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Phytochemical Investigation and Pharmacological Activity of Petroleum Ether Fraction of *Solidago canadensis* L. against COVID-19 Activity

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ABSTRACT

Coronavirus COVID-19 is a dangerous viral disease caused by a new Coronavirus (SARS-CoV-2), medicinal plants have been used in the treatment of a large number of diseases since ancient times, Solidago canadensis L. belongs to the asteraceae family, which includes approximately one hundred species, widely distributed in North American wildflowers and greater than a dozen species inhabiting South America, Europe and Asia. It has been cultivated in Iraq in the provinces of Babylon and Diyala recently. 750 gm has been extracted with ethanol 85% then fractionated after drying with petroleum ether, then GC/MS analysis by Shimadzu GCMS-QP2020 show the detection of 60 compounds. The results showed the presence of a variety of compounds; fatty acids like palmitic acid and linoleic acid methyl ester. Terpenes and terpenoids like α-pinene, bornyl acetate, myrcene, verbenol, terpinolene, germacrene-D, andrographolide, caryophyllene oxide, as well as vitamin E.

High throughput Cytopathic Effect (CPE) inhibitory tests for the COVID-19 virus on vero cells were developed in investigating new potential antiviral agents. A crystal violet uptake assay was used to measure the cytotoxic and antiviral effects.

The identified compounds in the petroleum ether fraction showed high antiviral activities against COVID-19 with Selective Index (SI)=Estimated CC_{50}/estimated IC_{50}=2,283.9, consequently, the tested samples are good candidates for further experiments as anti-COVID-19.

Key words: *Solidago canadensis* L, Terpenes, Terpenoids, Germacrene, Garyophyllene, Anti-COVID-19

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INTRODUCTION

Coronaviruses are cytoplasmic positive stranded RNA viruses with an envelope [1]. When the Severe Acute Respiratory Syndrome (SARS) outbreak shocked the world in 2002–2003, Coronaviruses garnered a lot of attention, it infect a wide range of animals and birds, producing respiratory and gastrointestinal infections, as well as hepatitis and neurologic disease in certain rare cases [2].

Human Coronaviruses (HCoVs) have been found in seven different strains; HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1 are four non-zoonotic viruses that produce widespread epidemics of upper respiratory tract illnesses, mostly in the winter [3].

According to WHO, there have been 258,164,425 confirmed cases of COVID-19 worldwide, with 5,166,192 fatalities as of November 21, 2021 [4].

Natural compounds have always piqued scientists' interest when it comes to drug development; traditional healers in many civilizations believe medicinal plants to be extremely effective in preventing and treating a variety of illnesses and disorders. Various traditional herbal treatments have been employed since the COVID-19 pandemic outbreak and have resulted in desirable health results among COVID-19 patients [5].

Medicinal plants have been used in the treatment of a large number of diseases since ancient times [6]. The human use of plants as medications has been dated at least in the middle paleolithic from a thousand years ago [7].

The potential for phytochemicals like terpenes to be used as effective antiviral medicines has recently gotten a lot of attention, especially because these compounds are plentiful and have minimal toxicity and cost [8].

The asteraceae family consists of about 25,000 species, of about 1,500 genres, they are incredibly very diverse subordinate plants, herbs, sub-shrubs or trees and 98% are composed of small plants. The most frequently studied species are *Solidago virgaurea* L, *Solidago gigantea* L, *Solidago canadensis* L. and *Solidago chilensis* [9].

Genus *Solidago* L. belongs to the Asteraceae family, which includes approximately one hundred species; it is widely distributed in North American wildflowers and greater than a dozen species inhabiting South America, Europe and Asia [10].

Solidago canadensis has been cultivated in Iraq in the provinces of Babylon and Diyala recently.

S. canadensis (*S. altissima*), tall goldenrod, it is an erect plant 1 m–2.5 m with a range of about 1 m.

The stems are pubescent all through with lanceolate, sharply pointed, three veined leaves, roughish above, pubescent below. Broadly plumose heads of yellow flowers form a big pyramidal panicle from August to November making for an appealing autumn border plant [11-13].

The genus *Solidago* possesses variant important secondary metabolites like saponins like canadensis saponin, polysaccharides, flavonoids glucosides like quercetin, rutin, phenolic acids like caffeic acid, chlorogenic acid, alkaloids like dictamnine-7-b-d-mannopyranoside and 8-methoxydictamnine-7-b-d-mannopyranoside [14-18].

Terpenes or terpenoids are a class of chemicals that are made up of multiple isoprene structural units; hence they are also called isoprenoids [19].

Terpenoids are usually found in the form of various oxygen containing derivatives, such as alcohols, aldehydes, carboxylic acids, ketones, esters and glycosides, in addition to terpene hydrocarbons [20].

Several monoterpenes like α-pinene and myrcene, monoterpenoids like verbenol and bornyl acetate, sesquiterpenes like germacrene D, β-elemene and β-caryophyllene, copaene and ylangene, sesquiterpenoids like viridoflorol have been detected in *S. Canadensis* [21-29]

S. canadenesis and other *Solidago* species exhibited variant pharmacological activities like anticancer activity, antimutagenic, diuretic, antibacterial, anti-inflammatory, antioxidant, gastroprotective, hypolipidimic, anti-tumor, hypoglycemic, antimalarial and antidiabetic [30-37].

Terpenes have a wide range of pharmacological activities in both animals and humans like analgesic, anti-inflammatory, antiviral and antibacterial properties [38,39].

Terpenes potential for usage against a wide spectrum of viruses has been established in several *in vitro* experiments like Herpes simplex virus, Anti-Infectious Bronchitis Virus (IBV), West nile virus and Human Immunodeficiency Virus 1 (HIV-1) [40-43].

The main objective of this study is to evaluate the antiviral activities of the petroleum ether fraction on the HCoV-229E strain.

Depending on these facts our research will focus on the ability to use the petroleum ether fraction to evaluate the antiviral activity of the terpenes in the plant.

MATERIALS AND METHODS

Collection of plant materials

S. canadensis plant (aerial part) was collected during flowering in October; the plant was washed thoroughly, dried under shade and grinded in a mechanical grinder to a fine powder.

Equipment and chemicals

The instruments used were a rotary evaporator (BUCHI Rotavapor R-205, Swiss), sonicator (Branson Sonifier, USA), all chemicals and solvents used were of analytical grade and obtained from Riedel-de Haen, Germany.

Experimental work

The experimental work is divided into:

Extraction of plant materials (aerial part): 750 grams of the shade dried pulverized *S. canadensis* plant materials were defatted through soaking the pulverized material in a glass beaker with 1000 ml of hexane at room temperature, the mixture was occasionally shaken by magnetic stirrer for 3 days. The extract was filtered and the residue obtained from the method i was extracted by soxhlet using aqueous ethanol 85% as a solvent for 24 h. The extract was filtered and the solvent was evaporated under reduced pressure using a rotary evaporator to get a dry extract, The residue about 27 gm was suspended in 400 ml water and partitioned with petroleum ether (BP 30–60), (3 × 300 ml). Then the petroleum ether layer filtered and evaporated to dryness [44,45].

Preliminary qualitative phytochemical analysis of crude extracts: Phytochemical analysis for the screening and identification of bioactive chemical constituents in the medicinal plants under study was carried out on crude extracts, as well as powder specimens using the standard procedures as described by Harborne [46].

Test for terpenoids (Salkowski test): 5 ml of each plant extract were mixed in 2 ml of chloroform followed by the careful addition of 3 ml concentrated (H_2SO_4). A layer of the reddish brown coloration was formed at the interface thus indicating a positive result for the presence of terpenoids.

GC-MS analysis for essential oils: GC/MS analysis was done using Shimadzu GCMS-QP2020 (Tokyo, Japan). The GC was equipped with Rtx-1 MS fused bonded column (30 m × 0.25 mm ID × 0.25 µm film thickness) (Restek, USA) and a split split less injector. The initial column temperature was kept at 45°C for 2 min (isothermal) and programmed to 300°C at a rate of 5°C/min and kept

constant at 300°C for 5 min (isothermal). The injector temperature was 250°C. Helium carrier gas flow rate was 1.41 ml/min. All the mass spectra were recorded applying the following condition: (Equipment current) filament emission current, 60 mA; ionization voltage, 70 eV; ion source, 200°C. Diluted samples (1% v/v) were injected with split mode (split ratio 1:15).

The gas chromatography mass analysis of petroleum ether fraction was performed on a Shimadzu QP2010 quadrupole. Gas Chromatography-Mass Spectrometer (GC-MS) instrument equipped with a carbowax (30 m × 0.25 mm ID; 0.25 mm film thickness) capillary column (intercut DB5MS, Japan). One microliter of the sample was injected into the column.

Helium was used as the carrier gas. Injector and detector temperatures were set at 280°C. The injection was performed in split mode (1:30). The column temperature was programmed initially at 50°C and then to increase at a rate shown in Table 1 to a final temperature of 280°C.

The compounds were separated at constant pressure (100 kPa) and peaks were identified by comparing the mass spectra with the mass spectral database. The identification of compounds was based on the comparisons of their mass spectra with the NIST library [47].

Table 1: The GC mass oven temperature program.

Rate	Temperature (°C)	Hold time (min)
-	50	5
50	100	2
9	280	2

Investigate the antiviral activity of the petroleum ether fraction isolated from *S. Canadensis*

The antiviral activity of petroleum ether fraction against human Coronavirus 229E was identified using the high throughput cytopathic effect CPE inhibition assay. The assay was developed to evaluate the range of efficacy; *i.e.* the 50% Inhibitory concentration (IC_{50}) and cytotoxicity for the chosen antiviral (CC_{50}), in cell culture systems, this assay is crucial for evaluating antiviral efficacy.

Cell line and culture: The 229E virus and Vero E6 cells (African green monkey kidney) were obtained from Nawah-Scientific, Egypt. Vero E6 cells were grown in DMEM medium (Dulbecco's Modified Eagle's Medium) supplemented with 10% fetal bovine serum and 0.1% antibiotic/antimycotic solution. The antibiotic and antimycotic solution was obtained from Gibco BRL, trypsin EDTA, fetal bovine serum and DMEM medium (Grand Island, NY, USA) [48].

Antiviral activity test: The antiviral activity and cytotoxicity evaluation was performed using the crystal violet assay [49]. The Vero E6 cells were seeded into a 96 well culture plate at a density of 2×10^4 cells/well one day before infection. The culture medium was removed the next day and the cells were washed with phosphate buffered saline. The infectivity of Coronavirus 229E was determined using the crystal violet method, which monitored CPE and allowed the percentage of cell viability to be calculated. 0.1 mL of diluted virus suspension of 229E virus containing $CCID_{50}$ (50 percent cell culture infective dose) of virus stock was added to mammalian cells. This dose was selected to produce the desired CPEs. For compound treatments, 0.01 mL of medium containing the desired compound concentration was added to the cells. Each test sample's antiviral activity was determined using a tenfold diluted concentration range of 0.1 µg/mL-100 µg/mL. The virus controls (virus infected, nondrug treated cells) and cell controls (non-infected, nondrug treated cells). For 72 hrs, culture plates were incubated at 37°C in 5% CO_2.

The development of the cytopathic effect was monitored by light microscopy. Following a PBS wash, the cell monolayers were fixed and stained with a 0.03% crystal violet solution in 2% ethanol and 10% formalin.

After washing and drying the optical density of individual wells was quantified spectrophotometrically at 540 nm/630 nm. The percentage of antiviral activities of the tested compounds was calculated using the following equation:

Antiviral activity=(Mean optical density of cell controls −Mean optical density of virus controls)/(Optical density of the test-Mean optical density of virus controls) × 100%, using the Dynex Immuno Assay System (DIAS) reader. Based on the results, the 50% CPE Inhibitory Dose (ID_{50}) was calculated.

Cytotoxicity test: Before this assay, we assessed the cytotoxicity; Vero cells were seeded at a density of 2×10^4 cells/well in a 96 well culture plate. The next day, the culture medium containing serially diluted samples was added to the cells and incubated for 48 hours before being removed and the cells washed with PBS. The following steps were carried out in the same manner as described above for the antiviral activity assay [50].

The 50% Cytotoxic Concentrations (CC_{50}) and the 50% Inhibitory Concentration (IC_{50}) were determined using GraphPad PRISM Software (Graph Pad Software, San Diego, USA).

GraphPad PRISM Software (Graph Pad Software, San Diego, USA) was used to calculate the 50% Cytotoxic Concentrations (CC_{50}) and 50% Inhibitory Concentrations (IC_{50}).

RESULTS

The weight of the dried extract was 3.5 gm and the result of the preliminary test for terpenoids was positive, the GC-MS analysis of petroleum ether fraction from *S. canadensis* is demonstrated (Figure 1) and the compounds are listed (Table 2).

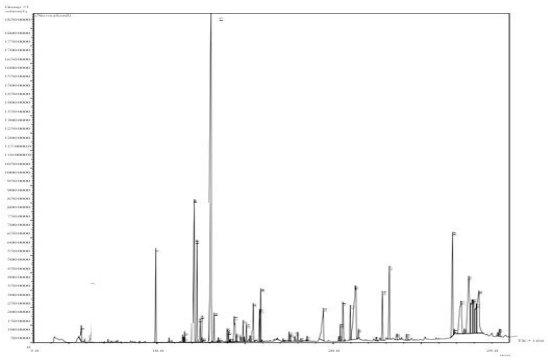
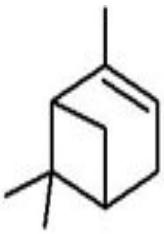
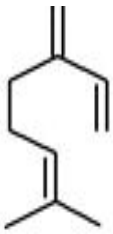
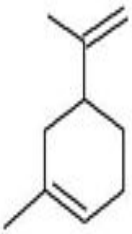
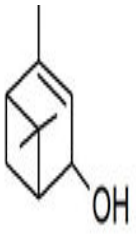
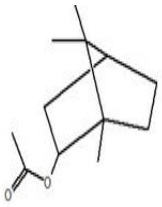
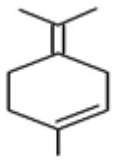
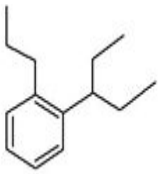
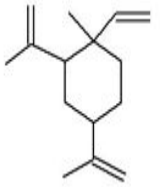
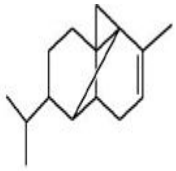
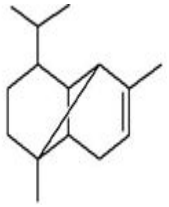
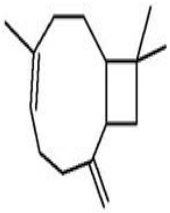
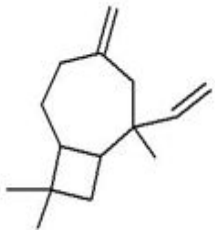
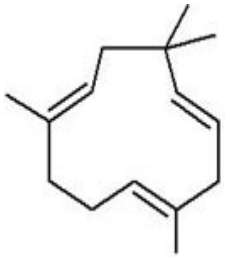
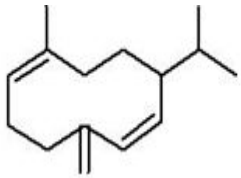
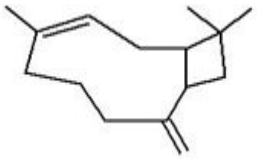
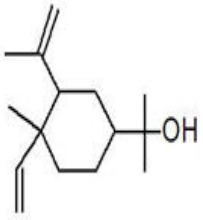
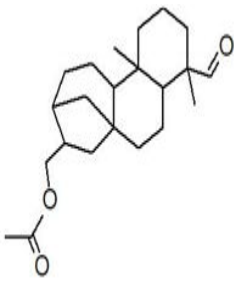


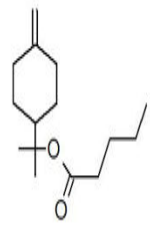
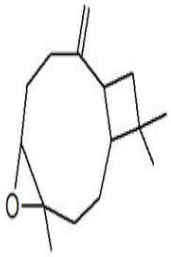
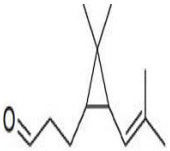
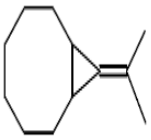
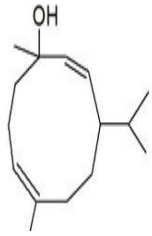
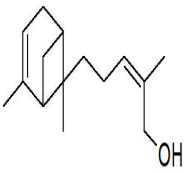
Figure 1: The GC mass chromatogram of the petroleum ether fraction of *S. canadensis* plant.

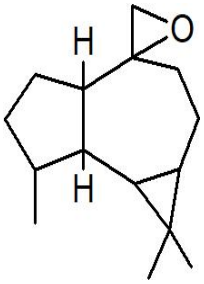
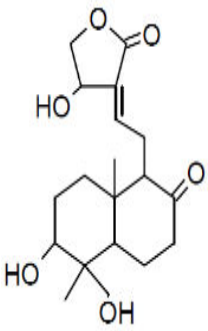
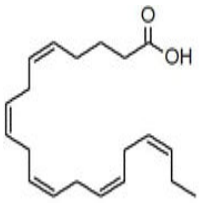
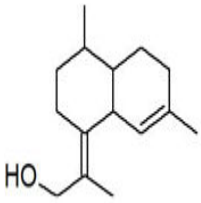
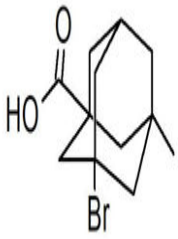
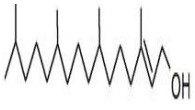
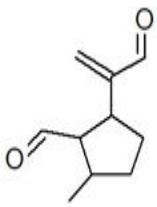
Table 2: The GC mass profile of *S. canadensis* L. aerial part.

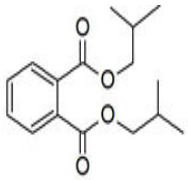
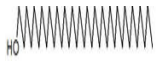
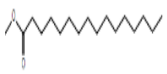
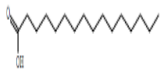
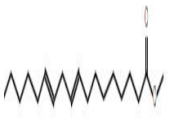
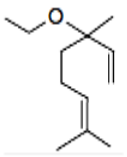
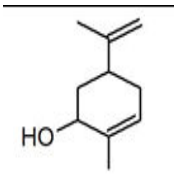
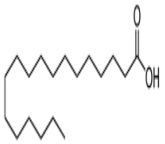
Peak number	Retention time	Area	Area %	Mol. weight	Mol. formula	Compounds name	Structure
1	5.649	2184956	0.48	136	$C_{10}H_{16}$	alpha-Pinene	
2	5.870	280275	0.06	136	$C_{10}H_{16}$	beta-Myrcene	
3	6.254	8277130	1.81	136	$C_{10}H_{16}$	(+)-Sylvestrene	
4	7.230	339490	0.07	152	$C_{10}H_{16}O$	cis-Verbenol	

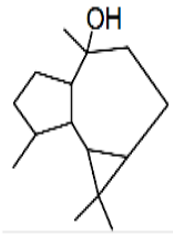
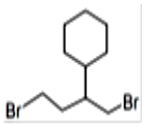
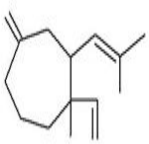
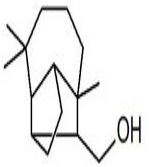
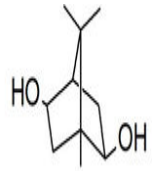
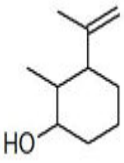
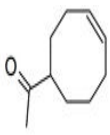
5	9.936	13218526	2.89	196	$C_{12}H_{20}O_2$	(-)-Bornyl acetate	
6	10.694	486752	0.11	136	$C_{10}H_{16}$	Terpinolene	
7	11.455	1184103	0.26	204	$C_{15}H_{24}$	beta.-Bourbonene	
8	11.517	828204	0.18	190	$C_{14}H_{22}$	Benzene, 1-(1-ethylpropyl)-2-propyl-	
9	11.566	1327065	0.29	204	$C_{15}H_{24}$	beta.-Elemene	
10	12.040	27558413	6.03	204	$C_{15}H_{24}$	(+)-Ylangene	
11	12.196	14691074	3.22	204	$C_{15}H_{24}$	Copaene	
12	12.376	2809466	0.62	204	$C_{15}H_{24}$	4,11,11-Trimethyl-8-methylenebicyclo [7.2.0] undec-4-ene	

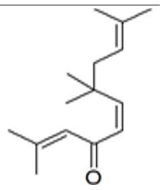
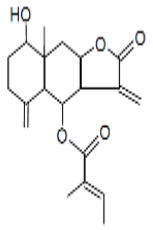
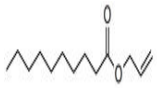
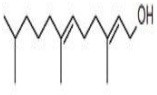
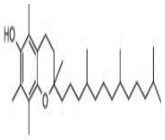
13	12.496	3757497	0.82	204	$C_{15}H_{24}$	Bicyclo [5.2.0] nonane, 4-methylene-2,8,8-trimethyl-2-vinyl-	
14	12.550	447213	0.10	204	$C_{15}H_{24}$	alpha.-Caryophyllene, alpha.-Humulene	
15	13.064	117884411	25.81	204	$C_{15}H_{24}$	Germacrene D	
16	13.224	3886668	0.85	204	$C_{15}H_{24}$	1R,3Z,9s-4,11,11-Trimethyl-8-methylenebicyclo [7.2.0] undec-3-ene	
17	13.463	565549	0.12	204	$C_{15}H_{24}$	Bicyclo [4.1.0]-3-heptene, 2-isopropenyl-5-isopropyl-7,7-dimethyl-	
18	13.967	2128997	0.47	222	$C_{15}H_{26}O$	Elemol	
19	14.051	1376543	0.30	346	$C_{22}H_{34}O_3$	Kauran-18-al, 17-(acetyloxy)-, (4.beta.)-	

20	14.158	448803	0.10	238	$C_{15}H_{26}O_2$	trans-beta-Terpinyl pentanoate	
21	14.372	6800969	1.49	220	$C_{15}H_{24}O$	Caryophyllene oxide	
22	14.511	1048911	0.23	152	$C_{10}H_{16}O$	trans-Chrysanthemal	
23	14.732	719321	0.16	164	$C_{12}H_{20}$	Bicyclo [6.1.0] nonane, 9-(1-methylethylidene)-	
24	14.874	3196901	0.70	360	$C_{24}H_{40}O_2$	10,12-Tricosadiynoic acid, methyl ester	
25	14.932	594225	0.13	222	$C_{15}H_{26}O$	Germacrene D-4-ol	
26	15.059	2471474	0.54	220	$C_{15}H_{24}O$	Bergamotol	

27	15.268	1058847	0.23	220	$C_{15}H_{24}O$	Aromadendrene oxide-(2)	
28	15.452	7595443	1.66	350	$C_{20}H_{30}O_5$	Andrographolide	
29	15.809	6656828	1.46	302	$C_{20}H_{30}O_2$	Icosapent	
30	15.873	7349568	1.61	220	$C_{15}H_{24}O$	Cis-Lanceol	
31	17.137	500030	0.11	272	$C_{12}H_{17}BrO_2$	3-Bromo-7-methyl-1-adamantanecarboxylic acid	
32	17.476	1370888	0.30	296	$C_{20}H_{40}O$	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	
33	17.561	1233530	0.27	166	$C_{10}H_{14}O_2$	Cyclopentane acetaldehyde, 2-formyl-3-methyl-. alpha. -methylene-	

34	17.808	732776	0.16	278	$C_{16}H_{22}O_4$	Palatinol IC	
35	17.972	1445865	0.32	536	$C_{37}H_{76}O$	1-Heptatriacotanol	
36	18.146	681054	0.15	190	$C_{15}H_{22}$	11,11-Dimethyl-spiro [2,9] dodeca-3,7-dien	
37	18.507	631046	0.14	270	$C_{17}H_{34}O_2$	Palmitic acid, methyl ester	
38	19.422	11378057	2.49	256	$C_{16}H_{32}O_2$	Palmitic acid	
39	20.301	753950	0.17	294	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid, methyl ester, Linoleic acid methyl ester	
40	20.386	2276448	0.50	268	$C_{17}H_{32}O_2$	7-Hexadecenoic acid, methyl ester, (Z)-	
41	20.528	8292856	1.82	182	$C_{12}H_{22}O$	Ethyl linalool	
42	20.954	5635379	1.23	152	$C_{10}H_{16}O$	cis-Carveol	
43	21.237	30424178	6.66	224	$C_{15}H_{28}O$	(Z)6,(Z)9-Pentadecadien-1-ol	
44	21.414	1362383	0.30	284	$C_{18}H_{36}O_2$	Octadecanoic acid, stearic acid	

45	22.421	773866	0.17	222	$C_{15}H_{26}O$	Viridiflorol	
46	22.775	7579156	1.66	310	$C_{11}H_{20}Br$	1,6-Dibromo-2-cyclohexylpentane	
47	23.168	11921464	2.61	210	$C_{13}H_{22}O_2$	3,7-Dimethyl-2,6-non-adien-1-ol acetate	
48	23.596	1371898	0.30	204	$C_{15}H_{24}$	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	
49	24.140	1043273	0.23	288	$C_{18}H_{37}Cl$	Octadecane, 1-chloro-	
50	26.755	22549397	4.94	410	$C_{30}H_{50}$	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-, Spinacene, Squalen	
51	26.851	651682	0.14	222	$C_{15}H_{26}O$	(-)-Isolongifolol	
52	27.236	17175198	3.76	178	$C_{10}H_{18}O_2$	Angelicoidenol, 1,7,7-trimethylbicyclo [2.2.1] heptane-2,5-diol	
53	27.421	845858	0.19	154	$C_{10}H_{18}O$	Isopropenyl-2-methyl cyclohexanol.	
54	27.669	26729205	5.85	152	$C_{10}H_{16}O$	1-(4-Cycloocten-1-yl) ethanone	

55	27.864	14315021	3.13	220	$C_{15}H_{24}O$	(5Z)-2,7,7,10-Tetramethyl-2,5,9-undecatrien-4-one.	
56	27.917	11657608	2.55	346	$C_{20}H_{26}O_5$	8-Hydroxy-8a-methyl-3,5-dimethylene-2-oxododecahydronaphtho [2,3-b] furan-4-yl (2E)-2-methyl-2-butenoate	
57	28.108	10347338	2.27	212	$C_{13}H_{24}O_2$	Decanoic acid, 2-propenyl ester, Allyl caprate	
58	28.246	19332679	4.23	210	$C_{15}H_{28}O$	(2E,6E)-3,7,11-Trimethyl-2,6-dodecadien-1-ol, farnesol	
59	29.315	832202	0.18	272	$C_{20}H_{32}$	(E,E,E)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene, alpha-springene	
60	29.418	1786871	0.39	430	$C_{29}H_{50}O_2$	Vitamin E	
Total		456804778	100.00				

The GC mass analysis shows the presence of many terpenes and terpenoids that fall within the monoterpenes and sesquiterpenes. The results also showed the presence of fatty acids as well as vitamin E.

The most important compounds detected were α-pinene, bornyl acetate, myrcene, verbenol, terpinolene, germacrene-D andrographolide, caryophyllene oxide, palmitic acid and linoleic acid methyl ester.

The cytotoxic effects of the petroleum ether fraction had to be tested on Vero cells to rule out non-specific activities and the maximal non-toxic concentration for the Vero cells was also calculated (Figures 2 and 3).

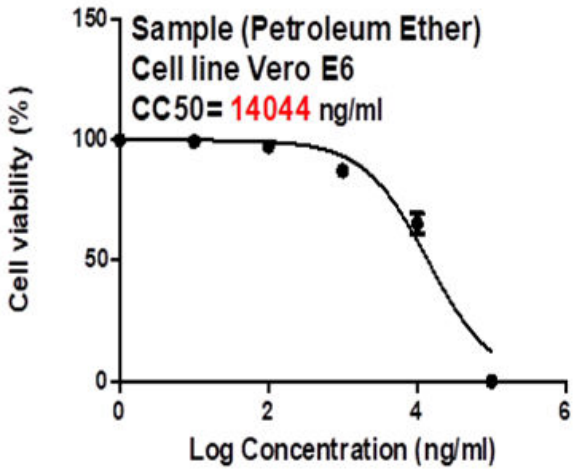


Figure 2: Cytotoxic concentration 50% CC_{50}.

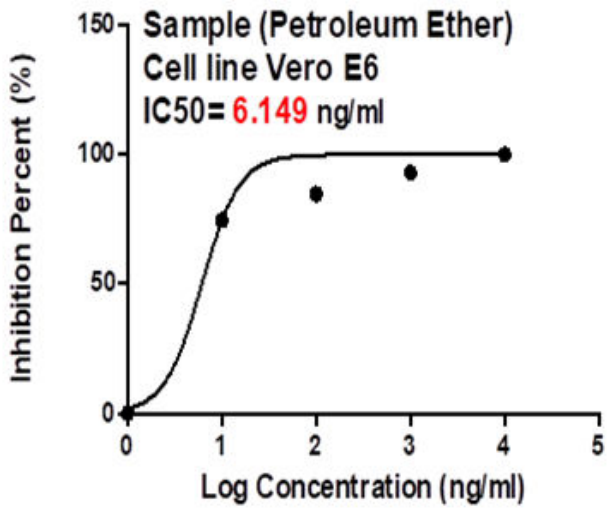


Figure 3: Inhibitory concentration 50% IC_{50}.

The results of the 50% Cytotoxic Concentrations (CC_{50}) and the 50% Inhibitory Concentration (IC_{50}) were determined using Graph Pad PRISM Software (Graph Pad Software, San Diego, USA).

Graphs of Cytotoxicity Concentration 50 (CC_{50}) and the 50% Inhibitory Concentration (IC_{50}) on Vero E6 cells and 229E.

DISCUSSION

Terpenes are secondary metabolites that are generated by a broad group of plants and animals. Terpenes have shown effectiveness against viruses, bacteria, fungus and protozoa, making them a prospective source of novel antimicrobial agents [51].

By dividing the supplied Antiviral Activity (AVA) value by the cytotoxicity (TOX) value (AVA/TOX), the Selectivity Index (SI) quantifies the window between cytotoxicity and antiviral activity. The greater the SI ratio, the more successful and safer a medicine would be *in vivo* during viral infection therapy. The ideal medicine would be cytotoxic only at very high doses but antiviral at extremely low concentrations, resulting in a high SI value (high AVA/low TOX) and the ability to destroy the target virus at concentrations much below its cytotoxic concentration. A compound's selectivity index is a commonly accepted metric for expressing a compound's *in vitro* performance in inhibiting viral reproduction [52].

The tested samples (petroleum ether) showed high antiviral activities against COVID-19 (229E virus). The determination of Selective Index (SI) for the tested sample is very important, for biologically active compounds it is recommended to obtain SI ≥ 10 [53].

The selective index=Estimated CC_{50}/Estimated IC_{50}=2,283.9. Consequently, the tested samples are good candidates for further experiments as anti-COVID-19.

The experiment shows that the CC_{50} of the petroleum ether fraction from *S. canadensis* was 14044 ng/ml while the IC_{50} was 6.149 ng/ml only, which indicates a potent activity against the Human Coronavirus 229E.

It has been found that the compounds in petroleum ether fraction have antiviral activity at a concentration below the cytotoxic concentration.

The GC mass analysis revealed the presence of about 60 compounds like terpenes, terpenoids, fatty acid, fatty acid esters and vitamin E.

Most of them possess pharmacological and biological activities, concerning the compounds with a relatively good concentration in the fraction, a-pinene, terpineol have high SI against Herpes Simplex Virus type 1 (HSV-1) *in vitro*, it has been suggested that when herpes virus was incubated with essential oils and isolated monoterpenes before host cell infection and may interfere with virion envelope structures or mask the viral structure required for adsorption or entrance into host cells [54]. It has been demonstrated using molecular docking techniques that several major monoterpenes found in essential oils for medicinal use (-pinene and -terpineol) can exert relatively strong binding and inhibit the active site of the Mpro protein, a key homodimeric cysteine protease enzyme that cleaves polyproteins into individual proteins required for SARS-CoV-2 replication and transcription [55].

Andrographolide is a bicyclic diterpenoid lactone, it has been found to have antiviral properties in several earlier researches against human immunodeficiency virus HIV, hepatitis-B virus, HPV16 pseudovirus and hepatitis-C virus, herpes simplex virus HSV-1, flaviviruses and pestiviruses Dengue virus (DENV1) and Chikungunya Virus (CHIKV) [56-63].

Several types of research report the mechanism of action of andrographolide against influenza virus. The Retinoic acid Inducible Gene-1 (RIG-1) like receptors signaling pathway have been identified as potential targets for andrographolide's antiviral effect against influenza A [64]. NF-kB was identified as a particular andrographolide binding target in a recent proteomic investigation [65]. It significantly suppresses the activation of NF-kB *in vivo* in a dose dependent manner, preventing NF-kB from binding to DNA [66].

Bornyl acetate a bicyclic monoterpene is a lung inflammatory disease preventative agent. It reduced the levels of proinflammatory cytokines *in vivo* and *in vitro* by inhibiting the activation of MAPKs and NF-kB signaling pathway, through repressing the activation of extracellular regulated protein kinases, c-Jun N-terminal kinase and p38 Mitogen Activated Protein Kinase (MAPK), also the number of total cells, neutrophils and macrophages, histologic changes in the lungs were decreased [67].

Ylangene a sesquiterpenoid, has anti-inflammatory properties it inhibits (inducible nitric oxide synthase) and COX-2 (cyclooxygenase-2) activity and promotes the pro-inflammatory proteins iNOS and COX-2 to be downregulated [68]. It has been found that oxygenated ylangene sesquiterpenoids be cytotoxic against HepG2, MDA-MB231 and A549 cancer cell lines With IC_{50} values of 16.0, 16.3 and 15.8 g/mL, respectively [69].

α-Copaene a sesquiterpene possesses antileishmanial activity, crude extracts and fractions rich in *α*-copaene (38.8%) from *Copaifera paupera*, had the most activity against *L. amazonensis* (IC_{50}=62.5 g/mL) and *L. infantum* (IC_{50}=65.9 g/mL) [70]. *α*- Copaene has been proved as non-genotoxic with a significant antioxidant activity [71].

Germacrene-D a sesquiterpene hydrocarbon is one of the major constituents of *Cedrus libani* (29.40%), Loizzo, Monica Rosa, et al., reported that leaves ethanolic extract exhibited an interesting antiviral activity against Herpes Simplex Virus type 1 (HSV-1) with no cytotoxic effect [72]. Essential oils with a high concentration of geranial and germacrene-D from 13 plants demonstrated antimicrobial activity towards *Candida albicans* [73]. Another study on the essential oils of *Lippia javanica* containing a high concentration of germacrene-D demonstrates potent antimicrobial activity against all the tested gram positive, gram negative bacteria and the pathogenic fungi strains [74]. In our study Germacrene-D was the major compound in the tested fraction about (25.81%).

Icosapent an Eicosapentaenoic Acid (EPA) fatty acid, Icosapent has proven successful in the treatment of individuals with moderate rises in blood triglyceride levels who have already been treated with statins, as well as in the treatment of patients with severe hypertriglyceridemia [75].

The use of icosapent ethyl as a part of supportive therapy in patients with mild to severe COVID-19 pneumonia has been documented in various clinical trials, in individuals with SARS-CoV-2 infection, icosapent ethyl provides anti-inflammatory benefits by lowering inflammatory markers such as CRP, ILs, granulocyte colony stimulating factor, interferons and TNFs, which promote inflammation and lung damaging [76,77].

Lanceol cis a sesquiterpene alcohol is the most prevalent component (20.22%) in *Rubus fruticosus* L. essential oil. Lanceol has been shown to have substantial antioxidant and antibacterial action against resistant strains obtained from patients suffering from respiratory infections [78]. Lanceol was also reported to possess antibacterial activity against *Helicobacter pylori* [79].

Palmitic acid a saturated fatty acid demonstrated antioxidant, potent inhibition of gastrointestinal motility and considerable anti-proliferative effects against different cancers cell lines [80]. Methyl Palmitate (MP) and MP like compounds possess anti-inflammatory and anti-fibrotic activity due to NF-kB suppression [81].

Ethyl linalool show bacteriostatic activity against gram negative *Escherichia coli* bacteria [82].

(-)-Carveol, a natural product, has significant gastroprotective activity involving multiple mechanisms of action, including cyto protection, anti-secretory, antioxidant and immune regulation [83].

(Z)6,(Z)9-pentadecadien-1-ol one of the major components of essential oil of *Nigella sativa* L. seeds (13.52%), possess antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, as well as antifungal activity against *Candida albicans* [84]. The presence of (Z)6,(Z)9-pentadecadien-1-ol as one of the major compounds in the methanol extract of *Psydrax dicoccos* leaves showed significant antifungal activity against *Candida albicans* and other fungal tested species, this activity has been attributed mostly to its antioxidant properties as a result of free radical scavenging [85].

Squalene a triterpene compound has been shown to have many biological and pharmacological activities such as antioxidant, immune stimulant, hepato protective, anticancer, antigenotoxic, atherosclerosis, anti-proliferative, anti-inflammatory, preventing skin oxidative damage caused by free radicals and an intermediate in cholesterol biosynthesis [86,87].

Angelicoidenol derivative like 2-O-b-D-glucopyranosyl-(+)-angelicoidenol is a mono-terpenoid compound known to inhibit Viral Protein R (Vpr) activity which is important for HIV viral replication [88]. But only weak anticancer activity against hepatoma cancer, epidermoid carcinoma, prostate cancer and breast cancer and melanoma cancer cell lines [89].

A silico study showed that farnesol was the best docking ligand from the 171 tested essential oils compounds for the SARS-CoV-2 target proteins; Main Protease (Mpro), endoribonuclease (Nsp15/NendoU), ADP-Ribose-1-Phosphatase (ADRP), RNA dependent RNA Polymerase (RdRp), Spike protein (rS) and Human Angiotensin Converting Enzyme (hACE2), which are essential for a viral infection to be effective indicating that it can limit viral replication when administered alone or in combination [90-92].

Natural products especially essential oils from several plants have shown antiviral activity to Coronaviruses, although the mechanism of action of these herbal products is mainly through the inhibition of viral replication [93]. Another study revealed that the antiviral activity was strongest in the pretreatment system, suggesting that the compounds' antiviral action is dependent on viral attachment and/or entrance prevention [94].

In general Essential oil components, on the other hand, may function synergistically with each other; they may enhance other antiviral medicines, or give some alleviation from COVID-19 symptoms.

CONCLUSION

Coronavirus COVID-19 is a dangerous viral disease caused by a new Coronavirus (SARS-CoV-2), medicinal plants have been used in the treatment of a large number of diseases since ancient times, *Solidago canadensis* L. belongs to the asteraceae family, which includes approximately one hundred species, widely distributed in North American wildflowers and greater than a dozen species inhabiting South America, Europe and Asia. It has been cultivated in Iraq in the provinces of Babylon and Diyala recently. 750 gm

has been extracted with ethanol 85% then fractionated after drying with petroleum ether, then GC/MS analysis by Shimadzu GCMS-QP2020 show the detection of 60 compounds. The results showed the presence of a variety of compounds; fatty acids like palmitic acid and linoleic acid methyl ester. Terpenes and terpenoids like α-pinene, bornyl acetate, myrcene, verbenol, terpinolene, germacrene-D andrographolide, caryophyllene oxide, as well as vitamin E.

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