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EFFICACY OF FERULA L. SPECIES EXTRACTS FROM TAJIKISTAN AGAINST INFLUENZA VIRUSES

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Abstract

One of the challenges facing both the domestic and international pharmaceutical industries is the limited selection and suboptimal efficacy of available antiviral drugs. The high mutational variability of influenza viruses contributes to rapid acquisition of drug resistance, which diminishes or nullifies the effectiveness of existing therapies, thereby necessitating the exploration for novel therapeutics. This study aimed to assess the antiviral activities of three species of the genus Ferula—indigenous to the Republic of Tajikistan—against influenza strains A/Vladivostok/2/09 (H1N1) and A/Almaty/8/98 (H3N2). The investigation revealed that phytochemical constituents—specifically, root gum, seed juice, and ethanol extracts—from the endemic species F. violacea and F. kuhistanica demonstrated a range of EC_{50} values from 0.38 to 5.50 µg/mL against the tested influenza viruses, outperforming standard antiviral medications. Remarkably, the seed extract from F. violacea exhibited an EC_{50} value against the H3N2 strain that was 81.8 times more effective than Tamiflu. Analysis of the selectivity index (SI) revealed that the root gum from F. violacea was highly effective against both strains of influenza. While the seed juice and ethanol extract showed heightened activity particularly against the H3N2 strain, their chemotherapeutic effectiveness against the H1N1 strain was notably lower. Conversely, the root gum from F. kuhistanica demonstrated high efficacy against the H1N1 strain but reduced efficacy against the H3N2 strain. The SI value for the root gum from F. gigantea was significantly low, approximating unity. Further analysis indicated that extracts from both aerial and underground parts of F. violacea and F. kuhistanica displayed superior anti-influenza activity against the H1N1 strain compared to Tamiflu. Our findings suggest that species within the Ferula genus, particularly F. violacea and F. kuhistanica, which are native to Tajikistan and Central Asia, respectively, exhibit significant antiviral properties. These results highlight the potential of these species as valuable sources for the development of novel antiviral drugs with enhanced efficacy.

Keywords: *Ferula, F. violacea, F. kuhistanica, F. gigantea, influenza A viruses (H1N1 and H3N2)*

I. INTRODUCTION

Viral and bacterial infections have continuously accompanied humanity, and since ancient times, humans have used plants to address health-related problems. There is evidence that the Egyptians utilized herbal medicines for this purpose around 3500 BC, which were later refined by the Greeks and Romans and are extensively documented in official medicinal texts known as pharmacopoeias[1]

Medicinal plants were used to treat viral diseases long before the discovery of viruses. Folk healers, unaware of viruses as etiological agents of infectious pathologies, employed plants and herbs to treat ailments such as smallpox, influenza, and flu-like diseases. Notably, for centuries, Native Americans who contracted smallpox used seasonings and infusions obtained from *Sarracenia purpurea* L. to treat this viral pathology [2, 3]. During the Spanish flu pandemic, herbal remedies derived from *Allium cepa* L., *Gelsemium sempervirens* (L.), *Eupatorium perfoliatum* L., *Actaearacemose* L. and *Asclepiastuberosa* L. were used to mitigate the severe course of the disease [4].

The use of medicinal plants as complementary and alternative medicine is increasingly widespread globally. According to the authors, between 10 to 50% of the population in developed countries regularly consumes plant products [5, 6]. In 2021, the global market for herbal medicines grew by 151.91 billion US dollars and is projected to exceed 300 billion by 2029, with an average annual growth rate of 11.16% [7].

One challenge in the modern pharmaceutical industry is the limited arsenal and low effectiveness of antiviral drugs. The insufficient variety of antiviral drugs represents only one aspect of this issue. Another significant concern is the drug resistance of viral pathogens, particularly the high mutational variability of influenza viruses, which can rapidly develop resistance, diminishing or completely negating the efficacy of drug therapies. This necessitates the continuous search for new medications [8].

A significant issue in modern medicine is the potential side effects of chemotherapeutic agents used to treat viral infections, particularly influenza. Currently, potential antiviral agents are either quite toxic or have low efficacy [9, 10, 11]. In this context, drugs that are effective against many viruses and possess additional beneficial properties hold an undeniable advantage, allowing for the combination of etiotropic and pathogenetic treatments. For this reason, substances of plant origin are of considerable interest because some, being relatively low toxic, exhibit a broad range of biological activities, including antimicrobial, antiviral, and immunomodulatory effects [12, 13].

Advances in plant chemistry, pharmacology, and medicine have significantly deepened our knowledge of the healing effects of many plant species. In economically developing or non-industrial countries, about 60-90% of the population uses herbal medicines for the prevention and treatment of both infectious and non-communicable diseases. Herbal medicines are also widely used in economically developed countries, particularly in Japan, China, and Brazil, mainly for prevention and palliative care [14, 15, 16].

One way to actively search for new highly effective remedies in the world of herbal compounds is through the systematic study of the experience of traditional medicine or ethnopharmacology, which has made a substantial contribution to the development of phytotherapy for infectious and non-infectious diseases [17, 18].

Ethnopharmacology or traditional medicine in Tajikistan has deep centuries-old roots dating back to the time of Avicenna. Back then, traditional healers in Tajikistan widely used medicinal plants to treat both non-communicable diseases and infectious pathologies [19, 20]. In this context, it should be noted that the flora of Tajikistan is remarkably diverse. According to experts, more than 9,000 plant species grow here, 1,132 of which are considered endemic; more than 100 of these plants are medicinal. Among them, the most valuable are representatives of the genus *Ferula* L. There are 39 species of this genus growing in Tajikistan, of which 6 species are endemic and grow at altitudes of 300 to 3600 meters above sea level. Endemic species include *F. violacea* Kor, *F. karategina* Lipsky ex Korn, *F. koso-poljanskyi* Kor., *F. linczevskii* Kor., *F. decurrens* Kor., and *F. botschantzevii* Kor. [21], whose biological activity remains unexplored [22, 23].

As is known, medicinal plants and their biologically active compounds have recently become one of the main directions in the development of effective and affordable medicines that meet current needs, since their plant metabolites can interfere with virus replication without affecting vital organs and tissues of the body [24, 25, 26, 27, 28].

Thus, conducting targeted exploratory research on medicinal plants and creating new effective phytopreparations, along with developing modern, environmentally friendly, resource-saving, and waste-free technologies, represents a promising direction in both scientific and practical aspects.

The main purpose of this study was to evaluate the antiviral activity of three species of the genus *Ferula*, found in the Republic of Tajikistan, against influenza strains A/Vladivostok/2/09 (H1N1) and A/Almaty/8/98 (H3N2).

II. MATERIAL AND METHODS

II.1. Plants. The roots and seeds of three species of the genus *Ferula* L. were tested for antiviral activity: *F. violacea* Kor., endemic to the Republic of Tajikistan; *F. kuhistanica* Kor., endemic to Central Asia and the Pamir-Alai; and *F. gigantea* B. Fedtsch., collected from various

Table 1. Characteristics of plants used in the study.

NO	Plant name	Local name	Part used	Plant collection location	Date of collection	Coordinates
1	*Ferula violacea* Kor.	Kastruf, roschak	root, seeds	Maikhura, Varzob Gorge	July 23, 2022	Latitude: 38.48319 Longitude: 68.490523 Elevation: 1180 MASL
2	*Ferula kuhistanica* Kor.,	Kamoli koohi	root	Vanj district, Badakhshan Mountainous Autonomous Region	July 21, 2022	Latitude: 37.47887 Longitude: 71.59682 Elevation: 2269 MASL
3	*Ferula gigantea* B. Fedtsch.,	Kamoli boozurg-jusa	root	The south-western slope of the Shughnan ridge, Badakhshan	July 21, 2022	Latitude: 37.47887 Longitude: 71.59682 Elevation: 2269 MASL

				Mountainous Autonomous Region		

regions of the Republic of Tajikistan. Plant specimens were identified based on Moscow University herbarium sheets[29] and were checked by staff of the Botanical Garden of the Academy of Sciences of Tajikistan (Table 1).

II.2. Gum and Extraction

Gum was obtained from the root by making cuts in the plant tissue. The exudate (gum) that appeared on the surface of the cuts was scraped off using a sterile scalpel and collected into a Petri dish. It was then dried in a thermostat set at 37°C for 24 hours.

The roots were first washed, dried, and then finely chopped using a sharp scalpel. The chopped roots were placed in a SPV-2 juicer (manufactured in Kharkov, 1984), and 30 mL of 70% ethanol was added. To expedite the drying process, the resultant juice was poured into Petri dishes and dried in a thermostat at 37°C for 24 hours.

Seeds were placed in the juicer hopper, and 30 mL of 70% ethanol was added. The resulting extract was then dried and placed in a thermostat at 37°C for 24 hours.

II.3. Viruses and chemicals.

Ten-day-old chicken eggs and 50% chicken red blood cell (cRBC) suspensions were sourced from the Almaty Chicken Factory farm (Almaty, Kazakhstan).

Phosphate-buffered saline (PBS, pH 7.4) was procured from Amresco (Solon, OH, USA). All water used in the experiments underwent purification using an E-pure water purification system with a minimum resistivity of 17.6 MΩ cm (Barnstead, Dubuque, IA).

Tamiflu (75 mg) was obtained from Roche-Korea (Seoul, South Korea) Co. Ltd.

Oseltamivir-resistant human influenza A virus A/Vladivostok/2/09 (H1N1) was acquired from the virus collection of the Smorodintsev Research Institute of Influenza, St. Petersburg, Russia. Influenza virus A/Almaty/8/1998 (H3N2) was obtained from the Research Institute for Biological Safety Problems, Gvardeyskiy, Kazakhstan.

The presence of the virus was determined either by titration using hemagglutination or by observing cytopathic effects (CPE) in chicken embryos induced by viral infection, and then expressed as 50% infectious doses (IED50) calculated based on the Reed-Muench method [30].

II.4. Extracts toxicity in fertilized chicken eggs

Ten-day-old fertilized chicken eggs (FCE) were procured from a licensed commercial vendor and subsequently incubated in a humidified incubator at 37°C. All studies adhered to local guidelines established by the Ministry of Education and Science of Kazakhstan and were approved by the Committee on Ethics of Animal Experiments at the Research and Production Center for Microbiology and Virology, Almaty, Kazakhstan (approval no.: 02-12-18).

Extract dilutions were prepared at concentrations of 10, 20, 40, 50, 70, and 100 μg/embryo. Each concentration (100 μL) was inoculated into the allantoic cavity of the 10-day-old FCEs. Subsequently, the eggs were incubated in a humidified environment at 37°C for 5 days, with viability assessed twice daily by candling. The 50% inhibitory concentration (IC_{50}), denoting the sample concentration resulting in 50% embryo survival, was calculated [31]

II.6. The ability of plant extracts to inhibit influenza virus replication.

The specific virus-inhibiting activity of the studied drugs was determined following established methods [32]. Briefly, different doses of the extract were mixed with an equal volume of 100 EID50/mL of the virus. After 30 minutes of incubation at 37°C, the mixture was inoculated into 10-day-old chicken embryos or MDCK cells. The viruses were cultured in the allantoic cavity of 10-day-old chicken embryos for 24-48 hours (depending on the virus strain) at 37°C, or for 48-72 hours in cell culture. The presence of the virus was determined through the hemagglutination reaction (HA). The suppression of virus reproduction was assessed by comparing the results of HA in experimental and control samples, with a saline solution used as a control at pH 7.2. Based on the experimental results, the average effective viral inhibitory concentration of the studied drug (EC_{50}) was determined.

As a criterion for the specific antiviral effect of the compounds, the chemical therapeutic index (CTI) was calculated, representing the ratio of the average toxic concentration of the substance (TC_{50}) to the average effective viral inhibitory concentration (EC_{50}).

II.5. Statistical analysis.

All results were calculated and expressed as the mean ± standard error of the mean (SEM). The results of three independent experiments are presented, each with four repetitions. Differences between more than two groups were analyzed for statistical significance using single-factor analysis of variance (ANOVA). Values of $p< 0.05$ were considered statistically significant.

The study was conducted at the Laboratory of Antiviral Protection, part of the Research and Production Center for Microbiology and Virology at the Institute of Microbiology and Virology, Almaty, Kazakhstan

III. THE RESULTS OF THE CONDUCTED RESEARCH

III.1. Assessment of cytotoxicity of the researched samples. Inhibitory concentration (IC50) the researched extracts

Prior to assessing antiviral activity, the potential cytotoxic effects of plant extracts on chicken embryos were evaluated. A series of experiments demonstrated that within a dose range of 1 to 100 µg/mL, the studied extracts did not induce cytotoxic cell changes or lethality in chicken embryos (Table 2). Consequently, subsequent research utilized doses of the preparations up to 100 µg/mL.

III.2. Study of the effective concentration (EC_{50}) of the researched extracts

The effective concentration 50 (EC_{50}), defined as the concentration resulting in a 50% protective effect relative to viral control, is a critical measure of a therapeutic drug or test compound's antiviral efficacy. Generally, a higher IC_{50} value indicates lower potency.

The EC_{50} of the endemic species *F. violacea* was of particular interest at the outset of this study, as prior research had not documented the biological activities of this species, including its antiviral effects. As detailed in Table 2, various preparations from *F. violacea*, including gum from the roots, seed juice, and ethanol extracts, exhibited diverse EC_{50} values against influenza virus strains, ranging from 0.38 to 5.50µg/mL. These values are significantly lower than those for Tamiflu, which shows EC_{50}s of 31.00 µg/mL for H3N2 and 10.70µg/mL for H1N1. Notably, the

Table 2. Antiviral activity of plant extracts. IC_{50} - inhibitory concentration 50; EC_{50} - effective concentration 50; SI -selectivity index: IC_{50} [µg/mL]/EC_{50} [µg/mL]. All results expressed as the

mean ± standard error of the mean. The results of three independent experiments are presented, each with four repetitions.

Sample	IC_{50}	A/Vladivostok/2/09 (H1N1)		A/Almaty/8/98 (H3N2)	
		EC_{50}	SI	EC_{50}	SI
Tamiflu	>350	10.70±0.040	>11.30	31.00±0.144	>32.70
F. violaceae Ethanol extract from roots	>100	0.50±0.015	>200.00	0.60±0.016	>166.67
F. violaceae Gum from roots	>100	0.70±0.012	>142.90	0.38±0.015	>263.00
F. violaceae Ethanol extract from seeds	>100	5.50±0.156	>18.20	0.50±0.014	>200.00
F. violaceae Juice from seeds	>100	5.50±0.144	>18.20	0.80±0.07	>125.00
F. kuhistanica Gum from roots	>100	0.38±0.012	>263.00	4.00±0.144	>25.00
F. gigantea Gum from roots	>100	>100	1	>100	1

gum from the roots of F. violacea was the most potent, with an EC_{50} of 0.38 µg/mL against H3N2. The ethanol extracts from the seeds and roots were also effective, particularly against H3N2 and H1N1 respectively, with EC_{50} values of 0.50 µg/mL. The least active were the ethanol extract from the roots and seed juice against H3N2, and gum from the roots against H1N1, with the highest EC_{50} measured at 5.50 µg/mL for the latter against H1N1. All EC_{50} values for *F. violacea* are markedly superior to those of Tamiflu.

The gum from the roots of *F. kuhistanica* also demonstrated differential activity against the tested strains, exhibiting a very low EC_{50} of 0.38 µg/mL against H1N1 and a relatively high EC_{50} of 4.00 µg/mL against H3N2. In contrast, gum from the roots of *F. gigantea* showed no measurable antiviral activity.

A comparative analysis between the EC_{50} values of these extracts and the widely used anti-influenza drugs Tamiflu revealed that in many instances, the plant extracts exhibited more pronounced antiviral activity against the employed viral strains. Specifically, the EC_{50} of Tamiflu for strain H1N1 was 10.70 µg/mL, whereas extracts from *F. violacea* and *F. kuhistanica* did not exceed 5.50µg/mL.

III.4. Evaluation of selectivity indices of plant extracts in antiviral therapy.

The selectivity of plant extracts in antiviral applications was quantified using the selectivity index (SI), calculated as the ratio of the cytotoxic concentration (CI_{50}) to the inhibitory concentration (IC_{50}). As depicted in Figure 2, gums from the roots of *F. violacea* and *F. kuhistanica* exhibited notably high selectivity for the viruses investigated in this study. Specifically, gum derived from the root of *F. violacea* demonstrated an enhanced chemotherapeutic potential against

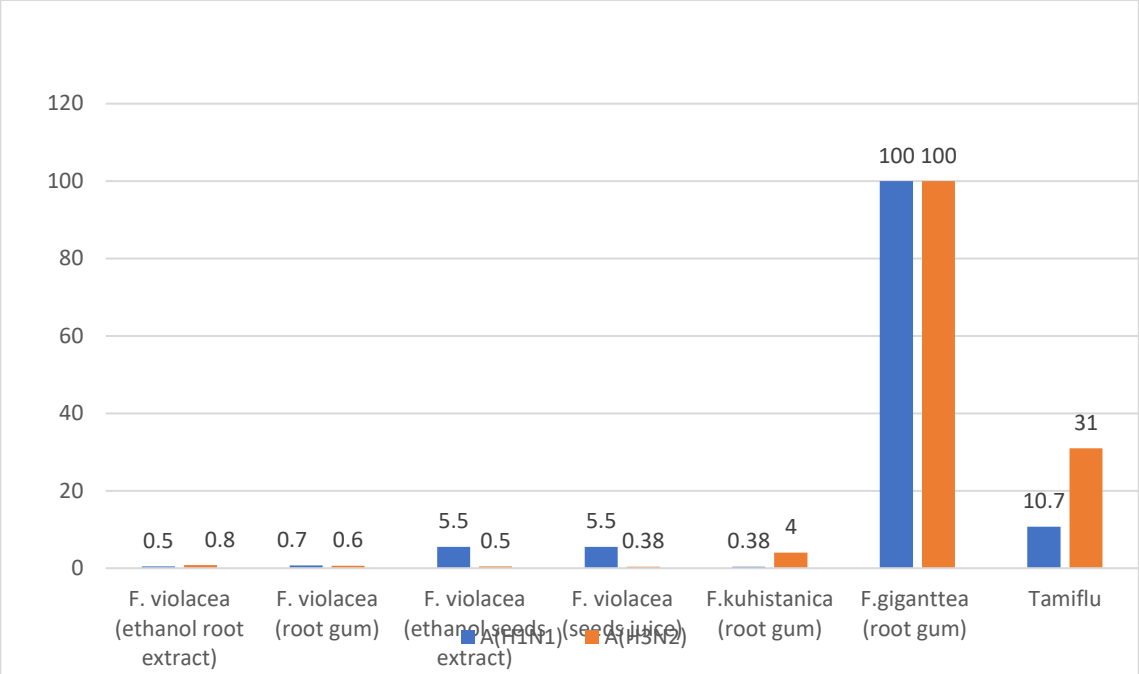

Fig.2. Selectivity index (SI50) of the studied gums and extracts in relation to influenza virus strains

the H3N2 strain, with an SI of 263. Likewise, gum from the root of *F. kuhistanica* achieved a high SI of 263 against the H1N1 strain.

Ethanol extracts from the roots and seeds of *F. violacea* were also distinguished by their high selectivity indices, each exceeding 200, indicative of a robust chemotherapeutic effect against both viral strains. In contrast, the lowest SI values for the H1N1 strain were recorded for the ethanol extract and seed juice from *F. violacea*, each with an SI of 18.20, suggesting lower selectivity and potential therapeutic efficacy.

Furthermore, the SI for gum from the root of *F. gigantea* was determined to be 1 for both viral strains, indicating a negligible chemotherapeutic effect against the influenza viruses tested. This stark contrast in SI values highlights the variability in antiviral efficacy among different botanical extracts and underscores the need for thorough biochemical and pharmacological characterization of potential antiviral agents derived from plant sources.

DISCUSSION

Influenza viruses have consistently been a major element of human history, closely intertwined with the evolutionary dynamics of wildlife and humanity. Historical accounts repeatedly underscore the severity of influenza outbreaks that have escalated into global epidemics and pandemics. Notably, the Spanish flu of 1918-1920 resulted in an estimated 25 to 50 million deaths worldwide. Similarly, the 1957 Hong Kong flu pandemic caused considerable human suffering and economic damage [33, 34].

Despite significant advancements in antiviral therapeutics and the development of various influenza vaccines, the incidence and mortality rates associated with influenza continue to be alarmingly high [35, 36, 37]. The periodicity and severity of these outbreaks are largely driven by the virus's ability to undergo antigenic drift—a process involving minor but critical changes in its antigenic structure that allow it to evade immune detection and facilitate the emergence of new, potentially widespread outbreaks [38, 39].

This persistent challenge highlights the urgent need for ongoing research into the discovery and development of novel, effective, and safe anti-influenza agents. Such efforts are essential to mitigate the recurring impact of influenza outbreaks on global health.

There is a very limited information on *Ferula* plants antiviral properties. The lipid soluble fraction of *Ferula ferulaeoides* (Steud.) Kor., showed a significant inhibitory effect against HBV in HBV-producing cell line HepG2.2.15. It reduced the HBsAg level and HBV replication by 87 and 36%, respectively [40]. *Ferula foetida* Regel oligo-gum resin containing phytochemicals with anti-viral properties. Some sesquiterpene coumarins of *Ferula* species were shown to be active against H1N1. It was demonstrated that 5′S-hydroxyumbelliprenin, 8′-acetoxy-5′S-hydroxyumbelliprenin, conferol, epi-conferdionefarnesiferol A, farnesiferol C, galbanic acid, ligupersin A, and methyl galbanate, isolated from CHCl3-soluble extract of *Ferula assa-foetida* L. displayed significant antiviral activity against H1N1 (IC_{50}: 0.26–0.86 μg/mL), which was more effective than amantadine (IC_{50}: 0.92 μg/mL) [41]. Findings from CPE-inhibitory assays showed it has a dose-dependent antiviral activity against HRV-2 for asafetida gum resin the gum resin of *F. assa-foetida*as well as its isolated sesquiterpene umbelliferons Microlobidene, Farnesiferol C, Farnesiferol B and Kellerin [42]. It was also shown that kellerin could significantly inhibit the cytopathic effects and reduce the viral titre of the herpes virus type 1 (HSV-1) DNA viral strain KOS at concentrations of 10, 5 and 2.5 μg/mL [43]. In addition, Sinkiangenorin E isolated from the seeds of *Ferula sinkiangensis*. K.M.Shen also had a significant inhibitory effect on H1N1 [44]. The sesquiterpene coumarin kellerin showed an antiviral effect against herpes virus type 1 (HSV-1) by the plaque-reduction assay. It could dramatically reduce the viral titer of the HSV-1 DNA viral strain KOS at concentrations of 10, 5, and 2.5 μg/mL and considerably lessen its cytopathic effects [45].

The objective of this study was to investigate the antiviral efficacy of gum and ethanol extracts from three *Ferula* species endemic to Tajikistan - *F. violacea*, and those endemic to Central Asia and the Pamir-Alai region, *F. kuhistanica* and *F. gigantea*. These extracts were tested against two antigenic formula of the influenza virus: A/ Vladivostok/2/09 (H1N1) and A/Almaty/8/98 (H3N2).

Comparative analyses included the well-known anti-influenza medications Tamiflu, which served as positive controls due to their low IC_{50} values and broad-spectrum antiviral capabilities [46, 47].

The initial phase of our study focused on determining the 50% inhibitory concentration (IC_{50}) of the samples under examination against various influenza virus strains. These samples demonstrated a significant level of inhibitory effect across influenza strains with diverse antigenic structures. Our comparative assessments revealed that the gums and ethanol extracts derived from

the roots and seeds of *F. violacea*, as well as gums from *F. kuhistanica* and *F. gigantea* were over three times more effective than Tamiflu.

Historically, the roots of the *Ferula* L. genus have been extensively utilized in both folk and scientific medicine [48, 49, 50, 51, 52, 53]. This prompted further investigations into the biological activity of this specific plant part in two other species—*F. gigantea* and *F. kuhistanica*—included in this study.

The main and key criteria for evaluating the effectiveness of antiviral drugs under development or potential sources of such drugs include their effective concentration (EC50) and selective (SI) or chemotherapeutic index (CTI). The results obtained by us give reason to believe that the samples studied by us are characterized by a sufficiently pronounced effective concen-tration. Differences in effective concentration indicators between the studied samples and the comparison drug - Tamiflu, which is widely used anti-influenza drugs, were revealed. Our results show that gums and extracts obtained from the root and seeds of the endemic species *F. violacea* and *F. kuhistanica* exhibit more pronounced antiviral activity than comparison drugs. So, the value of the squeeze from the seeds is *F. violacea* for strain H3N2 was 81.8 times higher than the same value for Tamiflu (31.00).

Further analysis showed that gum from the root of *F. kuhistanica*, as a squeeze from the seeds of *F. violacea* has a higher anti–influenza activity (0.38 mg/mL) against strain H1N1 than Tamiflu (10.7 mg/mL) - 28.2 times. However, the anti-influenza activity (4.0 mg/mL) of this gum against the H3N2 virus was only 7.8 times higher than the effective concentration Tamiflu.

It is known that one of the informative criteria for the effectiveness of an antiviral compound or a finished drug is the value of its chemotherapeutic index or selectivity index (SI), which is the ratio of the 50% toxic concentration of the drug to its 50% effective concentration [54, 55].

The results of our study showed that the studied gums and ethanol extracts have a sufficiently pronounced and selective or chemotherapeutic index compared with widely used antiviral drugs. Thus, gum from the root of *F. violacea* showed a high SI value for strain H3N2 - 23 times more than Tamiflu. At the same time, a similar sample obtained from the root of *F. kuhistanica* showed the same SI value for another virus used in the study, i.e. strain H1N1. However, this studied sample exceeded the SI value of Tamiflu by 8 times in this indicator. The average SI value for both influenza virus strains used in the study was characterized by ethanol extract from the root, pomace or juice and ethanol extract from *F. violacea* seeds. The lowest and approximately the same as the comparison drug – Tamiflu chemotherapeutic effect in relation to strain H1N1 extract and ethanol extract from *F. violacea* seeds were shown, as well as gum from the root of *F. kuhistanica* for strain H3N2.

The selective chemotherapeutic effect of individual studied samples in relation to a particular strain is noteworthy. Thus, samples obtained from *F. violacea* seeds with a low value (18.2) for strain H1N1 showed a sufficiently high value (up to 200) for strain H3N2. In turn, gum from the root of *F. kuhistanica* exhibits a high (more than 263) chemotherapeutic effect against strain H1N1 it turned out to be ineffective (25) for strain H3N2. Apparently, the variation in the value of the assumed SI in

relation to different variants of the influenza virus is related to their genetic variety, which requires additional research.

CONCLUSION

This study marks the inaugural examination of the antiviral activities of three species within the genus *Ferula*, namely, *F. violacea* endemic to Tajikistan, and *F. kuhistanica* and *F. gigantea*, both native to Central Asia and the Pamir-Alai region. We established the efficacy of both gum and ethanol extracts derived from the roots and seeds of *F. violacea* and *F. kuhistanica*. Notably, the gum from the roots of *F. violacea* demonstrated significant antiviral activity against both influenza virus strains tested, H1N1 and H3N2.The study also highlighted the distinctive antiviral capacities of the individual samples. The antiviral activity of the gums and ethanol extracts was characterized by exceptionally high EC_{50} and SI values, surpassing those of the standard comparison drug, Tamiflu. These findings suggest that *F. violacea* and *F. kuhistanica* possess promising potential for the development of new, effective antiviral agents against influenza. Further research is recommended to explore the mechanistic basis of the antiviral properties of these extracts and to evaluate their efficacy and safety in clinical settings.

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ETHICS DECLARATIONS

Ethics approval and consent to participate

Not applicable.

CONFLICTS OF INTEREST

The authors declare no conflict of interest