Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Efficacy and safety of add-on *Viola odorata* L. in the treatment of COVID-19: A randomized double-blind controlled trial

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ABSTRACT

Ethnopharmacological relevance: Severe Acute Respiratory Syndrome (SARS) due to the novel coronavirus has become the highest priority that threatens human health. This situation demands widespread vaccination and the innovation of new therapeutic methods. Despite drug discoveries, the need for approving new medicaments is felt because of adverse effects and lack of efficacy. Several medicinal plants including *Viola odorata* L. are recommended in traditional Persian medicine for alleviating respiratory infection symptoms. Recent studies showed anti-inflammatory, antioxidant, anti-asthmatic, antitussive, analgesic, and antibacterial activities of sweet violet. These enhance respiratory functions, reduce pulmonary inflammation, and decline mucous membrane edema. This study aimed to evaluate the efficacy of sweet violet syrup in alleviating the manifestations of COVID-19 infection.

Material and methods: A randomized parallel-group double-blind controlled trial was conducted at Al-Zahra general hospital, Isfahan, Iran. A total of 108 outpatients were enrolled in the study. The patients were randomly allocated to intervention and placebo groups, with 54 patients in each group. The allocation was concealed using sealed opaque envelopes. The intervention group received violet syrup and the control group received placebo syrup, an add-on to the conventional treatment.

The outcomes were COVID-19 manifestations, such as dyspnea, cough, myalgia, headache, and diarrhea, considered as outcomes of the study and were evaluated twice using a visual analog scale before the intervention and after 7 days, at the end of the study. Patients were followed daily by phone calls to monitor proper drug consumption and possible side effects.

Results: No significant difference was between groups regarding demographic characteristics and vital signs before and after the treatment. Although all symptoms have improved significantly in both groups, patients who received violet syrup recovered faster and the mean severity scores of cough ($P = 0.025$), myalgia ($P = 0.036$), headache ($P = 0.037$), and diarrhea ($P = 0.044$) decreased greater in comparison to control group.

Conclusion: This study, the first clinical trial on the effectiveness of *Viola odorata* on SARS-CoV-2 patients, showed that *Viola odorata* L. effectively controls prevalent manifestations of COVID-19 including cough, myalgia,

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headache, and diarrhea. Regarding this survey, the violet syrup can be mentioned as a complementary treatment for viral influenza-like infections in which cough, myalgia, headache, and diarrhea are prominent.

1. Introduction

The novel coronavirus disease 2019 (COVID-19) pandemic has become the highest priority that threatens human health(Fauci et al., 2020). Severe Acute Respiratory Syndrome CoronaVirus-2 (SARS-CoV-2), first identified in Wuhan, central China, is a highly infectious single-stranded RNA virus, considered zoonotic in origin, which can cause fatal illness in humans(Cui et al., 2019; Fazil and Nikhat, 2022; Lai et al., 2020). Up to July 2022, SARS-CoV-2 had infected more than 550 million people and killed about 6 million across the world. Further, the number of patients infected with SARS-CoV-2 in Iran has been estimated at up to 7 million with 141000 deaths (World Health Organisation, 2022). The COVID-19 global pandemic is responsible for unprecedented worldwide extensive economic, social, and health complications including respiratory, cardiovascular, mental, and neurological consequences such as anxiety, depression, tremors, or seizures(De Felice et al., 2020; Whittaker et al., 2020). COVID-19 causes a various range of symptoms from fatigue, myalgia, loss of appetite, diarrhea, fever, dyspnea, dry cough, and rhinorrhea to pneumonia or severe acute respiratory distress syndrome (ARDS)(Abebe et al., 2020; Figueiredo-Campos et al., 2020). Despite a large number of patients remaining asymptomatic(Figueiredo-Campos et al., 2020), older people or patients with pre-existing high-risk health conditions such as respiratory or cardiovascular conditions, hypertension, diabetes, or immunocompromised states are at increased risk for developing severe complications like ARDS or uncontrolled inflammatory state leading to multiple organ failure, or death(Bai et al., 2022; Weiss and Murdoch, 2020). This situation demands widespread vaccination and innovation of new therapeutic methods (Abebe et al., 2020). Pharmaceutical companies and health institutions announced efforts to develop effective vaccines and different treatments to blockade disease transmission and prevent potential complications in case of SARS-CoV-2 infection(Abebe et al., 2020; Vaz de Paula et al., 2020). During the pandemic, different types of natural products, medicinal herbs, and their derivatives have been employed in the treatment of COVID-19 (Chen et al., 2022; Yang et al., 2020). In Traditional Persian Medicine (TPM) several medicinal plants including sweet violet are recommended for alleviating respiratory infection symptoms(Abumansour, 1967; Avicenna, 2005; Jorjani, 2001). *Viola odorata* L. (sweet violet) (Fig. 1) is a medicinal plant with anti-inflammatory, antioxidant, anti-asthmatic, antitussive, analgesic, antioxidant, and antibacterial activities(Mittal et al., 2015; Mosavat et al., 2017; Nimrouzi et al., 2019; Qasemzadeh et al., 2015; Tafazoli et al., 2020).

It contains various biochemical compounds such as tannins, methyl salicylate, alkaloids, glycosides, coumarin(Mittal et al., 2015), flavonol tri and tetra glycosides, c-glycosides of apigenin, luteolin, flavonol-O-glycosides(Karioti et al., 2011), and phenylpropanoids including anthocyanins, flavonoids, flavonol glycosides (Kaempferol), poly-saccharides (18% mucilage) (Fig. 2)(Lamaison et al., 1991). These have been introduced as substances that enhance respiratory functions, reduce pulmonary inflammation, decline mucous membrane edema (Muhammad et al., 2013), and make sweet violet a potential medication for SARS-CoV-2 infection.

The main uses of *V. odorata* for respiratory problems are mentioned in different schools of traditional medicine such as traditional Chinese medicine, Ayurveda, and TPM. Likewise, the effects of violet are cited in various western herbal sources including British Herbal Pharmacopeia, European Pharmacopeia, and the Physician Desk Reference, where it is focused on as an expectorant and antitussive agent for bronchial catarrh (Mahboubi and Kashani, 2018). This study aimed to evaluate the efficacy of sweet violet syrup in alleviating the manifestations of COVID-19 infection.

2. Material and Methods

2.1. Study design and participants

This study was a randomized parallel-group double-blind controlled trial conducted from April 21st, 2021 to May 21st, 2021 at Al-Zahra general hospital, Isfahan, Iran. The trial was performed in accordance

Fig. 1. *Viola odorata* leaves and flowers.

with the principles of the Declaration of Helsinki approved by the ethics committee of Tehran University of Medical Sciences (TUMS) under the registration number IR.TUMS.MEDICINE.REC.1399.784. Moreover, it was registered at the Iranian Registry of Clinical Trials (IRCT20190409043215N2). We studied outpatients suffering from respiratory manifestations referring to the COVID-19 center. Prevalent COVID-19 manifestations were defined according to WHO guidelines, and recent pieces of evidence(Hong et al., 2020; Xu et al., 2020; Yifan and Jun 2020).

A total of 120 participants were screened and 12 patients met exclusion criteria. Of 108 patients who enrolled in the study, 54 patients were randomly allocated to the intervention group and 54 to the control group and were followed for 7 days. Statistical analysis has been performed for all 108 patients at the end of the evaluation. CONsolidated Standards of Reporting Trials (CONSORT) diagram was considered for the process of patient allocation (Fig. 3).

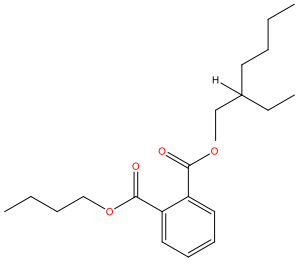
2.2. Inclusion and exclusion criteria

Inclusion in the study required the following criteria: (1) Patients diagnosed as SARS-CoV-2 infected based on clinical manifestations and/or lung CT scan with the approval of a radiologist and/or positive Polymerase Chain Reaction (PCR) test who did not need to be hospitalized and were treated as outpatients, (2) percentage of peripheral capillary oxygen saturation (SpO2) greater than or equal to 90%, (3) respiratory rate more than 24 per minute, (4) systolic blood pressure more than 90 mmHg, and (5) age range of 15–75 years in both sexes. Exclusion criteria were as follows: (1) Severe forms of COVID-19 (respiratory rate more than 24 per minute, or SpO2 less than 90%), (2) existing medical indications for non-per os, diabetes mellitus, diarrhea, or history of allergies to herbal products, medicinal plants, or natural medicaments.

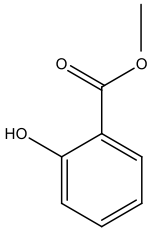
2.3. Study medication

Violet syrup, called Banafsheh syrup in TPM, contains an aqueous extract of *Viola odorata* L. flowers and *Saccharum officinarum* (brown sugar). This is manufactured and standardized under the trade name Banafsheh Syrup® by Barij Essence Pharmaceutical Company, Kashan, Iran. The drug has a production license from the Iranian food and drug administration (No: 6599807159453166). The batch number utilized in this study was 11212541. Banafsheh was obtained from the domestic herbal market and recognized by the herbarium of the faculty of pharmacy, Alborz University of Medical Sciences, Karaj, Iran under voucher number: V01-023.

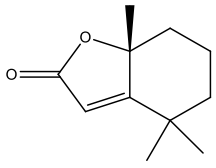
To prepare Banafsheh syrup, first dried violet flowers are soaked in water (room temperature) for 6 h. Thereafter, the mixture is heated to



Butyl 2-ethylhexyl phthalate ($C_{20}H_{30}O_4$)

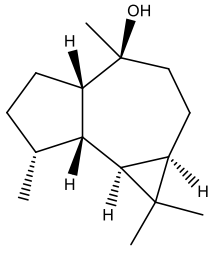


Methyl salicylate (C8H8O3)

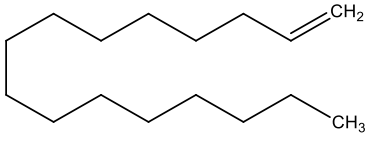


5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone

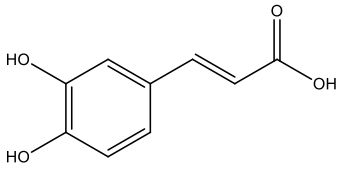
($C_{11}H_{16}O_2$)



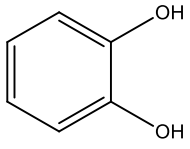
Viridiflorol (C15H26O)



1-Hexadecene ($C_{16}H_{32}$)



Caffeic acid (C9H8O4)



Pyrocatechol (C6H6O2)

Fig. 2. The chemical structure of the main compounds in *Viola odorata* L.

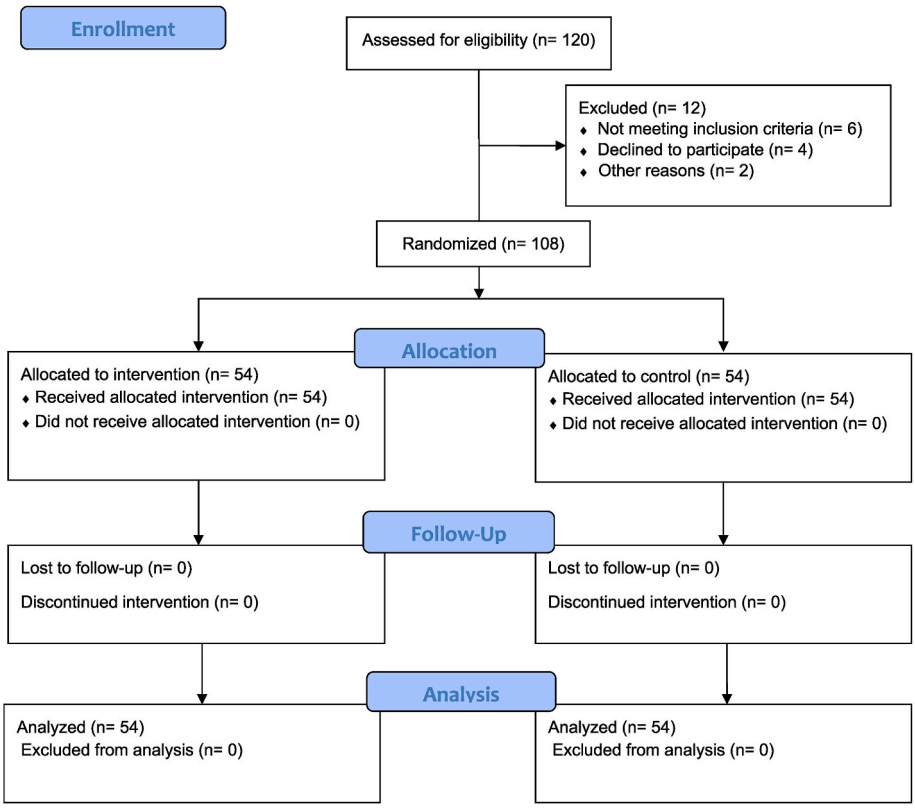


Fig. 3. CONsolidated Standards of Reporting Trials (CONSORT) diagram for the process of patient allocation.

boiling and then filtered. In the next phase, brown sugar is added to the extract, and heating is continued to concentrate the syrup. Violet syrup contains 9.22 mg total phenolic (as gallic acid) in every 5 mL of product. Syrup viscosity and amount of dry matter are 106 cP and 58% (w/w), respectively.

2.3.1. *High-performance liquid chromatography analysis of violet extract*

Sample solution: 5 mL of Violet Extract (VE) was made up to 10 mL with methanol and centrifuged after homogenization; 2 mL of this was passed through a 0.45-μm filter for High-Performance Liquid Chromatography (HPLC) analysis. Reference Standard Solutions (RSS) were made by dissolving hydroquinone, gallic acid, resorcinol, pyrocatechol, catechin, and caffeic acid standards in methanol.

The HPLC analysis was performed using Azure HPLC system (Knauer, Germany) equipment by P 6.1L pump and MWD 2.1L detector. An analytical column, Nucleodur C18 100-5 (150*4.6 mm, 5 μm particle size) was used at 35 °C to separate phenolic compounds in the sample solution. The injection volume was 20 μl. The column was monitored at wavelength 280 nm. The mobile phase consisted of 2%(v/v) acetic acid (A) and methanol (B) and the gradient program was set according to the method described by Jamshed H et al. with some modifications (Jamshed et al., 2019). The gradient elution was started with 5% B increasing to 30% at 10 min, then increased from 30% B to 100% B during the next 20 min at a flow rate of 0.8 ml/min. The chromatography peaks were confirmed by comparing their retention time with those of RSS.

2.4. *Intervention*

The design of the study was an add-on to the conventional protocol. For all the patients, standard care was administered according to the Iranian clinical management protocol for COVID-19, the ninth version (December 2020)(Ebrahimi, 2020). Based on the protocol, diagnostic procedures and necessary training were performed for mild to moderate COVID-19 patients who did not indicate hospitalization. In the next stage, the patients were ordered to rest in an isolated place at home. Supportive measures, including proper nutrition, supplement consumption, adequate fluids, proper ventilation of the room, and psychosocial support, were carried out. Also, symptomatic treatments such as those for fever and myalgia (acetaminophen or NSAIDs), weakness (reduction in activity, proper nutrition, and light exercises), cough (bromhexine or dextromethorphan), nausea and vomiting (dimenhydrinate), and diarrhea (replacement of water and electrolytes) were performed. Antiviral agents were prescribed where indicated.

In addition to the routine measures, the intervention group received violet syrup in the amount of 10 mL three times a day for 7 days. The control group received a placebo syrup of 10 mL three times daily for 7 days (the same as the intervention group).

Placebo syrup, manufactured by the company mentioned above\, contained water, sugar, and food coloring similar to the drug regarding color, consistency, and packaging. Violet syrup and a placebo were added to the standard treatment of the patients.

2.5. *Sample size calculation*

According to the previous studies on *Viola odorata* L. and by considering $\alpha = 0.05$ and $\beta = 0.1$ to achieve 90% power, and assuming a 5% loss to follow-up, the sample size was calculated at 54 in each group.

2.6. *Randomization, blinding, and allocation concealment*

In this study, the physicians, patients, and medical staff at the hospital remained blinded to the allocation. A balanced block technique with 4 blocks was used for randomization. Random number chains from 1 to 6 were produced to reach the desired sample size using SPSS software version 16. Prepared random assignment sequences for individuals were placed into sealed opaque envelopes and numbered with a 5-digit serial number by a third party who did not play a role in the design of the

study. All envelopes had a random dedicated serial number that was immediately opened after recruitment to allocate the participants to the drug/placebo groups. Moreover, the statistical analyst was totally blinded and the data were analyzed based on the labels A-B and specific codes for complete blinding and concealment. After the complete process of analysis, the codes were opened by the corresponding manager of the project.

2.7. Safety monitoring

According to the recommended dosage of *Viola odorata* L. (2–5 g per day of dry flower)(Andrea Peirce, 2007; Duke, 2002), the violet syrup dosage administered in this study was safe. This dosage has been tried in three different trials within the above-mentioned range as well (Porrostami and Ahmadian-Attari, 2020; Qasemzadeh, M. J. et al., 2015; Tavassoli et al., 2021). Besides, the drug is registered as a safe traditional antipyretic, antitussive, and anti-inflammatory agent for upper respiratory tracts by the Iranian food and drug administration. Moreover, important indices including vital signs (body temperature, heart rate, respiratory rate, and blood pressure) and laboratory tests (complete blood count and C-reactive protein) were assessed. In addition, any adverse effects were recorded via phone call during the study and through history-taking and physical examination at the final visit.

2.8. Outcome measures

Clinical improvement of COVID manifestations was the primary outcome of the study. This contained the following domains: dyspnea, cough, sputum, myalgia, headache, abdominal pain, dizziness, chest pain, fatigue, sneeze, rhinitis, ageusia, anosmia, anorexia, nausea and vomiting, and diarrhea. They were measured by a Visual Analogue Scale (VAS) chart according to the study of Skipper et al. (2020). Besides, before and after the intervention 10 mL of venous blood was taken from the patients for the following laboratory tests: White Blood Cell Count (WBC), neutrophil percentage, lymphocyte percentage, Red Blood Cell Count (RBC), Hemoglobin (HGB), Platelets count, C-Reactive Protein (CRP), and Erythrocyte Sedimentation Rate (ESR). Also, oxygen saturation, pulse rate, and systolic/diastolic blood pressure were measured as other indices for evaluation of infection intensity and improvement rate. Adverse reactions were monitored daily based on Common Terminology Criteria for Adverse Events (CTCAE) ver. 5.

2.9. Statistical analysis

Data were represented by frequency and percentage or mean and standard deviation for quantitative and qualitative variables, respectively. Data were analyzed using chi-square and Fisher's exact tests for qualitative variables, paired-sample *t*-test and independent-sample *t*-test for quantitative variables, and equivalent nonparametric test for non-normally distributed variables using SPSS software. P-value equal to 0.05 was considered a significant level.

3. Results

3.1. Phenolic identification of VE by HPLC

Considering that polyphenolic and flavonoid compounds of the violet flower have medicinal activities, in order to know the quality of VE used in making violet syrup, the primary extract was analyzed by HPLC. The analysis results showed the presence of hydroquinone, gallic acid, resorcinol, pyrocatechol, catechin, and caffeic acid in VE (Fig. 4).

3.2. Patient sample and characteristics

Most of the patients were in the range of 30–60 years old (min: 18, max: 78). They were approximately equal in sex and mostly married. Most of them were highly educated and self-employed. Sixty-five percent of the patients had no underlying disease and there was no

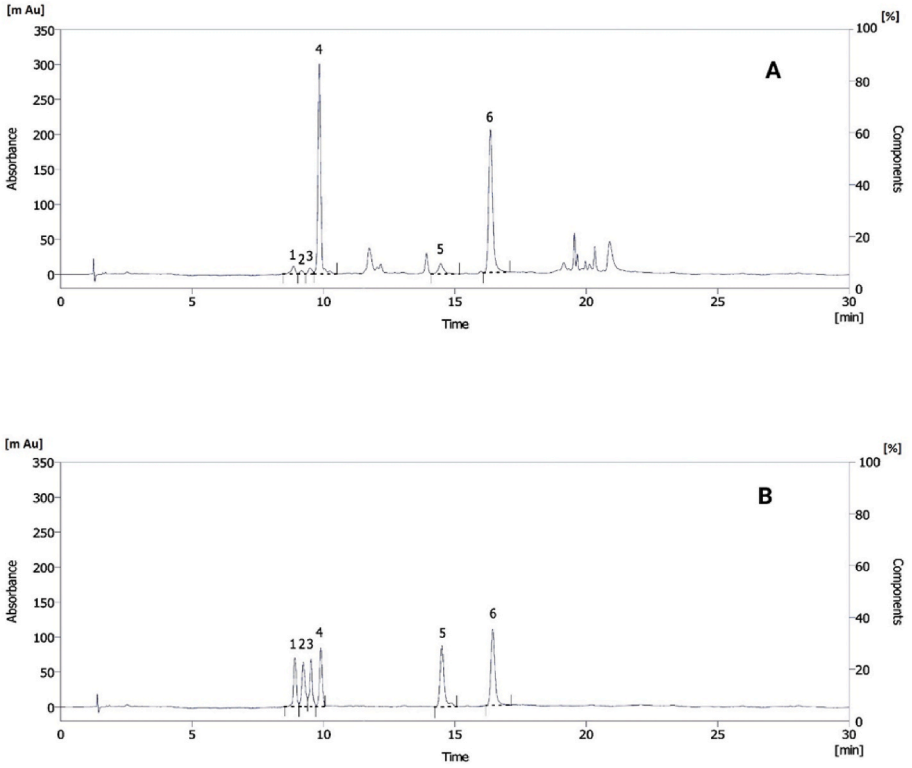


Fig. 4. HPLC chromatograms: violet exstract (A); reference standard solution (B) Including hydroquinone (1), gallic acid (2), resorcinol (3), pyrocatechol (4), catechin (5), and caffeic acid (6).

significant difference considering the demographic information of the patients between groups (Table 1).

3.3. Vital signs of the patients

At the beginning of the study, only 10% of the patients had a fever (oral body temperature more than or equal to 38 degrees centigrade). The mean oxygen saturation of the patients was 94%, the mean pulse rate was 90.5 per minute, and the mean systolic blood pressure was 114 mmHg. Statistical analysis showed that there was no significant difference between the two groups considering fever, oxygen saturation, pulse rate, and systolic blood pressure at the beginning of the study (Table 2). Also, after 7 days of follow-up, no remarkable changes have been reported in any vital signs. It is important to mention that vital signs at the beginning of the study and the end of the study were in the normal range.

3.4. Progression of COVID-related symptoms

Among all COVID-related symptoms investigated in this study, there was no significant difference between groups at the beginning of the study. By the 7th day, all symptoms have improved significantly (severity scores decreased) in both the intervention group and the control group. However, the mean severity score decreased greater considering cough (P = 0.025) (Fig. 5), myalgia (P = 0.036) (Fig. 6), headache (P = 0.037) (Fig. 7), and diarrhea (P = 0.044) (Fig. 8) in comparison with the control group (Table 3). In addition, considering the influence of confounding factors on the study outcomes, related regression models have been performed (such as age, sex, and initial symptom severity). However, no significant relationship was observed.

3.5. Laboratory findings

At the baseline, there was no significant difference between the laboratory findings of the 2 groups. After 7 days of intervention, despite the increment of WBC in both groups, the neutrophil percentage decreased and the lymphocyte percentage increased in both groups (only significant in the placebo group). No significant differences were observed considering WBC, neutrophil percentage, lymphocyte percentage, RBCs, hemoglobin, and ESR; however, platelet count and CRP were higher in the placebo group after intervention (P = 0.022 and P = 0.033, respectively) (Table 4).

4. Discussion

To our knowledge, this is the first clinical trial on the effectiveness of *Viola odorata* L. on SARS-CoV-2 patients. Despite the declining COVID-19 mortality rate, the number of affected people rises from time to time and region to region. These people impose a significant financial burden on the health care system, occupy hospital beds, and suffer from cough, myalgia, headache, and diarrhea. Thus, investigating therapeutic strategies to reduce the above-mentioned issues is still valuable(Ma et al., 2021).

Chemical constituents of *Viola odorata* L. flower and aerial parts are previously well-documented. The results of this study can be used to scrutinize the probable mechanisms of action of this medicinal plant on viral proliferation and inflammatory processes in the lung. Detected prominent ingredients such as caffeic acid and pyrocatechol in VE analysis in this survey have been investigated before in experimental studies.

Caffeic acid has an antiviral effect and significantly inhibits the replication of HCoV-NL63 in a cell type-independent manner and specifically blocks virus attachment(Weng et al., 2019). In addition, recent investigations proposed the effect of caffeic acid against 5 proteins of SARS-CoV-2(Adem et al., 2021). Furthermore, pyrocatechol exhibits anti-inflammatory effects by inhibiting the expression of inflammatory

Table 1
Patients' demographic information.[a].

Characteristic		Group		P value
		Placebo	Violet syrup	
Age (years old)	>30	6 (11.3)	11 (21.2)	0.295
	31–45	26 (49.1)	17 (32.7)	
	46–60	16 (30.2)	17 (32.7)	
	<61	5 (9.40)	7 (13.5)	
Sex	Male	28 (51.9)	27 (50.0)	0.847
	Female	26 (48.1)	27 (50.0)	
Educational status	No formal education	0 (0.00)	0 (0.00)	0.891
	Primary education	15 (27.8)	15 (27.8)	
	Secondary education	14 (25.9)	12 (22.2)	
	Tertiary education	25 (46.3)	27 (50.0)	
Marital status	Single	11 (20.8)	21 (40.4)	0.029
	Married	42 (79.2)	31 (59.6)	
Occupational status	Skilled employed	11 (21.6)	15 (29.4)	0.160
	Unskilled employed	7 (13.7)	1 (2.00)	
	Unemployed	16 (31.4)	16 (31.4)	
	Self employed	17 (33.3)	19 (37.3)	
Past medical history	None	45 (83.3)	40 (74.1)	0.540
	Cardiac disease	1 (1.90)	3 (5.60)	
	HTN	3 (5.60)	4 (7.40)	
	Pulmonary disease	1 (1.90)	3 (5.60)	
	DM	3 (5.60)	2 (3.70)	
	Kidney disease	1 (1.90)	0 (0.00)	
	Multiple sclerosis	0 (0.00)	2 (3.70)	
Patients' manifestations at baseline	Dyspnea	35 (64.8)	40 (74.1)	0.296
	Cough	47 (87.0)	46 (85.2)	0.781
	Sputum	32 (60.4)	31 (57.4)	0.775
	Myalgia	34 (64.2)	36 (66.7)	0.784
	Headache	31 (57.4)	35 (66.0)	0.359
	Abdominal pain	17 (32.1)	15 (28.3)	0.672
	Dizziness	20 (37.0)	30 (55.6)	0.054
	Chest pain	28 (51.9)	22 (40.7)	0.247
	Fatigue	42 (77.8)	41 (75.9)	0.820
	Sneeze	25 (46.3)	19 (35.2)	0.240
	Rhinitis	24 (44.4)	18 (33.3)	0.236
	Ageusia	23 (42.6)	23 (42.6)	1.000
	Anosmia	23 (42.6)	23 (42.6)	1.000
	Anorexia	33 (61.1)	35 (64.8)	0.690
	Nausea and vomiting	18 (34.0)	18 (33.3)	0.945
	Diarrhea	12 (23.1)	11 (20.4)	0.735

[a] Data is represented by number (%).

Table 2
Vital signs of the patients.[a].

Sign	Time	group		P value
		Placebo	Violet syrup	
Fever	Before treatment	7 (13.0)	4 (7.4)	0.344
	After treatment	54 (100.0)	54 (100.0)	>0.05
Oxygen saturation	Before treatment	94.94 ± 2.13	94.56 ± 1.87	0.319
	After treatment	95 ± 3.43	94.7 ± 1.42	0.802
Pulse rate	Before treatment	91.91 ± 15.72	89.3 ± 14.04	0.367
	After treatment	85.67 ± 17.46	76.8 ± 13.5	0.230
Systolic blood pressure	Before treatment	112.2 ± 10.79	115.85 ± 9.94	0.077
	After treatment	123.33 ± 12.25	127.5 ± 8.58	0.398

[a] Data is represented by number (%) or mean ± standard deviation.

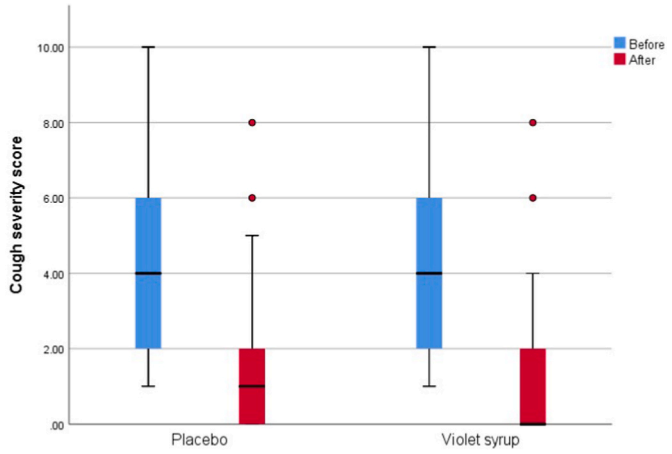


Fig. 5. Cough severity scores in the drug and placebo groups before and after the intervention.

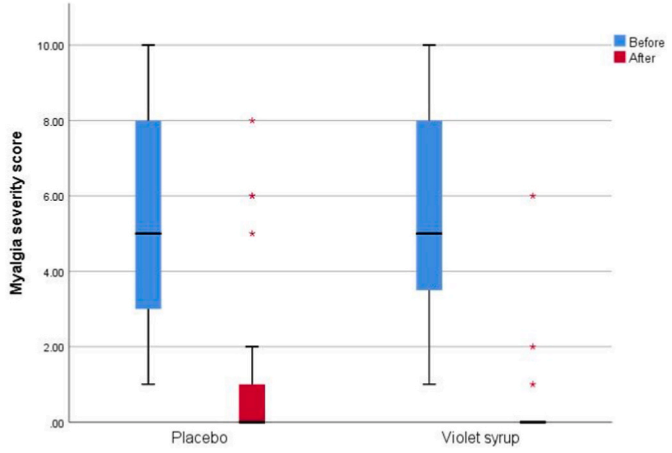


Fig. 6. Myalgia severity scores in the drug and placebo groups before and after the intervention.

cytokines(Funakoshi-Tago et al., 2020).

Cyclotides are among the prominent compositions in the Violaceae family. Recent investigations indicated that cyclotides exhibit antiviral activities via disruption of the viral envelope(Mammari et al., 2021). A

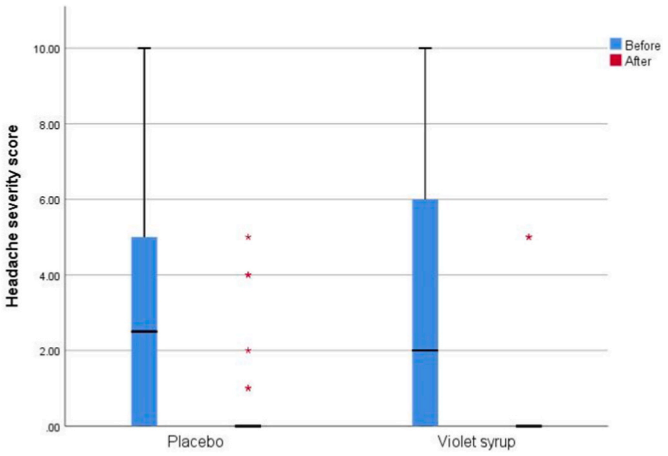


Fig. 7. Headache severity scores in the drug and placebo groups before and after the intervention.

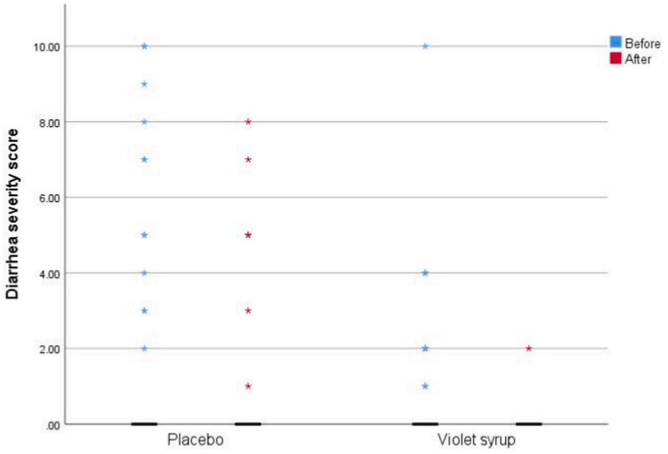


Fig. 8. Diarrhea severity scores in the drug and placebo groups before and after the intervention.

part of the therapeutic effect of violet syrup can be due to the presence of these compounds. In this regard, a clinical trial has been recently conducted on the effectiveness of cyclotide-rich syrup (composed of cyclotides of three herbs other than violet) as a prevention of disease complications in people exposed to COVID-19 virus (IRCT20160131026298N4) which has positive and hopeful results (unpublished data)(S, 2020).

Additionally, saponins with bronchosecretolytic effects would be associated with the therapeutic activities of violet syrup. They reduce the surface tension of lung secretions which facilitates their separation from the mucous membranes. Mucilage, another major substance of VE, can form a protective film over the inflamed mucous membranes and helps to reduce cough and inflammation(Bone K, 2013; Nosal'ova et al., 1992).

A previous clinical study showed that violet syrup enhances the cough suppression activity of salbutamol in children with intermittent asthma. In this double-blind randomized controlled trial, adding violet syrup to salbutamol completely controlled cough in more than 60 percent of the cases. In this study, cough suppression occurred only in 32% of people receiving a placebo and salbutamol ($P < 0.001$). Reduction in wheezing in pulmonary auscultation was also significantly different between both groups ($P < 0.001$). Beyond the expectorant and antitussive activity of saponins and mucilages, the authors suggested that inhibition of total immunoglobulin E and reduction of interleukin (IL)-4 and IL-13 levels can be proposed as the mechanisms of action of

Table 3
COVID-related symptoms.[a].

Symptom	Time	Group		P value
		Placebo	Violet syrup	
Dyspnea	Before treatment	4.23 ± 1.99	4.78 ± 2.66	0.151
	After treatment	1.04 ± 1.61	1.22 ± 2.41	0.230
Cough	Before treatment	4.66 ± 2.73	4.52 ± 2.75	0.725
	After treatment	1.71 ± 2.08	1.21 ± 2.19	0.025
Sputum	Before treatment	3.53 ± 2.06	3.35 ± 2.61	0.668
	After treatment	1.09 ± 1.34	1.4 ± 2.44	0.718
Myalgia	Before treatment	5.62 ± 2.69	5.78 ± 2.83	0.716
	After treatment	1.28 ± 2.34	0.47 ± 1.43	0.036
Headache	Before treatment	3.07 ± 3.32	3.21 ± 3.39	0.838
	After treatment	0.62 ± 1.37	0.33 ± 1.37	0.037
Abdominal pain	Before treatment	3.71 ± 2.39	3 ± 2.45	0.392
	After treatment	0.79 ± 1.67	0.63 ± 1.77	0.316
Dizziness	Before treatment	4.4 ± 2.09	4.03 ± 2.93	0.248
	After treatment	0.53 ± 0.92	0.89 ± 1.88	0.754
Chest pain	Before treatment	4.25 ± 2.34	4.45 ± 3.07	0.476
	After treatment	1.05 ± 2.38	1.08 ± 1.98	0.347
Fatigue	Before treatment	4.69 ± 3.53	4.33 ± 3.51	0.605
	After treatment	1.59 ± 2.38	1.33 ± 2.64	0.210
Sneeze	Before treatment	3.76 ± 2.74	2.84 ± 2.12	0.094
	After treatment	0.74 ± 1.1	0.78 ± 2.33	0.070
Rhinitis	Before treatment	1.89 ± 3.02	1.2 ± 2.13	0.176
	After treatment	0.62 ± 1.31	0.4 ± 1.38	0.221
Ageusia	Before treatment	6.3 ± 2.91	6.09 ± 3.44	0.897
	After treatment	2.26 ± 2.84	1.83 ± 3.56	0.054
Anosmia	Before treatment	6.78 ± 2.58	7.04 ± 3.23	0.883
	After treatment	2.21 ± 2.7	2.55 ± 3.78	0.087
Anorexia	Before treatment	6.76 ± 2.41	5.94 ± 3.13	0.706
	After treatment	0.96 ± 1.58	0.74 ± 1.82	0.586
Nausea and vomiting	Before treatment	4.83 ± 2.5	3.33 ± 2.68	0.269
	After treatment	0.08 ± 0.29	0.22 ± 0.44	0.747
Diarrhea	Before treatment	1.40 ± 2.90	0.63 ± 1.67	0.979
	After treatment	0.74 ± 2	0.07 ± 0.37	0.044

[a] Data is represented by mean ± standard deviation.

Table 4
Laboratory findings.[1].

Test	Time	Group		P value
		Placebo	Violet syrup	
White blood cells (× 10^3)	Before treatment	6.753 + 3.23	6.56 + 3.64	0.801
	After treatment	7.98 + 3.02**	6.9 + 1.42***	0.326
Neutrophil (%)	Before treatment	59.11 + 11.89	61.89 + 11.89	0.292
	After treatment	57.78 + 8.85	53.70 + 11.53	0.387
Lymphocyte (%)	Before treatment	30.49 + 10.69	28.21 + 11.02	0.343
	After treatment	30.66 + 8.19*	35.11 + 10.08	0.293
Red blood cells (× 10^3)	Before treatment	4.76 + 0.51	4.84 + 0.49	0.524
	After treatment	4.67 + 0.35*	4.72 + 0.59***	0.832
Hemoglobin (mg/dl)	Before treatment	13.39 + 1.64	13.33 + 1.53	0.862
	After treatment	13 + 1.26***	13.61 + 1.52**	0.341
Platelets (× 10^3)	Before treatment	210.18 + 8.718	186.4 + 53.49	0.136
	After treatment	298.7 + 91.99	216 + 48.99	0.022*
C-reactive protein (CRP)	Before treatment	23.24 + 24.12	19.09 + 12.89	0.060
	After treatment	26.50 + 25.92	18.60 + 13.90	0.033*
Erythrocyte sedimentation rate (ESR)	Before treatment	15.07 + 27.74	12.94 + 18.49	0.339
	After treatment	11.50 + 17.94	6.40 + 14.39	0.410

1. Data is represented by mean ± standard deviation; stars show the within-group analysis significant difference, significance level: *P < 0.05, **P < 0.01, and ***P < 0.001.

violet(Qasemzadeh et al., 2015). A better understanding of the mechanism of action of *Viola odorata* in the treatment of inflammatory conditions of the lung has been made possible by recent animal studies. In an animal-model study that evaluated the prophylactic and therapeutic roles of violet in the treatment of formalin-induced lung damage, both aqueous extract of Viola flower and hydrocortisone significantly and similarly reduced hemorrhage area, alveolar wall thickness, alveolar septum rupture, and epithelial lining alteration of bronchioles(Koochek et al., 2003). *Viola tricolor* L. with similar active ingredients has also been shown to decrease IL-4 levels and increase INF-ɣ levels in a mouse model of allergic asthma Induced by ovalbumin (Harati et al., 2018). Recent investigations show the role of T helper-2 cytokines especially IL-4 in lung damage from COVID-19 infection(Vaz de Paula et al., 2020). It seems that some parts of the therapeutic activities of violet syrup are related to the effects of the VE on lung tissue IL-4.

Based on the inflammatory root of COVID-19 myalgia(Hasan et al., 2021), the anti-muscle-pain effect of violet syrup would be due to its anti-inflammatory activities. Moreover, the analgesic activity of Viola extracts (especially aqueous extract) has been previously shown in rats. This article revealed that oral VE can reduce both central and peripheral pains(Antil et al., 2011). This evidence could also justify the effect of violet syrup on headaches.

Violet also has been shown as an anti-diarrheal medicinal plant in an animal study. Janbaz KH et al. showed the relaxation of K+ (80 mM)-induced contractions and shifted Ca2+ concentration-response curves toward the right in isolated jejunum similar to verapamil as a confirmation for Ca2+ channel blocking activity. Those researchers concluded that this finding provides an objective rationale for the folkloric use of *Viola odorata* to control diarrhea(Janbaz et al., 2015).

There are limitations to this study. In order to determine the efficacy of the treatment of viral proliferation, virological tests can accurately clarify the specific mechanisms of action of the intervention. Since the virological survey requires high-tech and expensive instruments and it was beyond the budget of our study, this was a limitation to evaluate virological clearance. Additionally, due to COVID-19-induced anxiety and fear, convincing patients to follow the standard protocol was somewhat difficult. Hence, patients were followed by frequent phone calls. Although this study showed significant clinical effects of violet syrup on COVID manifestations, further studies with larger sample sizes seem to be beneficial.

5. Conclusion

This study showed that *Viola odorata* L. effectively controls prevalent manifestations of COVID-19 including cough, myalgia, headache, and diarrhea. Regarding this survey, violet syrup can be mentioned as a complementary treatment for viral influenza-like infections in which cough, myalgia, headache, and diarrhea are prominent. Probable

ingredients responsible for these functions are caffeic acid, pyrocatechol, cyclotide, saponin, and mucilage. The underlying mechanisms for those effects seem to be a reduction in lung inflammation.

CRediT authorship contribution statement

Mohammad Sadegh Adel Mehraban: Formal analysis, Investigation, Project administration, Funding acquisition. **Meysam Shirzad:** Conceptualization, Methodology, Software, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Leila Mohammad Taghizadeh Kashani:** Resources, Writing – original draft, Funding acquisition. **Mohammad Mahdi Ahmadian-Attari:** Conceptualization, Data curation, Writing – review & editing. **Ali Akbar Safari:** Validation, Data curation. **Narges Ansari:** Investigation, Supervision, Project administration, Funding acquisition. **Hossein Hatami:** Conceptualization, Methodology, Supervision. **Mohammad Kamalinejad:** Conceptualization, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Abbreviations:

Traditional Persian Medicine TPM
Coronavirus Disease 2019 COVID-19
Severe Acute Respiratory Syndrome CoronaVirus-2 SARS-CoV-2
Acute Respiratory Distress Syndrome ARDS
Violet Extract VE
Visual Analogue Scale VAS
Common Terminology Criteria for Adverse Events CTCAE

References

Abebe, E.C., Dejenie, T.A., Shiferaw, M.Y., Malik, T., 2020. The newly emerged COVID-19 disease: a systemic review. Virol. J. 17 (1), 1–8.
Abumansour, H.M., 1967. Abniah 'an Haqaeqe Al-Adviah. Tehran University Press, Tehran.
Adem, Ş., Eyupoglu, V., Sarfraz, I., Rasul, A., Zahoor, A.F., Ali, M., Abdalla, M., Ibrahim, I.M., Elfiky, A.A., 2021. Caffeic acid derivatives (CAFDs) as inhibitors of SARS-CoV-2: CAFDs-based functional foods as a potential alternative approach to combat COVID-19. Phytomed 85, 153310.
Andrea Peirce, C.P., 2007. PDR for Herbal Medicines, fourth ed. Medical Economics.
Antil, V., Kumar, P., Kannappan, N., Diwan, A., Saini, P., Singh, S., 2011. Evaluation of the analgesic activity of Viola odorata aerial parts in rats. J. Nat. Pharm. 2 (1), 24. -24.
S, S., 2020. Pilot clinical trial to evaluate the effectiveness of Herbal Cyclotide complex syrup as a prevention of disease complications in people exposed to COVID-19 virus. Bagheiat-allah Univ. Med. Sci.
Avicenna, 2005. Canon of Medicine Dar Ehya toras al-arabi. Beirut.
Bai, Z., Li, P., Wen, J., Han, Y., Cui, Y., Zhou, Y., Shi, Z., Chen, S., Li, Q., Zhao, X., Wang, Z., Li, R., Guo, Y., Zhan, X., Xu, G., Ding, K., Wang, J., Xiao, X., 2022. Inhibitory effects and mechanisms of the anti-covid-19 traditional Chinese prescription, Keguan-1, on acute lung injury. J. Ethnopharmacol. 285, 114838.
Bone, K., S, M., 2013. Principles and Practice of Phytotherapy, Modern Herbal Medicine, second ed. Elsevier Ltd.
Chen, Y., Liu, C., Wang, T., Qi, J., Jia, X., Zeng, X., Bai, J., Lu, W., Deng, Y., Zhong, B., He, W., Xing, Y., Lian, Z., Zhou, H., Yan, J., Yang, X., Yu, H., Zhou, J., Zhou, D., Qiu, L., Zhong, N., Wang, J., 2022. Efficacy and safety of Bufei Huoxue capsules in the management of convalescent patients with COVID-19 infection: a multicentre, double-blind, and randomised controlled trial. J. Ethnopharmacol. 284, 114830.
Cui, J., Li, F., Shi, Z.-L., 2019. Origin and evolution of pathogenic coronaviruses. Nat. Rev. Microbiol. 17 (3), 181–192.
De Felice, F.G., Tovar-Moll, F., Moll, J., Munoz, D.P., Ferreira, S.T., 2020. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the central nervous system. Trends Neurosci. 43 (6), 355–357.
Duke, J.A., 2002. Handbook of Medicinal Herbs. CRC Press.
Ebrahimi, Y., 2020. Iranian National Guideline for Diagnosis and Treatment of COVID-19 in Outpatient and Inpatient Services, ninth ed. Iranian Ministry of Health.
Fauci, A.S., Lane, H.C., Redfield, R.R., 2020. Covid-19—navigating the uncharted. Mass. Med. Soc.
Fazil, M., Nikhat, S., 2022. Therapeutic and palliative role of a unani herbal decoction in COVID-19 and similar respiratory viral illnesses: phytochemical & pharmacological perspective. J. Ethnopharmacol. 115526.
Figueiredo-Campos, P., Blankenhaus, B., Mota, C., Gomes, A., Serrano, M., Ariotti, S., Costa, C., Nunes-Cabaço, H., Mendes, A.M., Gaspar, P., 2020. Seroprevalence of anti-SARS-CoV-2 antibodies in COVID-19 patients and healthy volunteers up to 6 months post disease onset. Eur. J. Immunol. 50 (12), 2025–2040.
Funakoshi-Tago, M., Nonaka, Y., Tago, K., Takeda, M., Ishihara, Y., Sakai, A., Matsutaka, M., Kobata, K., Tamura, H., 2020. Pyrocatechol, a component of coffee, suppresses LPS-induced inflammatory responses by inhibiting NF-κB and activating Nrf2. Sci. Rep. 10 (1), 1–17.
Harati, E., Bahrami, M., Razavi, A., Kamalinejad, M., Mohammadian, M., Rastegar, T., Sadeghipour, H.R., 2018. Effects of viola tricolor flower hydroethanolic extract on lung inflammation in a mouse model of chronic asthma. Iran. J. Allergy, Asthma Immunol. 409–417.
Hasan, L.K., Deadwiler, B., Haratian, A., Bolia, I.K., Weber, A.E., Petrigliano, F.A., 2021. Effects of COVID-19 on the musculoskeletal system: clinician's guide. Orthop. Res. Rev. 13, 141.
Hong, J.-M., Hu, L.-H., Zhong, Q.-S., Zhu, L.-C., Hang, Y.-P., Fang, X.-Y., Sun, H.-B., Huang, Z.-H., Xu, J., Chen, Y.-H., 2020. Epidemiological characteristics and clinical features of patients infected with the COVID-19 virus in Nanchang, Jiangxi, China. Front. Med. 7.
Jamshed, H., Siddiqi, H.S., Gilani, A.U.H., Arslan, J., Qasim, M., Gul, B., 2019. Studies on antioxidant, hepatoprotective, and vasculoprotective potential of Viola odorata and Wrightia tinctoria. Phytother Res. 33 (9), 2310–2318.
Janbaz, K.H., Khan, W.U., Saqib, F., Khalid, M., 2015. Pharmacological basis for the medicinal use of viola odorata in diarrhea, bronchial asthma and hypertension. Bangladesh. J. Pharmacol. 10 (4), 836–843.
Jorjani, 2001. Zakhireye Kharazm Shahi (Treasure of Kharazm Shah). Iranian Medical Academy, Tehran.
Karioti, A., Furlan, C., Vincieri, F.F., Bilia, A.R., 2011. Analysis of the constituents and quality control of Viola odorata aqueous preparations by HPLC-DAD and HPLC-ESI-MS. Anal. Bioanal. Chem. 399 (4), 1715–1723.
Koochek, M., Pipelzadeh, M., Mardani, H., 2003. The effectiveness of Viola odorata in the prevention and treatment of formalin-induced lung damage in the rat. J. Herbs, Spices, Med. Plants 10 (2), 95–103.
Lai, C.-C., Shih, T.-P., Ko, W.-C., Tang, H.-J., Hsueh, P.-R., 2020. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): the epidemic and the challenges. Int. J. Antimicrob. Agents 55 (3), 105924.
Lamaison, J., Petitjean-Freytet, C., Carnat, A., 1991. La fleur de violette: étude comparée de Viola lutea huds., V. Calcarata L. et V. Odorata L. Plant. Med. Phytother. 25 (2–3), 79–88.
Ma, Q., Xie, Y., Wang, Z., Lei, B., Chen, R., Liu, B., Jiang, H., Wang, Y., Liu, Q., Yang, Z., 2021. Efficacy and safety of ReDuNing injection as a treatment for COVID-19 and its inhibitory effect against SARS-CoV-2. J. Ethnopharmacol. 279, 114367.
Mahboubi, M., Kashani, L.M.T., 2018. A Narrative study about the role of Viola odorata as traditional medicinal plant in management of respiratory problems. Adv. Integr. Med. 5 (3), 112–118.
Mammari, N., Krier, Y., Albert, Q., Devocelle, M., Varbanov, M., OEMONOM, 2021. Plant-derived antimicrobial peptides as potential antiviral agents in systemic viral infections. Pharmaceuticals 14 (8), 774.
Mittal, P., Gupta, V., Goswami, M., Thakur, N., Bansal, P., 2015. Phytochemical and pharmacological potential of viola odorata. Int. J. Pharmacogn. 4, 693.
Mosavat, S.H., Marzban, M., Bahrami, M., Parvizi, M.M., Hajimonfarednejad, M., 2017. Sexual headache from view point of Avicenna and traditional Persian medicine. Neurol. Sci. 38 (1), 193–196.
Muhammad, N., Saeed, M., Qayum, M., Khan, H., 2013. Anti-microbial Screening o f Viola betonicifolia. Middle. East. J. Sci. Res. 15, 55–60.
Nimrouzi, M., Daneshfard, B., Tafazoli, V., Akrami, R., 2019. Insomnia in traditional Persian medicine. Acta. Med. Hist. Adriat. 17 (1), 45–54.
Nosal'ova, G., Strapková, A., Kardosová, A., Capek, P., Zathurecky, L., Bukovská, E., 1992. Antitussive Wirkung des Extraktes und der Polysaccharide aus Eibisch (Althaea officinalis L., var. robusta). Pharm. Times 47 (3), 224–226.
Porrostami, K., A, S., Ahmadian-Attari, M.M., 2020. The effect of Viola Syrup with acetaminophen syrup in comparison to acetaminophen syrup and placebo on the fever of children A Randomized and double blind clinical trial. Alborz Univ. Med. Sci.
Qasemzadeh, M.J., Sharifi, H., Hamedanian, M., Gharehbeglou, M., Heydari, M., Sardari, M., Akhlaghdoust, M., Minae, M.B., 2015. The effect of Viola odorata flower syrup on the cough of children with asthma: a double-blind, randomized controlled trial. J. Evid. Based. Complementary. Altern. Med. 20 (4), 287–291.
Skipper, C.P., Pastick, K.A., Engen, N.W., Bangdiwala, A.S., Abassi, M., Lofgren, S.M., Williams, D.A., Okafor, E.C., Pullen, M.F., Nicol, M.R., 2020. Hydroxychloroquine in

nonhospitalized adults with early COVID-19: a randomized trial. Ann. Intern. Med. 173 (8), 623–631.
Tafazoli, V., Shahriari, M., Heydari, M., Nikbakht, H.A., Zarshenaas, M.M., Nimrouzi, M., 2020. The effect of Viola Odorata L. Oil for fever in children: a randomized triple-blinded placebo-controlled clinical trial. Curr. Drug Discov. Technol. 17 (5), 696–703.
Tavassoli, S., Eftekhari, K., Karimi, M., Ghobadi, A., Shati, M., Naddaf, A., Abbassian, A., 2021. Effectiveness of viola flower syrup compared with polyethylene glycol in children with functional constipation: a randomized, active-controlled clinical trial. Evid. Based. Complement. Alternat. Med. eCAM 2021, 9915289.
Vaz de Paula, C.B., de Azevedo, M.L.V., Nagashima, S., Martins, A.P.C., Malaquias, M.A. S., Miggiolaro, A.F.R.d.S., da Silva Motta Júnior, J., Avelino, G., do Carmo, L.A.P., Carstens, L.B., 2020. IL-4/IL-13 remodeling pathway of COVID-19 lung injury. Sci. Rep. 10 (1), 1–8.
Weiss, P., Murdoch, D.R., 2020. Clinical course and mortality risk of severe COVID-19. Lancet 395 (10229), 1014–1015.
Weng, J.R., Lin, C.S., Lai, H.C., Lin, Y.P., Wang, C.Y., Tsai, Y.C., Wu, K.C., Huang, S.H., Lin, C.W., 2019. Antiviral activity of Sambucus FormosanaNakai ethanol extract and related phenolic acid constituents against human coronavirus NL63. Virus Res. 273, 197767.
Whittaker, A., Anson, M., Harky, A., 2020. Neurological manifestations of COVID-19: a systematic review and current update. Acta Neurol. Scand. 142 (1), 14–22.
World Health Organisation, 2022. Health Emergency Dashboard (covid-19). https://covid19.who.int. (Accessed 10 September 2022).
Xu, J., Ma, X.-P., Bai, L., Wang, M., Deng, W., Ning, N., 2020. A systematic review of etiology, epidemiology, clinical manifestations, image findings, and medication of 2019 Corona Virus Disease-19 in Wuhan. China. Med. 99 (42), e22688.
Yang, Y., Islam, M.S., Wang, J., Li, Y., Chen, X., 2020. Traditional Chinese medicine in the treatment of patients infected with 2019-new coronavirus (SARS-CoV-2): a review and perspective. Int. J. Biol. Sci. 16 (10), 1708.
Yifan, C., Jun, P., 2020. Understanding the clinical features of coronavirus disease 2019 from the perspective of aging: a systematic review and meta-analysis. Front. Endocrinol. 11, 557333.