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Investigation on chemical composition, antioxidant activity and SARS-CoV-2 nucleocapsid protein of endemic *Ferula longipedunculata* Peşmen

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SUMMARY: The essential and fatty oils were investigated and a quantitative analysis of the root, green and stem parts of *F. Longipedunculata* was performed by GC-MS and HPLC-TOF/MS and their antioxidant (DPPH method) activities and potential binding of phytochemicals against SARS-CoV-2 nucleocapsid were determined using Molegro Virtual Docker software. In the root part of the plant, the prominent components of oil were *β*-phellandrene (53.46%), ocimene (6.79%), 4-terpineol (5.94%) and santalol (5.03%). According to the quantitative results, vanillic acid (141.35 mg/kg), ferulic acid (126.19 mg/kg) and 4-hydroxybenzoic acid (119.92 mg/kg) were found in the roots; quercetin-3-*β*-*O*-glycoside (1737.70 mg/kg), quercetin (531.35 mg/kg) and ferulic acid (246.22 mg/kg) were found in the in the green part; and fumaric acid (2100.21 mg/kg), quercetin-3-*β*-*O*-glycoside (163.24 mg/kg), vanillic acid (57.59 mg/kg) were detected in the stem part. The antioxidant activity of all parts of the plant was higher than the control with BHT. Silibinin, rutin, and neohesperidin exhibited a stronger affinity than nucleotides. In the silico analysis, many of the phytochemicals were attached with strong hydrogen-bonds and electrostatic effects to the amino acids to which nucleotides are bound. The results indicated that the plant showed antioxidant effects and can be effective against SARS-CoV-2 thanks to the different phytochemical compounds it contains.

KEYWORDS: *Antioxidants; Chemical composition;* Ferula longipedunculata *Peşmen; COVID 19; SARS-CoV-2*

RESUMEN: ***Investigación sobre la composición química, la actividad antioxidante y la proteína nucleocápsida del SARS-CoV-2 de la endémica* Ferula longipedunculata *Peşmen.*** Se analizó el aceite esencial y la grasa de la raíz, la parte verde y el tallo de F. *Longipedunculata* mediante GC-MS y HPLC-TOF/MS y sus actividades antioxidantes (método DPPH) y posible unión de fitoquímicos contra el SARS-CoV-2 nucleocápside utilizando el software Molegro Virtual Docker. En la parte de la raíz de la planta, los componentes prominentes del aceite fueron *β*-felandreno (53,46%), ocimeno (6,79%), 4-terpineol (5,94%) y santalol (5,03%). Los resultados cuantitativos mostraron los siguientes valores: ácido vainílico (141,35 mg/kg), ácido ferúlico (126,19 mg/kg) y ácido 4-hidroxibenzoico (119,92 mg/kg) en la raíz, quercetina-3-*β*-*O*-glucósido (1737,70 mg/kg), quercetina (531,35 mg/kg) y ácido ferúlico (246,22 mg/kg) en la parte verde y ácido fumárico (2100,21 mg/kg), quercetina-3-*β*-*O*-glucósido (163,24 mg/kg) y ácido vainílico (57,59 mg/kg) en la parte del tallo, respectivamente. La actividad antioxidante de todas las partes de la planta fue mayor que el control de BHT. La silibinina, la rutina y la neohesperidina exhibieron una afinidad más fuerte que los nucleótidos. En el análisis silico, muchos de los fitoquímicos se pueden unir con fuertes enlaces de hidrógeno y con efectos electrostáticos a los aminoácidos a los que se unen los nucleótidos. Los resultados indicaron que la planta tiene un efecto antioxidante y puede ser eficaz contra el SARS-CoV-2 gracias a los diferentes compuestos fitoquímicos que contiene.

PALABRAS CLAVE: *Antioxidante; Composición química; COVID-19;* Ferula Longipedunculata *Peşmen; SARS-CoV-2*

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1. INTRODUCTION

The Apiaceae family consists of flowering and aromatic plants which are best known for their characteristic flowers, fruits (Heywood, 2007) and volatile substances (Widodo *et al*., 2014). *Ferula longipedunculata* Peşmen, Apiaceae, is a wild plant which is indigenous to Turkey. It grows in the central Anatolia region of the country. This plant has been used in Turkish folk medicine for stomach pain and as a wound healing remedy. Also, the roots and leaves of the *Ferula* plant are consumed as tea in Antolia in order to increase the aphrodisiac effect and sperm count. It has also been reported to be used to increase milk yield and fertility in goats and sheep (Pakdemirli, 2020). The *Ferula* species has been the subject of many studies on the chemicals often used in the characterization of compounds identified in the world as well as in the medical field. In the biochemical analysis, coumarins, methanolic, benzoic acid, antibacterial sesquiterpenes, ferulenol, terpenoids, steroidal esters, methanol, ethanol, sulfides, sinkiangenorin C have been found in many compounds and have been reported to be used in medicine (Duran *et al*., 2020; Li *et al*., 2015; Yang *et al*., 2006).

Antioxidants are gaining importance in the human health and food industry worldwide. Antioxidants are substances that prevent the easy degradation of the structure even in small quantities and the deterioration of the structure of oxidized substances (Brewer, 2011). Antioxidants are the main defense mechanism in the body and act as free-radical scavengers. They are manufactured inside the body and involve catalase, dismutase and peroxidase enzymes. BHT is the most widely used antioxidant and is a lipophilic organic compound, chemically a derivative of phenol, which is beneficial for antioxidant activity. Its aims to decelerate the effect of free-radical deterioration in several areas, especially the food, biomedical, rubber, plastic, oil, and petroleum industries (Yehye *et al*., 2015)

SARS coronavirus-2 (SARS-CoV-2) is a pathogen which is easily transferred from human to human. It is the main cause of the worldwide pandemic with serious diseases and death rates (Raoult *et al*., 2020). The coronavirus nucleocapsids (N) play a delicate role in improving the activity of virus transcription and assembly. Therefore, they were suggested as targets for drugs to combat CoVs (McBride *et al*., 2014). Plants are rich sources of natural compounds with antiviral effects (Sytar *et al.,* 2021). The therapeutic potential of many phytochemicals has been reported with *in silico* techniques to combat coronavirus (Adem *et al*., 2020; Galanakis *et al*., 2020). Molecular docking studies are actively used to describe biologically active compounds with the potential to bind the SARS-CoV-2 Nucleocapsid protein. However, no biotechnologically detailed studies on *Ferula longipedunculata* Peşmen plant have been found.

The aim of this study was to investigate the affinities of the phytochemicals found in the Endemic *Ferula longipedunculata* Peşmen towards SARS-CoV-2 nucleocapsid in *silico*. The constituents of the root, stem and green parts of the plant were investigated as the main reason for the chemical composition, antioxidant activities and SARS-CoV-2 nucleocapsid of *Ferula longipedunculata* Peşmen.

2. MATERIALS AND METHODS

2.1. Plant Material

Parts of *Ferula longipedunculata* Peşmen were collected from the Berit mountain province, (Figure 1), central Anatolia, Turkey during the flowering stage (June 15, 2015). After identification of the plant by Prof. Dr. Ömer Saya, a voucher (No. 1416) was deposited in the KOSAF herbarium of Turkey. The collected plant materials were air-dried in the shade.

2.2. Extraction Procedure

122 g (root), 82 g (stem) and 75 g (green-aerial) parts of the plant were dried at room temperature and cut into small pieces before being macerated three times (24h each time) with methanol/H_2O (80%). After filtration and evaporation, the obtained extract was partitioned with solvents in increasing polarity: chloroform, ethyl acetate and *n*-butanol. Each extract was evaporated under reduced pressure. The obtained extract contained (6.1 g root) $CHCl_3$, (0.9 g stem) EtOAc and (1.3 g green part) *n*-BuOH. Antioxidant activity analyses were performed with 10 grams of each plant material set on a balloon flask and 100 ml methanol and acetone solvents were added to each one. Extraction was then carried out for two hours, using conventional extraction methods (Khan *et al*., 1988).

FIGURE 1. *Ferula Longipedunculata* growing collection location Berit mount, Kahramanmaraş, Turkey. longitude: 37° 30' 93' 70'' E; latitude: 42° 031' 22'' N; altitude: 2100-2409 m above sea level

2.3. Isolation of the essential oils

The air-dried root of *F. longipedunculata* was subjected to methanol-distillation for 2 hours, using a Clevenger-type apparatus, according to the method recommended by the (European Pharmacopia procedure, 1983) to produce oils. The obtained essential oil was dried and after filtration, and stored at 4 °C until analysis.

2.4. Gas Chromatography (GC)

Fatty acids were analyzed by GC-MS (Agilent Technologies 7890A model GC system, 5975C inert MSD with Triple-Axis Detector/USA) using a BPX-20 capillary column (30 m x 0.25 mm, 0.25 µm film thickness; 5% phenyl polysilphenyl IN-siloxane), 70 eV ionization voltage, and FID detector. The oven temperature was between 50 and 120 ºC at 5 ºC/min and 120-240 ºC at 10 ºC/min and held for 5 minutes. 1.0 µL of diluted extracts 300:1 was injected in the split mode. The injector and detector temperatures were adjusted to 220 ºC and 290 ºC, respectively. Helium was used as carrier gas at a flow rate of 1 mL/min. The samples were determined with 1/1000 dilutions (Demirtas and Sahin, 2013).

2.5. Gas Chromatography/Mass spectrometry (GC/MS)

GC/MS analysis was performed by gas chromatography-mass spectrometer using a BPX20 column with autosampler and column (30 m x 0.25 mm x 0.25 µm film). A GC/MS detection system was used for electron ionization (ionization energy 70 eV). Helium was used as carrier gas at a a flow rate of 1.3 mL/min and diluted to 1/1000 (Demirtas and Sahin, 2013).

2.6. Molecular Docking Study

The docking studies used Molegro Virtual Docker software. The Crystal Structure of the N-terminal RNA binding domain of the SARS-CoV-2 nucleocapsid protein (PDB ID:6M3M) was downloaded from the online PDB database (www.pdb.org), and prepared for molecular docking using Molegro Virtual Docker Tools. The score function used was the MolDock score with the coordinates of the position X: 8.50 Y: -34.91 and Z:-28.06 at 16 Å3 radius, and 0.30 grid resolution. The docking region of the protein was selected according to previously reported studies (Dinesh *et al*., 2020; Kang *et al*., 2020). The 3D structure of the phytochemicals was downloaded from the website https://www.ncbi.nlm.nih.gov/pccompound, and geometrically optimized utilizing MarvinSketch 19.27 software.

2.7. Quantitative analysis by HPLC-TOF/MS

A HPLC analysis was performed with an Agilent Technology 1260 Infinity HPLC System equipped with 6210 Times of flight (TOF) LC/MS detector and ZORBAX SB-C18 (4.6 x100 mm, 3.5 µm) column. Mobile phases A and B were ultra-pure water

with 0.1% formic acid and acetonitrile, respectively. The flow rate was 0.6 mL/min and column temperature was 35 ºC. Injection volume was 10 µL. The solvent program was as follow: 0-1 min 10% B; 1-20 min 50% B; 20-23 min 80% B; 23-30 min 10% B. Ionization mode of HPLC-TOF/MS instrument was negative and operated with a nitrogen gas at 325 ºC, nitrogen gas flow of 10.0 L/min, nebulizer of 40 psi, a capillary voltage of 4000 V and finally, fragmentor voltage of 175 V. For sample analysis, dried crude extracts (200 ppm) were dissolved in methanol at room temperature. Samples were filtered through a PTFE (0.45µm) filter with an injector to remove particulates (Demirtas and Sahin, 2013; Abay G *et al*., 2015).

2.8. DPPH radical-scavenging activity

Different methods can be used to evaluate antioxidant activity but a rapid, simple and inexpensive method to measure the antioxidant capacity of food is DPPH, which is widely used to test the ability of compounds to act as free-radical scavengers or hydrogen donors and to evaluate antioxidant activity (Kedare SB *et al*., 2011).

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for the investigation of the free-radical scavenging activity of the extracts (Nabavi *et al*., 2008). Different concentrations of extract were added to the same volume of a methanol and acetone solution of DPPH (100 mM). Absorbance was recorded at 517 nm after 30 min in the dark at room temperature for reaction to take place. All tests were carried out three times. BHT was used for standard controls. The inhibition of free-radical DPPH in percent (I%) was calculated as follows:

$$I\% = [(A_{blank}-A_{sample})/A_{blank}]x100,$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound.

3. RESULTS and DISCUSSION

3.1. Chemical composition of the fatty acids

The analysis of fatty acid compositions of root, green and stem parts of *F. longipedunculata* plant was performed using gas chromatography (GC-MS). The results obtained from the GC and GC–MS analysis of the fatty acids of the plant are presented in Table 1. 16, 6 and 4 components of the root, green and stem parts, respectively, were identified as fatty acids representing 100%. For all parts (root, green and stem) the major compound was linoleic acid at 70.37, 35.38 and 53.58%, respectively. Our research showed that the stem part had more fatty acid than the root and green parts. A literature search showed that *Ferula* oils are rich in fatty acids (El-feraly and Khan, 2001; Garg and Agarwal, 1988; Nagatsu *et al*., 2002).

3.2. Chemical composition of the essential oil

The GC-MS analysis of the essential oil of the *F. longipedunculata* root part is presented in Table 2. Eighteen compounds, representing 99.9% of the essential oil, were identified and characterized. Monoterpene *β*-phellandrene (53.46%) was the major compound in this plant. Other major monoterpene compounds included ocimene (6.79%), 4-terpineol (5.94%) and sesquiterpene santalol (5.03%).

After comparing the chemical composition of *Ferula longipedunculata* essential oil with other species of the *ferula* genus some differences and similarities were found. The main key components of the essential oil of *Ferula persica* were dillapiole (57.3%) and elemicine (5.6%) (Javidnia *et al*., 2005). Guaiol (58.76%), (E)-nerolidol (10.16%) and α-eudesmol (3.05%) were found to be the major (key) compounds of the oil of *Ferula ferulaoides* (Shatar, 2005). These components were not present in *Ferula longipedunculata* essential oil.

In the essential oil analysis of *Ferula elaeochytris* with GC-MS, nonane (27.1%), *α*-pinene (12.7%) and germacrene B (10.3%) were obtained as the main compounds (Başer *et al.*, 2000). In a study conducted in Iran, the essential compounds of the *Ferula szowitsiana* plant were obtained as *α*-pinene (12.6%), germacrene D(12.5%) and *β*-pinene (10.1%) (Rustaiyan *et al*., 2006). As expected, compounds such as *α*-pinene and *β*-pinene were not obtained as the main compounds for the *F. longipedunculata* plant. In addition, *α*-pinene was identified in the *Ferula longipedunculata.*

Moreover, the major components in the oil of *F. gummosa* were found to be *β*-pinene (50.1%), *α*-pinene (18.3%), 3-carene (6.7%), *α*-thujene (3.3%) and sabinene (3.1%) (Eftekhar *et al*., 2004).

Table 1. The fatty acid composition of the root, green and stem parts of *Ferula longipedunculata*

No	Compounds[a]	RT	% in oil		
			Root	Green	Stem
1	γ-cadinene	17.742	0.35	-	-
2	Acoradien	19.762	0.54	-	-
3	Bisabolene	20.203	0.70	-	-
4	Bicyclo[3.3.1]nonane-2,6-diol	20.947	0.46	-	-
5	Sesquisabinene hydrate	22.475	1.04	-	-
6	p-Mentha-2,8-diene, 1-hydroperoxide	22.961	0.70	-	-
7	2,3-Dimethylhydroquinone	23.562	0.93	-	-
8	trans-8-Hydroxy-bicyclo(4,3,0)non-3-ene	23.682	0.41	-	-
9	3-Hydroxy-2-(2-methylcyclohex-1-enyl)propionaldehyde	24.380	1.03	-	-
10	Pentadecanoic acid, methyl ester	24.912	1.44	-	-
11	6-[1-(Hydroxymethyl)vinyl]-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-2(1H)-naphthalenone	25.301	2.85	-	-
12	Palmitic acid methyl ester	27.579	8.62	22.16	24.76
13	Cyclododecane methanol	31.092	0.53	-	-
14	Linoleic acid, methyl ester	32.173	70.37	35.38	53.58
15	9- octadecanoic acid, methyl ester	32.248	9.30	-	-
16	Linolenic acid, methyl ester	32.276	-	25.04	12.47
17	Octadecanoic acid, methyl ester	32.562	0.74	4.26	-
18	3-Heptadecen-5-yne	26.926	-	6.01	-
19	Phytol	32.431	-	7.15	-
20	Stearic acid, methyl ester	32.563	-	-	9.19

[a]Compounds are listed in order of their elution from the BPX-20 capillary column, RT-retention time. min.

Table 2. Chemical composition of the essential oil of *F. longipedunculata* root parts

No	Compounds[a]	RT	% Composition
1	*α*-Thujene	12.411	0.8±0.43
2	*α*-Pinen	12.711	1.41±0.05
3	*β*-Phellandrene	14.003	53.46±0.64
4	beta-Myrcene	14.328	0.91±0.05
5	*α*-Terpinen	15.387	1.46±0.06
6	*β*-Cymene	15.657	4.12±0.08
7	*α* -Pinen	15.887	1.89±0.13
8	Ocimene	16.295	6.79±0.01
9	γ-Terpinen	16.822	3.98±0.12
10	2,3-Heptadien-5-yne, 2,4-dimethyl-	17.556	1.99±0.11
11	2,3,4,5-Tetramethylcyclopent-2-en-1-ol	20.681	1.17±0.05
12	4-Terpineol	21.163	5.94±0.01
13	Santalol	30.721	5.03±0.05
14	Epiglobulol	32.844	2.18±0.02
15	alpha-Caryophyllene	33245	2.47±0.08
16	6-[1-(Hydroxymethyl)vinyl]-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-2(1H)- naphthalenone	34.330	0.94±0.03
17	6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2one	34.579	2.32±0.04
18	alpha-Bisabolol	37.069	3.13±0.01
Total			99.99

[a]Compounds are listed in order of their elution from the BPX-20 capillary column, RT-retention time. min.

The genera *Ferula* is rich in its essential content, which is also named Ferula oil. Of the genera, *F. assafoetida, F. gummosa* and *F. badrakema* contain essential oils. Those essential oils give a strong aromatic smell to the plant species. Furthermore, these oils have been documented to possess antifungal and antibacterial activities (Sahebkar and Iranshahi, 2011). Among the components of the essential oil, alpha-pinene and beta-pinene are of the major compounds (Benevides *et al*., 2001; Kim *et al*., 2006).

3.3. Identification and quantification of phenolic acids by HPLC-TOF/MS

The *n*-BuOH extract was obtained from the root, green and stem parts of *Ferula longipedunculata* and analyzed by HPLC-TOF/MS. The identification was performed based on their retention times and mass spectrometry by comparison with those of different standards. The results show the presence of 43 compounds including 17 organic and phenolic acids (Table 3), 26 flavonoids and phenolics (Table 4). Some phenolics were detected in a very small amount and barely reached detection limits (trace) because their concentration had not been seen. The main compounds of *F. longipedunculata* were fumaric acid, quercetin-3-*β*-D-glucoside, quercetin, ferulic acid, vanillic acid, and 4-hydroxybenzoic acid. The highest amounts were determined as vanillic acid in the root part, quercetin-3-*β*-D-glucoside in the green part and fumaric acid in the stem part. The green part of the plant contains more flavonoids than other parts of the plant. In terms of the phenolic acid richness of the plant parts, it was determined as stem, green and root part, respectively. As a result, *F. longipedunculata* is rich in flavonoids and phenolic compounds.

3.4. DPPH radical-scavenging Activity

The antioxidant activity may be due to different mechanisms, such as the decomposition of peroxides, prevention of chain initiation, reducing capacity, prevention of continued hydrogen abstraction, free-radical scavenging and binding of transition metal ion catalysts (Mao *et al*., 2006). The radical scavenging activity of organic extracts was determined from the reduction in the optical absorbance at 517 nm due to the scavenging of stable DPPH free radicals. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen contribution ability. DPPH is a stable free radical and accepts an elec-

Table 3. Quantitative results of organic and phenolic acids in plant extracts (mg phenolic/kg plant)

Organic and phenolic acids	RT	Root	Green	Stem
Gallic acid	2.4	Trace	Trace	Trace
Fumaric acid	3.2	75.49±2.14[a]	6.63±0.51[a]	2100.21±5.15[a]
Gentisic acid	4.5	24.73±1.04	120.21±2.89	16.51±1.53
Chlorogenic acid	5.5	5.13±0.01	72.35±1.95	16.19±1.06
4-Hydroxybenzoic acid	7.0	119.92±0.29	183.42±1.24	34.20±0.84
Protocatechuic acid	7.1	22.05±0.39	74.22±1.79	23.43±0.85
Caffeic acid	7.6	6.72±0.27	82.00±1.25	6.42±0.47
Vanillic acid	7.9	141.35±0.68	239.88±1.34	57.59±0.96
Syringic acid	8.1	116.57±0.52	214.24±1.14	50.87±0.38
4-Hydroxybenzaldehyde	9.4	13.25±0.57	89.11±1.01	Trace
Ellagic acid	9.7	Trace	127.58±2.08	Trace
Sinapic Acid	10.5	Trace	2.04±0.28	Trace
Ferulic Acid	10.6	126.19±0.72	246.22±1.7	nd
p-Coumaric acid	12.1	Trace	Trace±	Trace
Protocatechuic acid ethyl ester	12.8	Trace	Trace	Trace
Salicylic acid	13.1	51.14±1.88	185.69±1.3	11.13±1.23
Cinnamic acid	15.2	9.62±0.04	10.77±1.26	nd

RT-retention time. min, [a]Values expressed are means ± S.D. of three parallel measurements
nd: not detected

Table 4. Quantitative results of flavonoids and phenolics in plant extracts (mg phenolic/kg plant)

Flavonoids and phenolics	RT	Root	Green	Stem
Catechin	5.8	10.36±0.66	11.02±1.01	nd
Rutin	9.2	Trace	10.08±0.51	Trace
Polydatine	9.6	Trace	Trace	Trace
Scutellarin	9.7	Trace	16.45±1.80	Trace
Quercetin-3-*β*-D-Glucoside	9.8	7.39±0.53	1737.70±36.5	163.24±2.97
Naringin	10.5	Trace	213.29±3.67	19.02±2.04
Diosmin	10.6	38.70±0.82	46.38±1.63	47.57±1.28
Taxifolin	10.6	Trace	Trace	Trace
Hesperidin	10.8	Trace	278.38±1.07	Trace
Apigetrin	10.9	Trace	Trace	Trace
Neohesperidin	11.1	Trace	2.55±0.16	Trace
Myricetine	11.9	Trace	Trace	Nd
Baicalin	12.0	Trace	Trace	Trace
Fisetin	12.1	Trace	Trace	Trace
Morin	13.0	12.95±1.97	17.20±0.31	14.51±0.97
Resveratrol	13.0	Trace	Trace	Trace
Quercetin	14.0	10.54±0.67	531.35±2.45	3.93±0.6
Silibinin	15.1	Trace	Trace	nd
Apigenin	15.6	Trace	Trace	Trace
Naringenin	15.7	Trace	Trace	Trace
Kaempferol	15.7	Tr	60.51±2,51	tr
Diosmetin	16.1	Trace	Trace	Trace
Neochanin	17.7	Trace	Trace	Trace
Eupatorin	18.9	Trace	Trace	Trace
Wogonin	19.8	Trace	Trace	Trace
Biochanin A	20.5	Trace	Trace	nd

RT-retention time. min. [a]Values expressed are means ± S.D. of three parallel measurements
nd: not detected

tron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al.*, 1997).

The DPPH radical-scavenging activity of *F. Longipedunculata* root oil and its methanol and acetone extract are shown in Table 5. The methanol root extract at 0.1 mL concentration had the highest antioxidant value (98.5%). In the acetone solvent, it was found that the parts of green and stem at 0.3 mL concentration had the highest antioxidant value (86.8%). Among the solvent extracts from different parts of *F. longipedunculata,* the lowest concentration of methanol extract had the best antioxidant activity, whereas the stem part of the acetone extract showed the lowest activity. Interestingly, the results of the DPPH free-radical scavenging assay showed that the extracts had higher activities than the positive control (BHT) in all concentrations and higher activities in lower concentrations in methanol extracts as seen in Table 5. The reason for the high antioxidant activity is due to the phenolic compounds it possesses. The extract of *F. assafoetid*a exhibited a good antioxidant activity in all models studied. The extracts had good Fe^{2+} chelating ability, DPPH radical and nitric oxide scavenging activity (Dehpour *et al*., 2009). *Ferula-assafoetida* leaves are free-radical scavengers and may act as primary antioxidants, which react with free radicals by donating hydrogen (Nabavi *et al*., 2011). Research shows that the *ferula-assa-foetida* leaves have different kind of flavonoides, phenolic compounds (Dehpour *et al*., 2009). All these compounds probably contribute to the main reason for its significant radical-scavenging activity. Research-

TABLE 5. DPPH free radical scavenging activity of *F. longipedunculata* root, green and stem parts (methanol and acetone extract)

		DPPH free radical scavenging (%)					
		***F. longipedunculata* extract**			**BHT**		
		Concentration (ml)			Concentration (ml)		
Parts of Plant	Solvents	0.1	0.2	0.3	0.1	0.2	0.3
Root part	Methanol	98.5	98	98.3	90.7	92.1	97.9
Green part	Methanol	97.2	97	96.6	90.7	92.1	97.9
Stem part	Methanol	98.1	97.9	97.7	90.7	92.1	97.9
Root part	Acetone	79.5	80.6	82.8	77.6	75.1	74.8
Green part	Acetone	80.6	82	86.8	77.6	75.1	74.8
Stem part	Acetone	79.1	85.4	86.8	77.6	75.1	74.8

BHT (Butylated hydroxytoluene): as control, DPPH: 1,1-diphenyl-2-picryl hydrazyl

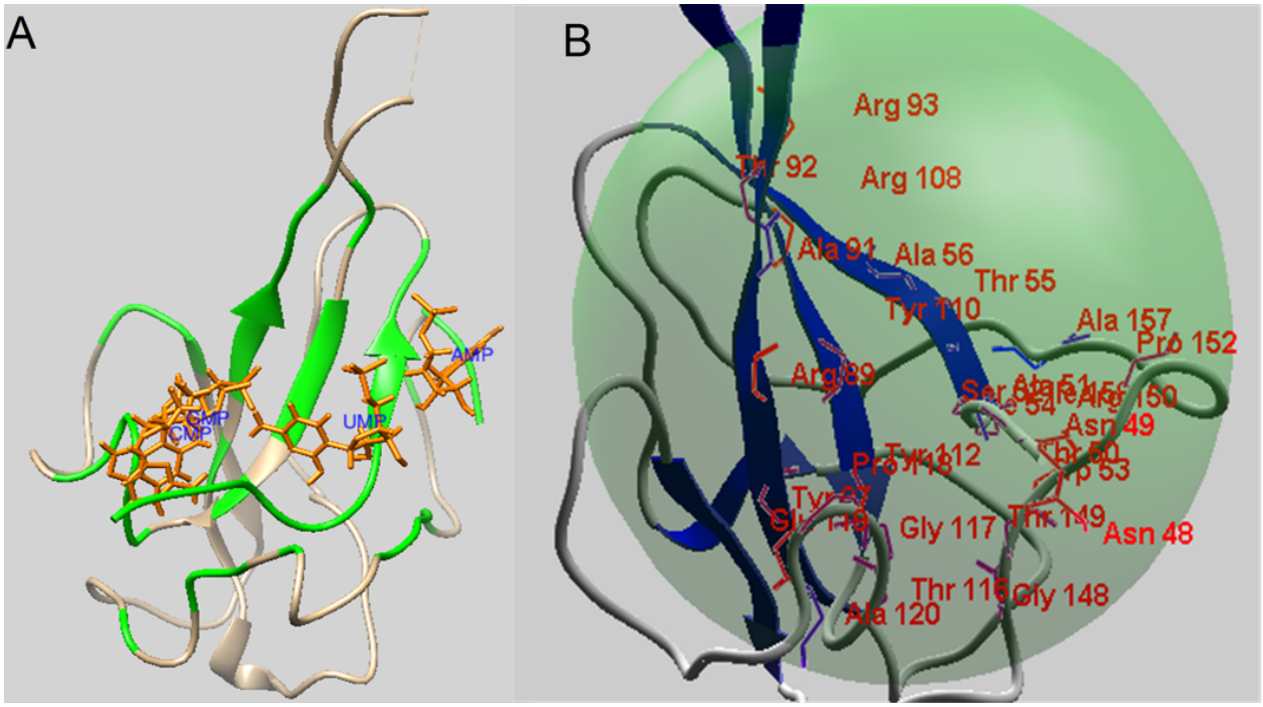
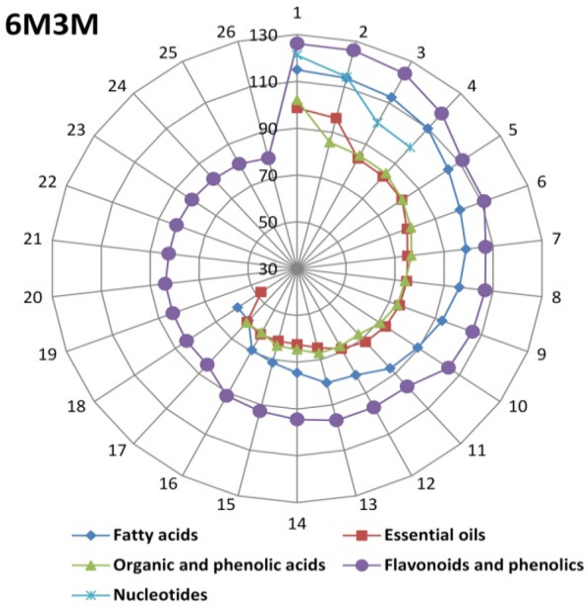


FIGURE 2. The docking region of SARS-CoV-2 nucleocapsid protein. (A: Active site (green) and binding of nucleotides (orange); B: Amino acid residues at docking cavity)

ers recently obtained better results regarding natural antioxidant compounds like gallic acid, coenzyme Q10, rosmarinic acid, tannins and flavonoids from medicinal herbs rather than artificial antioxidants (Tavafi and Ahmadvand, 2011). Natural antioxidants compared to artificial antioxidants are much safer and more beneficial and also have fewer side effects (Craft *et al*., 2010).

3.5. Docking Results

The SARS-CoV-2 nucleocapsid is a vital protein in the RNA genomic packing, viral transcription, and assembly in an infectious cell (Raoult *et al*., 2020). Therefore, it is considered an excellent target to battle against SARS-CoV-2. The possible interaction areas with nucleotides and RNA of the SARS-CoV-2 N protein N-terminal domain were previously determined (Dinesh *et al*., 2020; Kang *et al*., 2020). The site selected for docking, the binding sites of nucleotides and some amino acids are shown in Figure 2. Uridine 5'-monophosphate (UMP), adenosine 5'-monophosphate (AMP), cytidine 5'-monophosphate (CMP), and guanosine 5'-monophosphate (GMP) were used to compare the binding domain and affinity scores of



No	Fatty acids	Essential oils	Organic and phenolic acids	Flavonoids and phenolics	
1	Linolenic acid	6-[1 (Hydroxymethyl)vinyl]-4,8a-dimethyl-	Chlorogenic acid	Silibinin	19.Myricetine
2	9-octadecanoic acid	6-[1-(Hydroxymethyl)vinyl]-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-2(1H) naphthalenone	Sinapic acid	Rutin	20.Kaempferol
3	Linoleic acid	Epiglobulol	Caffeic acid	Neohesperidin	21. Quercetin
4	Phytol	alpha-Bisabolol	Ferulic acid	Naringin	22. Biochanin A
5	Palmitic acid	alpha-Caryophyllene	Ellagic acid	Diosmin	23. Apigenin
6	Pentadecanoic acid	Santalol	Syringic acid	Hesperidin	24. Morin
7	3-Heptadecen-5-yne	3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one	p-Coumaric acid	Scutellarin	25. Taxifolin
8	Bisabolene	Ocimene	Cinnamic acid	Apigetrin	26. Neochanin
9	6[1(Hydroxymethyl) vinyl]4,8adimethyl-4a,5,6,7,8,8a hexahydro-2(1H) naphthalenone	2,3-Heptadien-5-yne, 2,4-dimethyl-	Protocatechuic acid ethyl ester	Polydatine	**Nucleotides**
10	Sesquisabinene hydrate	2,3,4,5Tetramethylcyclopent-2-en-1-ol	Vanillic acid	Baicalin	**1) AMP**
11	Cyclododecane methanol	beta-Myrcene	Gallic acid	Quercetin -3-β-D Glucoside	**2) GMP**
12	Acoradien	α-Thujene	4-Hydroxybenzoic acid	Wogonin	**3) UMP**
13	γ-cadinene	β-Phellandrene	Gentisic acid	Fisetin	**4) CMP**
14	3-Hydroxy-2(2methylcyclohex-1 enyl)propionaldehye	γ-Terpinen	Fumaric acid	Resveratrol	
15	p-Mentha-2,8-diene, 1hydroperoxide	β-Cymene	Protocatechuic acid	Naringenin	
16	trans-8-Hydroxy-bicyclo(4,3,0)non-3-ene	α-Terpinen	4Hydroxybenzaldehyde	Catechin	
17	Bicyclo[3.3.1]nonane-2,6-diol	4-Terpineol	Salicylic acid	Diosmetin	
18	2,3Dimethylhydroquinone	α-Pinen		Eupatorin	

FIGURE 3. Radar graphic representation of molecular docking results of phytochemicals and list of molecules

phytochemicals. Our study shows that several phytochemicals present in the endemic *Ferula longipedunculata* Peşmen presented significant predicted binding activity towards the SARS-CoV-2 nucleocapsid protein. Figure 3 shows the binding affinity information of our phytochemicals, and details of their estimated binding scores were demonstrated in Table 6. Also, many of the phenolics present in endemic plant have

Table 6. Details of docking results of some phytochemicals

Name	MolDock Score	Hydrogen Bond	Amino acids involved in hydrogen bonding	Electrostatic	Nucleotides bound to the same region
Silibinin	-126.107	-10.155	Arg150, Tyr 112, Asn 49, Asn 48, Gly117, Thr 149		UMP, CMP, GMP
Rutin	-126.039	-16.549	Arg108, Tyr110, Thr58, Tyr 173, Gln 161, Leu 160, Aln 161, Ala174		AMP, UMP, CMP, GMP
Neohesperidin	-124.043	-13.436	Arg 89, Tyr112, Ser52, Tyr110, Thr 92, Thr 149		UMP, CMP, GMP
Naringin	-118.464	-12.895	Tyr 112, Thr 50, Thr 149, Asn 48, Asn 49		UMP, CMP, GMP
Diosmin	-111.507	-9.955	Arg 150, Tyr 110, Tyr 112, Ser 52, Ala 56		UMP, CMP, GMP
Hesperidin	-111.133	-17.762	Asn 49, Ser 52, Phe 54, Arg 150, Arg 89, Tyr 112, Arg103, Tyr 110		UMP, CMP, GMP
Scutellarin	-107.010	-9.490	Tyr 112, Thr 149, Asn 48, Thr 50, Asn 49, Arg89		UMP, CMP, GMP
Apigetrin	-106.803	-18.390	Tyr 112, Ser 52, Phe54, Arg150, Tyr110, Arg89, Thr 149, Thr 50		UMP, CMP, GMP
Polydatine	-106.120	-14.276	Leu 160, Ala 174, Gln 161, Thr 58, His 60, Tyr 173, Gln 161		AMP
Chlorogenic acid	-102.058	-14.141	Ser 52, Thr 50, Gly117, Thr149, Arg 89, Tyr 112	Arg 150	UMP, CMP, GMP
Sinapic Acid	-85.529	-12.355	Arg 89, Arg 90, Asp 129	Arg 89	CMP, GMP
Linolenic acid	-114.959	-1.023	Arg 89, Tyr 112	Arg 89	GMP, CMP
9- octadecanoic acid	-113.834	-2.468	Tyr 110, Arg 108	Arg 108, Arg 93	AMP, UMP
6-(1-Hydroxymet hylvinyl)-4,8a-dimethyl	-98.624	-5.959	Gly 117, Thr 149, Thr 50		GMP, CMP
6[1(Hydroxymethyl) vinyl]4,8a-dimethyl-4a,5,6,7,8,8ahexahy-dro-2(1H)-naphthalenone	-96.182	-7.907	Tyr 173, Thr 58, Gln 161, Lue 160		AMP
AMP	-121.197	-13.876	Arg93, Arg108, Ala 56, Thr 58, Tyr 173, His 60	Arg 108, Arg 93	
GMP	-114.272	-3.803	Arg89, Tyr112, Thr50, Thr 149	Arg 89	
UMP	-100.274	-7.321	Tyr 110, Arg 150, Tyr 112, Ser 52, Phe 54	Arg 93, Arg 108	
CMP	-99.163	-14.516	Tyr 112, Asn49, Ala51, Thr 149	Arg 89	

significant binding affinity with this target. Some of the flavonoids and phenolics are silibinin, rutin, neohesperidin, naringin, diosmin, hesperidin, scutellarin, apigetrin, and polydatine. Table 6 presents the binding score and amino acid residues that make their hydrogen bond. Figure 4 demonstrates the possible binding modes of some phytochemicals. Silibinin exhibited the highest binding energy at the active site of SARS-CoV-2 nucleocapsid protein. It formed hydrogen bond interactions Arg 150, Tyr 112, Asn 49, Asn 48, Gly 117, Thr 149. Active site residues Gln 192, Thr 190, Arg 188, His 164, Gln 189, Glu 166, Gly 143, Ser 144, and Cys 145 participated in hydrogen bond interactions with rutin. Chlorogenic acid and sinapic acid with -106.120 and -85.529 MolDock scores exhibited the most effective phenolic acids against the target as *in silico*. The computer analysis results suggest that two phenolic acids had electrostatic potential in the interaction. The results of the prepared study shown that Ser 52, Thr 50, Gly 117, Thr 149, Arg 89, and Tyr 112 were critical residues in the hydrogen bonding of chlorogenic acid with protein. It also interacts electrostatically with Arg 150. The docking results in Table 6 demonstrate that chlorogenic acid interacted with the region where UMP was connected. Arg 89, Arg 90, Asp 129 amino acids were responsible for sinapic acid-binding in the SARS-CoV-2 nucleocapsid protein. It acted electrostatically with the Arg 89 ami-

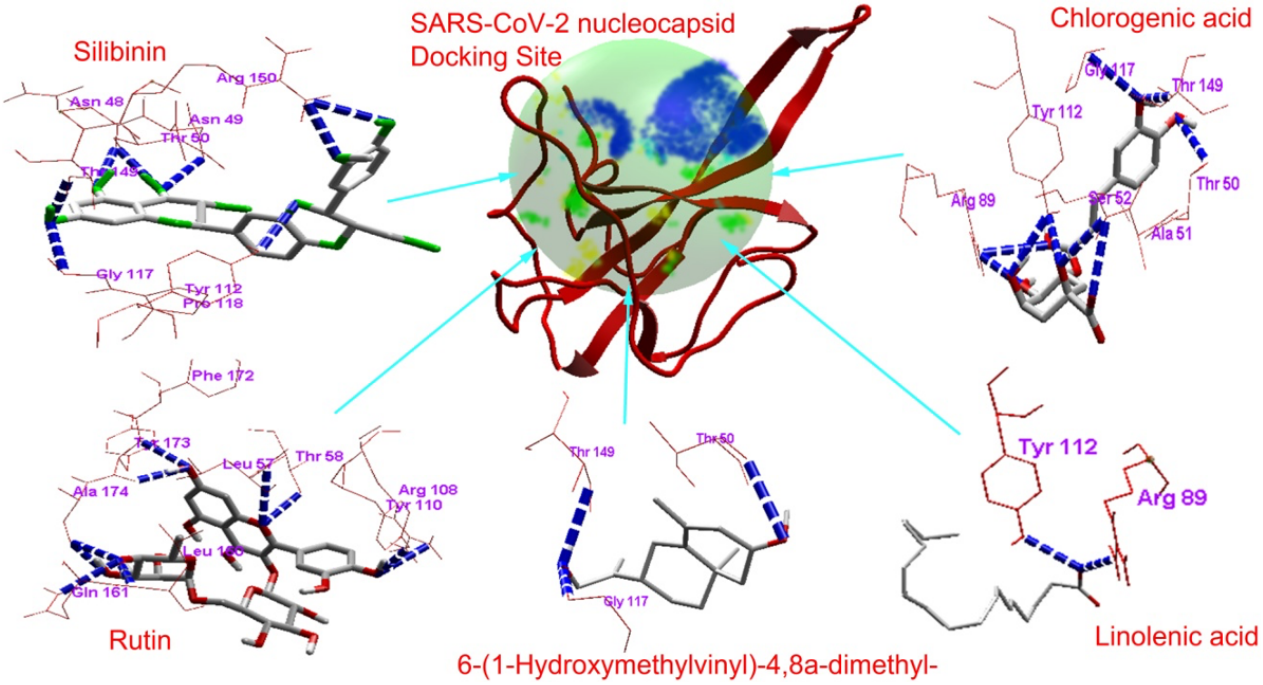


FIGURE 4. Protein binding site and 3D animation of possible binding of some phytochemicals (The dotted blue line shows hydrogen bonds)

no acid, in which GMP and GMP interacted as electrostatic. Two compounds, linolenic acid and 9-octadecanoic acid, showed the highest docking scores (-114.959 and -113.834, respectively) among all the fatty acids. Linolenic acid formed hydrogen bonds with Arg 89 and Tyr 112, and made an electrostatic interaction with Arg 89. This phytochemical was found to share the same region with CMP and GMP in the target protein. 9-octadecenoic acid showed a hydrogen bond with Tyr 110 and Arg 108, and was found to have electrostatic interaction with Arg 108 and Arg 93. It interacted with the same amino acids as AMP and UMP nucleotides.

6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-, the most active compound in the essential oils, formed hydrogen bonds with Gly 117, Thr 149, and Thr 50. The hydrogen bond interaction of 6-[1-(Hydroxymethyl)vinyl]-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-2(1H)- naphthalenone was formed with Tyr 173, Thr 58, Gln 161 and Lue 160 residues of protein. Both compounds made hydrogen bonds with similar amino acids to nucleotides GMP, UMP, and CMP.

4. CONCLUSIONS

F. longipedunculata flowers were investigated for their chemical composition. The extracts from the different plant parts exhibited well. The results of the present work indicate that the antioxidant activity of the methanol and acetone extracts of *Ferula longipedunculata* is higher than the control, such as BHT. The methanol and acetone extracts of the plant might be an alternative additive in foods, medicine and cosmetics, instead of toxic artificial antioxidants. The different results achieved in this study may be caused by factors such as the use of different parts of the plant, environmental and genetic differences and species diversity. These results interestingly encourage to continue the work to isolate the active molecules responsible for the antioxidant and assessment of biological activity of each compound individually and the need for in-depth studies on the plant extract.

The study also provided important insights into the first step of the COVID-19 infection, viral entry into cells, and defined potential phytochemicals for antiviral intervention. Although confirmation with an infectious virus is pending, our results indicate that natural compound responses raised against SARS-S could offer some protection against COVID-19 infection, which may have implications for outbreak control.

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