, 585.938, 292.969, 146.484 µg/mL). Cytotoxicity tests with tetrazolium salts [XTT, MTT (3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium-bromide)] may give false negative results because of the extracts can reduce the tetrazolium salts in the absence of living cells, it is also indicated that the tested compounds (or extracts) can interact with the tetrazolium salts [32]. Also, it was found that different antioxidant molecules containing free thiol groups could reduce the tetrazolium salts.

Furthermore, it has been stated that various plant extracts containing compounds such as polyphenols (resveratrol: an antioxidant substance contained in wine) and flavonoids (quercetin, luteolin, kaempferol) are strongly reduced tetrazolium salts (MTT or XTT) in the absence of living cells [33-38]. For these reasons, while evaluating the cytotoxic effects of extracts on living cells, it has been reported that these interactions should be ruled out by controlling the direct chemical interactions of extracts with XTT dye [38]. Taking into account these reported warnings, to check whether the extracts entered into chemical interactions directly with XTT or not, 50 µL of EMEM (without serum) was dispensed on to5-8 wells in 3-12 columns (these wells containing only extracts, no cells).

Each 50 µL Vero cells suspensions containing $1 \times 10^5$ in per mL were dispensed on to 8 wells of the second column (CC) and the line of A, B, C, D wells in 3-12 columns (5000 wells per well). Thus, the final concentrations of the extracts in the wells were 50000.000, 25000.000, 12500.000, 6250.000, 3125.000, 1562.500, 781.250, 390.625, 195.313, 97.656 µg/mL. To determine whether ACV has a toxic effect or not on Vero cells, the procedure described below is applied: First, a working solution of 750 µg/mL concentration was prepared using serum-free EMEM from a stock solution of ACV (1000 µg/mL). 200 µL of the working solution for ACV (750 µg/mL) was dispensed onto each of the 8 wells in the 3rd column. 100 µL working solution from each 8 well of the 3rd column was transferred to a 4th column and serial dilution based $log_2$ were done from 3rd to 12th column (750.00, 375.00, 187.50, 93.75, 46.88, 23.44, 11.72, 5.86, 2.93, 1.46 µg/mL). 50 µg/mL of Vero cell suspension containing $1 \times 10^5$ cells per milliliter (5000 cells per well) was put into each of the wells in 2-12th columns.

Thus, the final concentrations of ACV in the wells were 500.00, 250.00, 125.00, 62.50, 31.25, 15.63, 7.81, 3.91, 1.95, 0.98 µg/mL. After microplates containing plant extracts and ACV were incubated at 37 °C in a 5% $CO_2$ humidified incubator for 3 days, 50 µL of a suspension from a mixture of 5 mL commercially available XTT reagent and 0.1 mL PMS (N-methyl dibenzopyrazine methyl sulfate) were dispensed into each well of the microplate.

The microplates were gently shaken to disperse the dye into the wells uniformly. The microplates were incubated for a further 3 h to form the XTT formazan product. Optical densities (ODs) were recorded in an ELISA reader (Multiskan EX, Labsystems) at a reference wavelength of 490 nm and a reference wavelength of 630 nm to obtain ODs averages from the wells. To rule out the direct chemical interactions of the extracts with XTT, the mean ODs values of the different extract concentrations in the cell-containing wells were removed from the mean ODs values of the extracts at the same concentration without cells.

The tests were performed in triplicate, and the results were shown as mean cytotoxicity ratio according to CC. The percentage of cytotoxicity was calculated as [(A-B)/A × 100], where A and B are the absorbances of control and treated cells, respectively [39]. The calculated cytotoxic effect percentages were plotted against corresponding concentrations of the tested extracts (or ACV). 50% Cytotoxic Concentration ($CC_{50}$) values, defined as the concentration reducing the ODs of the cells treated with extracts (or ACV) by up to 50% compared to CC, were determined by using GraphPad Prism Version 5.03 statistical software with non-linear regression analysis [40]. Maximum non-toxic concentration (MNTC) of the extracts (or ACV) were determined by comparing the CC to ODs. These MNTC were used to determine the antiviral activity of extracts and ACV.

**Antiviral Activity Assay:** The dilutions of 10 times more concentrated than the MNTC determined of the extracts and ACV against the Vero cells were prepared. Then starting from these concentrations, the dilutions prepared according to the log2 base were checked for the antiviral activity against HSV-1 which was diluted 100 $DCID_{50}$ by the XTT method [41]. Suspensions of trypsin-treated Vero cells were prepared at a concentration of $1 \times 10^5$ cells/mL using 2% FBS-containing EMEM (maintenance medium). From these prepared cell suspensions, 70 µL (7000 cells/well) were seeded on to 96-well culture plates (except 8-well plates used as MC). 100 µL maintenance medium was dispensed on to 8 well used as MC. After incubation at 37 ºC in 5% $CO_2$ for 4 h; each 20 µL of HSV-1 suspensions which was diluted as 100 $DCID_{50}$/0.1 mL by using maintenance medium were put on to per wells (except the first column used as MC and second column used CC). 8 wells in the 3rd column of the microplate were used as Virus Control (VC). Each 20 µL maintenance medium were put on to 8 wells in the 2nd column of the microplate used for CC, and the plate was incubated for a further 2 h. Dilutions containing 2% FBS at 10 × MNTC were prepared from stock solutions of the extracts (100 mg/mL).

Subsequently, 2-fold dilutions [(10×MNTC)/2, (10×MNTC)/4, (10×MNTC)/8, (10×MNTC)/16, (10×MNTC)/32, (10×MNTC)/64, (10×MNTC)/128, (10×MNTC)/256] were prepared using the maintenance medium from the extract solutions in 10 × MNTC. After two hours of incubation, each 10 µL extract solutions prepared at 10×MNTC were put into per 8 wells of the 4th column. Each 10 µL of the extract concentrations (10×MNTC)/2, (10×MNTC)/4, (10×MNTC)/8, (10×MNTC)/16, (10×MNTC)/32, (10×MNTC)/64, (10×MNTC)/128, (10×MNTC)/256 were put on to the remaining 8 columns of the microplates (*i.e.*, wells on columns 5, 6, 7, 8, 9, 10, 11 and 12). Each 10 µL maintenance media were put on to wells of the column used as CC and VC. The same procedures were applied to ACV using another microplate. A dilution including 2% FBS at 10×MNTC was prepared from a stock solution of ACV (1000 µg/mL). Subsequently, 2-fold dilutions [(10×MNTC)/2, (10×MNTC)/4, (10×MNTC)/8, (10×MNTC)/16, (10×MNTC)/32, (10×MNTC)/64, (10×MNTC)/128, (10×MNTC)/256] were prepared using the maintenance medium from the ACV solution at 10 × MNTC.

Next, the steps in determining the antiviral activity of the extracts were followed. The plates were incubated at 37 ºC in 5% $CO_2$ for 3 days. After incubation, 50 µL of a suspension from a mixture of 5 mL commercially available XTT reagent and 0.1 mL PMS were dispensed on to each well of the microplate. Plates were incubated for a further 2 h to form the XTT formazan product. ODs were recorded in an ELISA reader (Multiskan EX, Labsystems) at 490 nm test wavelength and 630 nm reference wavelengths, and ODs averages from 8 wells were recorded. Percentage protection rates of different extract (or ACV) concentrations against virus 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 [39]. $EC_{50}$ values, defined as an extract or ACV concentration which protects 50% of infected cells, were determined by using GraphPad Prism Version 5.03 statistical software with non-linear regression analysis. The selectivity index (SI) of the extracts and ACV was calculated from the $CC_{50}/EC_{50}$ ratio. Experiments were done triplicates.

**RESULTS AND DISCUSSION:** The limited efficacy of the current treatment of HSV-1 infection increases the need for specific therapies including drugs with novel viral targets and mechanisms of action. Since approximately 40% of modern drugs are derived from natural sources [6], this study has been carried out to determine the natural source of compounds that can potentially be included in a formulation to be used for treatment against HSV-1. The cytotoxic and antiviral activities of the methanol and aqueous extracts from the flowers and stems of *S. junceum*, which was used as herbal medicine in the treatment of stomach ulcers in Turkish folk medicine, were evaluated by colorimetric XTT test. The results of the cytotoxic effects and antiviral activities for the extracts and ACV are given in **Table 1**.

As shown in **Table 1**, extracts with $CC_{50}$ values in the range of 3862.03-31095.09 42 µg/mL were found to be non-toxic to Vero cells according to Rukunga and Simon's criteria [42].

According to the Rukunga and Simons classification [42]; Extracts having a $CC_{50}$ value of less than 2 µg/mL are cytotoxic, extracts having a $CC_{50}$ value between 2-89 µg/mL are partially (moderate) cytotoxic, and extracts having a $CC_{50}$ of more than 90 µg/mL are nontoxic. Cerchiara *et al*., [15] determined chemical composition of aromatic water obtained from *S. junceum* flowers by using real samples of individual analytes as reference with Solid Phase Micro Extraction / Gas Chromatography / Mass Spectrometry (SPME/GC/ MS), and they performed quantitative measurements of some components by High Performance Liquid Chromatography/Diode Array Detector (HPLC/DAD) method. They were tested the cytotoxic activities of the essential components of aromatic water [(nerolidol, 1-octen-3-ol, farnesene, linalool, lynalyl acetate, and 2,4-bis (1,1-dy-methylethyl) phenol) on melanoma RPMI 7932 and normal keratinocyte NCTC 2544 cell lines by trypan blue dye method. As a result of the test, it was found that the basic components of *S. junceum* aromatic water were not cytotoxic to the normal NCTC 2544 cells used as controls, whereas it was showed a significant growth inhibitory effect on the RPMI 7932. In this study, although plant extracts were used as material, to be similar to the findings of Cerchiara *et al.*, [15] we determined that methanol and aqueous extracts prepared from *S. junceum*'s flowers and stems have no cytotoxic effect on normal Vero cells.

**TABLE 1: TOTAL RESULTS OF CYTOTOXICITY AND ANTIVIRAL ACTIVITY ASSAYS FOR METHANOL AND AQUEOUS EXTRACTS PREPARED FROM *S. JUNCEUM*'S FLOWERS AND STEMS, AND ACV[a]**

| Plant name | Used part | Extract type | Toxicity | | Antiviral activity | |
|---|---|---|---|---|---|---|
| | | | MNTC (µg/mL) | $CC_{50}$ (µg/mL) | $EC_{50}$ (µg/mL) | SI |
| *Spartium junceum* | Flower | Methanol | 3125.000 | 11285.26 | 1673.41 | 6.74 |
| | | Aqueous | 781.250 | 31095.89 | 1290.88 | 24.09 |
| | Stem | Methanol | 781.250 | 3862.03 | 795.31 | 4.86 |
| | | Aqueous | 781.250 | 14875.68 | 1217.52 | 12.22 |
| Acyclovir (ACV) | | | 62.500 | 3766.02 | 0.034 | 110.77 |

[a]Cytotoxicity and antiviral activity were measured by XTT test; MNTC: Maximum non-toxic concentration; $CC_{50}$: 50% cytotoxic concentration; $EC_{50}$: 50% Effective Concentration; Sample concentration required to inhibit up to 50% of the CPE induced by the virus; SI (Selective index): $CC_{50}/EC_{50}$

According to the antiviral activity of the extracts, it has been found that all extracts showed different degrees of anti-HSV-1 activity. Chattopadhyay *et al*., [43] reported that an SI value of 10 or greater should be generally considered as an indicator of positive antiviral activity, although other factors were taken into account such as a positive control (such as ACV) as a low SI value. According to these criteria, the strongest antiviral activity against HSV-1 were obtained from flower aqueous extract with $EC_{50}$ =1290.88 µg/mL and SI = 24.09, this was followed by stem aqueous extract ($EC_{50}$ = 1217.52 µg/mL, SI = 12.22), flower methanol extract ($EC_{50}$ = 1673.41 µg/mL, SI = 6.74) and stem methanol extract ($EC_{50}$= 795.31 µg/mL, SI= 4.86) **Table 1**. The $EC_{50}$ and SI values of ACV,

used as a standard drug in the clinical treatment of HSV infections, were 0.034 µg/mL and 110.77, respectively **Table 1**.

The alkaloid pattern of different parts of *S. junceum* (flower, bud, leaf, and branch) was investigated. Cyticin, N-methyl cytosine, anagirin, rhydropholine and epi-baptifolin have been identified as major compounds in various parts of the plant. Also, traces of spartein were found in the buds of the plant [44]. Greinwald *et al*., [44] reported that there were significant differences in the amount of alkaloid between the different parts of the plant, the alkaloid content varied depending on the seasons, while there was a limited variation in the amount of alkaloid according to geographical origin. In the various parts of *S. junceum*, especially the flowers, quinolinidine alkaloids (cyticin, N-methylcithin, anagirin) were also isolated [16-18, 45]. Some other alkaloids, especially quinolizide alkaloids, were reported to show antiviral activity against herpes viruses [46-47]. Chrysin, chrysin 7-glucoside and chrysin 7-gentiobioside [19] from flower, luteolin and genistein [19] from aerial parts along with some flavonoids including apigenin- 7-O-β-D- glucopyranoside, genistein-8-C-β-D-gluco-pyranoside, 5, 8-dihydroxy-4-methoxy- 6, 7-methylenedioxyiso-flavo-ne, carth-amidin-7-O-α-Lrhamnopyranoside, 3β, 16β, 22β, 24-tetra-hydroxyolean-1-ene-3-β-yl-[β-D-glucopyranosyl- (1 → 2)] - α-L-rhamnopy-ranoside (junceoside) [20] were determined from *S. junceum*.

It was determined that *S. junceum* contains flavonoid glucoside (luteolin 4'-beta-glucoside) [20-21] and proanthocyanidin [21]. It has been reported that flavonoids, especially luteoline and luteolin glycosides, have antiviral effects against various viruses including herpes simplex virus, dengue virus, hepatitis B virus, human cytomegalovirus, respiratory syncytial virus, parainfluenza virus and adenovirus [26-28, 41, 48-52]. A proanthocyanidin-enriched extract prepared from *Myrothamnus flabellifolia* Welw. showed antiviral activity against HSV-1 by inhibiting viral adsorption and penetration [23], oligomeric proanthocyanidins isolated from *Rumex acetosa* L. have been determined to inhibit HSV-1 binding to cells [22]. Blia *et al*., [20] determined that the aerial parts of *S. junceum* contain triterpenoid saponin (junceoside).

Yeşilada and Takaishi [12] isolated a new oleanane type saponin having strong anti-ulcerogenic activity from the flowers of *S. junceum*. In previous studies, triterpenoid saponins have been reported to have anti-herpes virus activity [24-25]. GC and GC/MS investigated the volatile oil isolated by hydrodistillation method from fresh flowers of S. junceum. 24 main components were identified from the samples; the main components of the oil were found to be cairomonal compounds [tricosan (22.9%), tetracosan (8.9%) and pentacosan (16.1%)] which accounted for about 48% of the total composition [53].

The volatile oil isolated by hydro-distillation method from the fresh flowers of *S. junceum* collected from the medicinal plant garden of the Faculty of Pharmacy in the city of Siraz, near the city of Siraz in Persian province in Iran, was investigated by gas chromatography-Mass spectrophotometer (GC-MS) method. GC-MS analysis of the essential oil revealed the presence of 30 components, mainly linalool (26.18%), tetradecanoic acid (22.83%), camphor (13.50%) and lauric acid (13.09%), which accounted for about 75.60% of the total composition. Ghasemi *et al*., [54] reported the presence of 30 components including linalool, tetradecanoic acid, camphor and lauric acid in volatile oil of *S. junceum*.

In particular, it has been reported that human herpes viruses such as HSV-1 and HSV-2 are sensitive to the inhibitory effect of plant essential oils [55-56]. The antimicrobial, antioxidant and cytotoxic activities of aromatic water obtained from *S. junceum* flowers were investigated and it was determined that aromatic water had a significant antioxidant activity [15]. Yeşilada *et al*., [57] stated that flavonoid-rich fractions obtained from *S. junceum* flowers showed strong antioxidant activity.

Some studies have been carried out to compare the antioxidant and antiviral activities of plant extracts and natural substances, and these studies have shown that the extracts are having good antioxidant activity and compounds usually show strong antiviral activity against various viruses. Aruoma *et al*., [58] demonstrated that extracts from dried rosemary and dried plants of various plants growing naturally in the Provence region of France show potential antioxidant and anti-HIV activity.

Methanol, chloroform, ethanol, n-butanol and water fractions of *Euphorbia thymifolia* L. has shown to have potent antioxidant and anti-HSV-2 activity [59]. Some fractions, flavonoids, and proanthocyanidins obtained from *Crataegus sinaica* were found to have anti-HSV-1 and antioxidant activities [60]. Different degrees of Anti-HSV-1 activities obtained flowers and stems of methanol, and aqueous extracts from *S. junceum* in the study can be connected to one or more of the various components (alkaloids, flavonoids, triterpenoid saponins, essential oils, *etc*.) which are indicated to be responsible for anti-HSV-1 activity as mentioned above by different researchers.

This study is the first result to evaluate the anti-HSV-1 activity of *S. junceum*.

**CONCLUSION:** In this study, it was revealed that methanol and aqueous extracts obtained from the flowers and stems of *S. junceum* tested for anti-HSV-1 activities by colorimetric XTT test have different degrees of anti-HSV-1 activity. However, the chemical composition of the extracts should be determined once again and the pure compounds to be obtained are not tested, it is also a fact that there is a significant deficiency regarding which component is responsible for the extract from the antiviral activity and that the mechanisms of action are not determined. Therefore, these deficiencies will be eliminated in future studies. Furthermore, during the determination of the cytotoxicity of the extracts, it was determined that the extracts were directly involved in chemical interactions with XTT. To rule out these interactions, it is recommended that some cell-free wells of microplates be separated to control XTT interactions with extract, or a more reliable test such as SRB will be used.

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