The potency of major *Teucrium polium* active ingredients as the Main Protease (M^{pro}) of COVID-19

ABSTRACT

The GC-MS and docking method were used to investigate active coronaviral protein (the Main Protease (M^{pro}). This coronaviral protein was tested using a methanol extract made from *T. polium* leaves. According to the GC-MS results, the plant's methanolic extract contains eight major natural substances. The lowest Binding Energy (LBE) and the inhibition constant (Ki) have been used to identify and classify the pharmacological potential of these lead drugs. The affinity of these compounds with coronaviral protein has been investigated in order to determine their use at the active sites of the receptor, $3CL^{pro}$ (PDB ID: 6LU7). 3-Hydroxytetradecanoic acid, 3-Dodecenedioic acid, D-Glucuronic, 3-Hydroxysebacic acid, and Dodecen-3-ol showed greater affinity to $3CL^{pro}$ than the supporting control drugs. Furthermore, when the interactions of these components with the amino acids of $3CL^{pro}$ are evaluated, 3-Hydroxytetradecanoic acid has the highest LBE score and Ki value when compared to the approved medication as well as all other compounds under examination. As a result, these potential compounds may be modern coronavirus inhibitors. Such computational findings require additional *in vitro* and *in vivo* research.

Keywords: Teucrium polium; COVID-19; GC-MS; $3CL^{pro}$; 3-Hydroxytetradecanoic acid

INTRODUCTION

The high cost of conventional medicines, as well as their reported serious side effects, have prompted researchers to look for alternative treatment options for a variety of diseases [1]. Several phytochemicals have recently been described to have potent antiviral efficacy, allowing them to inhibit the rapid replication of COVID-19 [2]. These very active compounds provide hope for the discovery of new inhibitors in nature [3]. In overall, natural substances are more chemically varied than biopharmaceutical compounds, they have lower production costs, and have milder or non-existent adverse effects when compared to synthetic drugs [4].

Medicinal herbs are widely used in Jordanian traditional medicine to treat many diseases because they are high in bioactive components [5]. For example, the *Teucrium polium L.* (family *Lamiaceae*) is a useful herb since it is widely prescribed by many traditional Arabic herbalists because of its diuretic, diaphoretic, antipyretic, anti-inflammatory, antispasmodic, tonic, anorexic, antihypertensive, analgesic [6, 7], antibacterial [8, 9], and antidiabetic effects [8, 9]. *T. polium* includes a variety of compounds, namely diterpenoids, iridoids, flavonoids, terpenoids, and sterols, according to phytochemical studies [12,13].

The ongoing industrialization causes uprooting, low yield, less cultivation, and destruction [14]. As a result, preservation as well as conservation of the *T. polium's* existing flora and fauna are required. As a result, phytochemical analysis is required not only for the discovery of analytical markers, but also because of the development of a valid and reliable, precise, appropriate, as well as efficient technique again for characterization of *T. polium* biography. Thorough chemoprofiling of *T. polium* leaves utilizing liquid chromatography necessitates a lengthy run time, a time-consuming analytical procedure, and a large volume of organic solvents for separation process [15].

The main objective of this study was to evaluate the phytoconstituents architecture using GC-MS analysis and also to look into mechanism-based research that could explain *T. polium's* pharmacological properties. This study demonstrates a GC-MS-based phytochemical

constituents and identifies a significant bioactive mechanism involved for the *T. polium's* positive attitude. Furthermore, the current study seeks to screen and evaluate the potential inhibitory activity of *T. polium*-derived phytochemicals against SARS-CoV-2 ($3CL^{pro}$), potentially presenting new identified compounds towards the novel pandemic coronavirus disease (COVID-19). Furthermore, this proposed process is straightforward, brief, precise, relevant, and economical, requiring only a small amount of organic solvent for sample processing.

MATERIAL and METHODS

Collection of *T. polium* leaf

At the start of the spring semester in 2021, Jordanian *T. polium* plant wild-growing leaf were collected from the Karak governorate. Al-Eisawi [14] explained a method for obtaining such leaves. The leaves of the plant were left to dry in the shade, isolated to a constant weight at room temperature, crushed, as well as stored in a dark place.

Extraction Process

One gram of Jordanian *T. polium* leaves was restored with 10 mL of methanol as well as stirred at room temperature for five days. For 10 minutes, the supernatant has been centrifuged at 4500 rpm. 3.0 mL of the supernatant has been transferred to a 10 mL test tube and distilled at room temperature. Before being injected into the GC-MS, the residues had been reformed in 100 L of N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) solution.

GC-MS (Gas Chromatography-Mass Spectrometry) Analysis

The GC-MS analysis was carried out on an Agilent technology model 6890 GC equipped with the Split-splitless injector and HP-5MS capillary column coated with a 5% phenylmethylpoly-siloxane film (30 m 0.25 mm, 0.25 m film thickness). The agile amount 6890 GC was outfitted with a 5973C mass spectrometer in the Inert MSD style (Mass Spectrometer with mass Selective Detector and GC-MS). The column oven's temperature was conditioned as

follows: The temperature began at 60 °C and was gradually increased to 300 °C with a ramp of 15 °C/min before remaining at 300 °C for 7 minutes before all elution was completed. After 15 seconds, the split valves had been opened to purge the injector for 3 minutes. Both injections (1 L) were made with a 10 L syringe. As a carrier gas, 99.999 percent pure helium gas was used at a flow rate of 1.0 mL/min [16].

Molecular Docking Process

The main protease's X-ray crystal structure was obtained from the RCSB database (PDB ID: 6LU7). To remove the heteroatoms, water, and prepare the protein further, Biovia Discovery Studio Visualizer 16.1 was used. The major *T. polium* active ingredients' 2D chemical structures have been downloaded from the PubChem database. The MM2 force field was therefore implemented at the ligands using PerkinElmer Chem 3D 17.1 software and saved as a PDB file. AutoDock 4.2 is a component of computational software that is used to prepare ligands and proteins as well as to generate the docking procedure [17, 18]. The polar hydrogens and Kollman charge were first added to the main protease. The active ingredients were then revitalized using Gasteiger charges. The grid box was 50*50*50 in size, and the coordinates have been -10.244, 17.966, 66.508 (as x, y, and z, respectively) with a spacing of 0.375. The Mpro was defined as rigid for the docking parameter, whereas drugs are flexible. The genetics algorithm run has been set to 150, as well as the Lamarckian genetic was chosen to proceed with the docking, while all other parameters were left at their default values [19, 20].

RESULTS

GC-MS Analysis of the Plant Extract

The extracted compositions of *T. polium* were studied using GC–MS. Each component in the methanolic extract was measured and identified by making comparisons mass fragmentation trends to standards such as Wiley 9 library spectral data as well as NIST standards (Figure 1). Table 1 shows the detailed information for 25 peaks; 1 component has an area percent of 22.66%, 3 components have an area percent among 9.59 and 9.78 %, 1 component has an area percent of 6.74 %, 1 component has an area percent of 5.69%, 1 component has an area percent of 2.70 %, 2 components have area precent of 1.09 % and 1.73 %, and 18 components have area percent of less than 1 %.

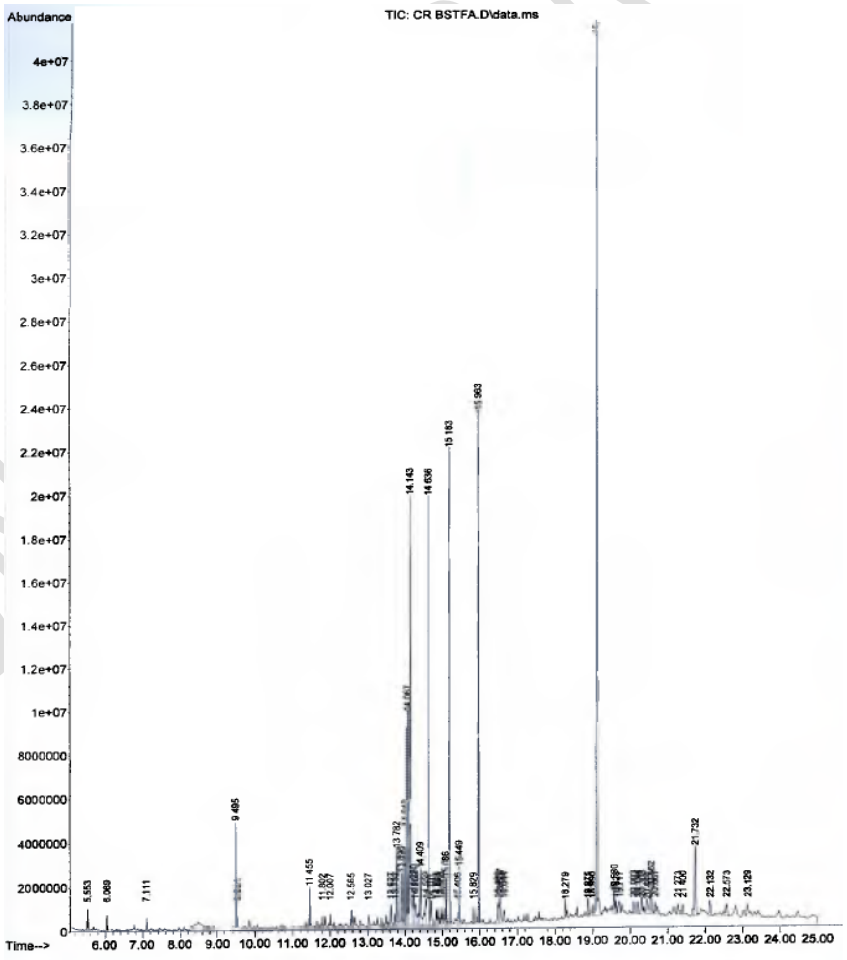


Figure 1: GC-MS chromatogram of the *T. polium* methanolic extract

Table 1: GC-MS Results of Phytonutrients of the *T. polium*

No.	Components	Retention Time (min)	Composition Percentage (%)
1.	Ethylamine	5.55	0.52
2.	Cyanuric Acid	6.06	0.65
3.	1,3-Propanediol	9.49	1.73
4.	Androstan-3-One	11.23	0.02
5.	Malic Acid	11.45	0.87
6.	N-Butyrlglycine	13.02	0.04
7.	Dodecen-3-ol	14.14	6.74
8.	L-Ascorbic acid	14.14	2.70
9.	3-OH-Tetradecenedioic Acid	14.63	9.70
10.	3-Hydroxysebacic acid	14.63	9.59
11.	Undecenedioic Acid	14.96	0.43
12.	3-Dodecenedioic acid	15.18	9.78
13.	Palmitic Acid	15.45	0.75
14.	Glucuronic	15.96	5.69
15.	Silane	16.26	0.13
16.	Isobutyric	16.53	0.48
17.	Hexadecanoic Acid	18.58	0.18
18.	Phenobarbitol	18.87	0.28
19.	α-D-Glucopyranose	19.11	22.66
20.	Chloral Hydrate	19.62	0.12
21.	D-Glucose	20.30	0.21
22.	Melibiose	20.36	0.23
23.	Maltose	21.16	0.04
24.	α-Tocopherol	22.26	0.06
25.	β-Sitosterol	24.88	0.02

Molecular Docking

The *T. polium* contains a variety of natural phytochemical compounds. These natural phytochemical compounds have been studied with in silico technique to examine the alleged anti-corona behavior. The utilized AutoDock algorithm can reveal the potential binding associations of the 8 major known compounds found in the methanolic extract of the *T. polium*. Docking simulation employs a grid-based energy evaluation method, in which pre-calculated interaction energies are being used as lookup tables to allow rapid assessment of ligand-protein interactions. Even so, unless complex side chains are treated beyond the grid, the execution of this grid-based method will entail strict processing of the target molecule. *T. polium* includes numerous natural phytochemical compounds. The alleged anti-corona actions of these natural phytochemical compounds were investigated using an in silico methodology. The AutoDock algorithm can help to describe binding associations of the eight major known chemical components in the *T. polium* methanolic extract. Docking simulation makes use of a grid-based energy evaluation technique in which pre-calculated pleasant relationship are used as lookup tables to allow for faster assessment of ligand-protein connections. Nonetheless, unless complex side chains have been treated beyond the grid, the grid-based technique will require strict processing of the target molecule.

Utilizing molecular docking just on $3CL^{pro}$ protein, the affiliation of eight *T. polium* phytoconstituents well with M^{pro} target SARS-CoV-2 proteins was investigated. The interacting atoms best binding capacity to $3CL^{pro}$ active site residues has indeed been determined. The study included FDA-approved HIV inhibitors such as Remdesivir, Hydroxychloroquine, and Chloroquine as positive controls [22]. Table 2 shows LBE (Lowest

Binding Energy) scores and Ki (Inhibition constant) values for the major *T. polium* phytocompounds with target protein.

Table 2: LBE scores and K_i values for the eight *T. polium* phytoconstituents and FDA approved HIV inhibitors with $3CL^{pro}$ (6LU7)

Group	No.	Compounds	6LU7 ($3CL^{pro}$)				
			LBE (kcal/mol)	**K_i (µM)**	**vdW + Hbond + desolv Energy (kcal/mole)**	**Electrostatic Energy (kcal/mole)**	**Torsional Free Energy (kcal/mole)**
Phytoconstituents of *T. polium*	1	1,3-Propanediol	-2.58	12950	-3.58	-0.19	+1.19
	2	Dodecen-3-ol	-4.92	247.68	-7.81	-0.09	+2.98
	3	L-Ascorbic acid	-3.91	1370	-5.63	-0.07	+1.79
	4	3-Hydroxytetradecanoic acid	-5.40	110.65	-9.38	-0.19	+4.18
	5	3-Hydroxysebacic acid	-5.12	175.66	-8.12	-0.29	+3.28
	6	3-Dodecenedioic acid	-5.38	380.36	-8.30	-0.24	+3.88
	7	D-Glucuronic	-5.20	153.64	-6.38	-0.61	+1.79
	8	α-D-Glucopyranose	-3.81	1600	-5.29	-0.31	+1.79
Positive Controls	1	Remdesivir	-4.59[a]	431.87[a]	-9.06[a]	-0.01[a]	+5.07[a]
	2	Chloroquine	-4.68[a]	373.83[a]	-6.92[a]	-0.14[a]	+2.39[a]
	3	Hydroxychloroquine	-4.22[a]	812.81[a]	-7.06[a]	-0.14[a]	+2.98[a]

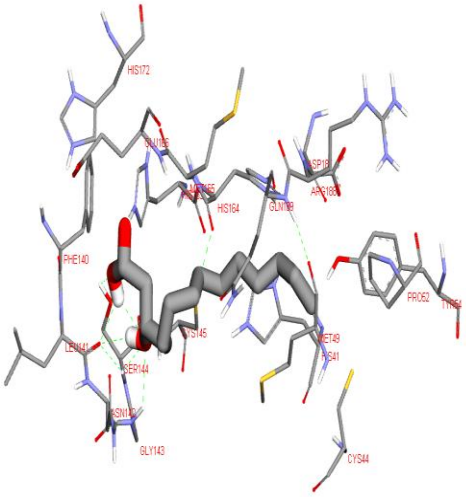
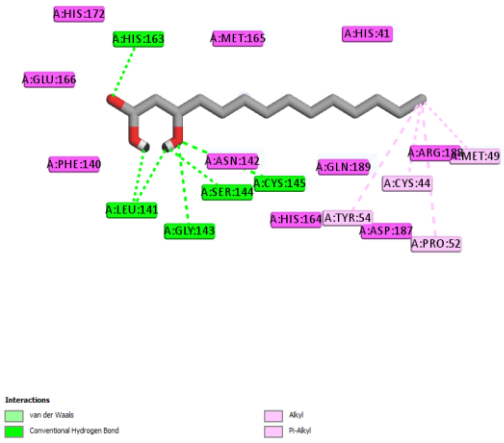
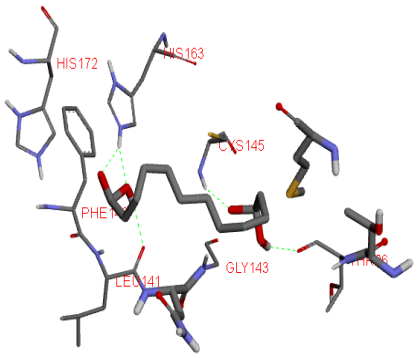
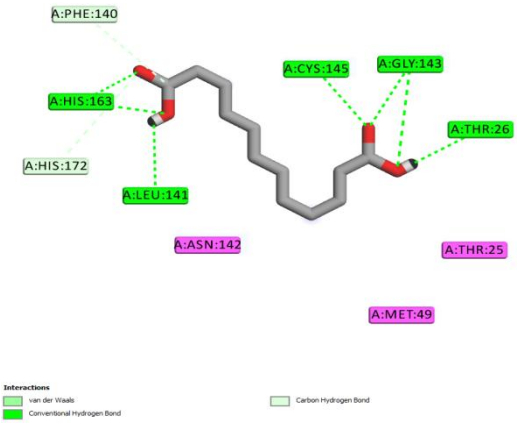
[18] [a]

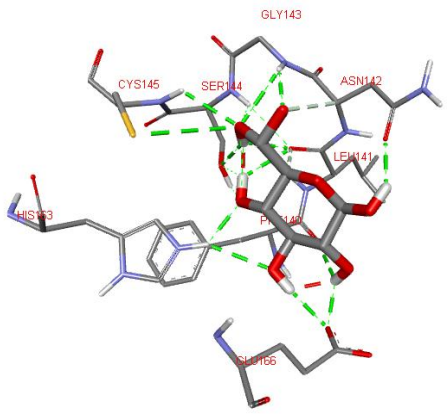
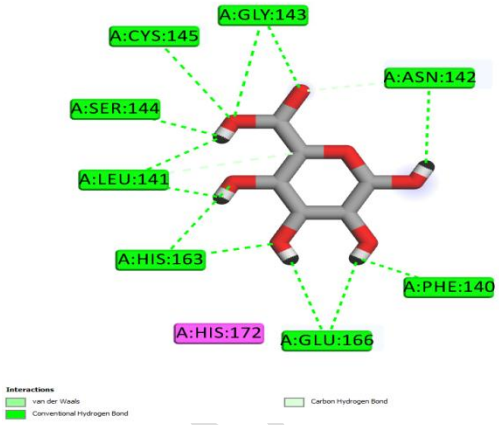
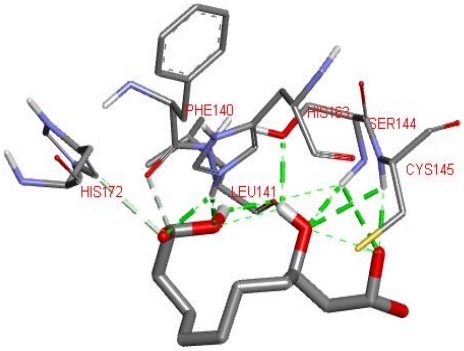
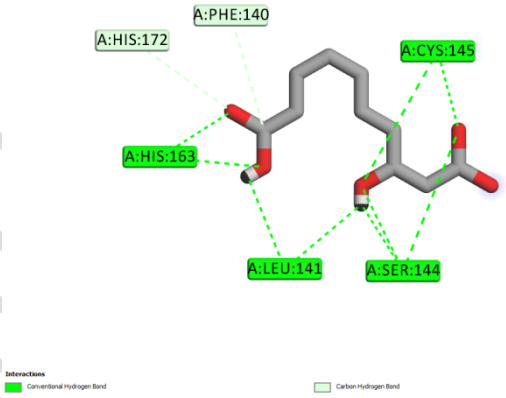
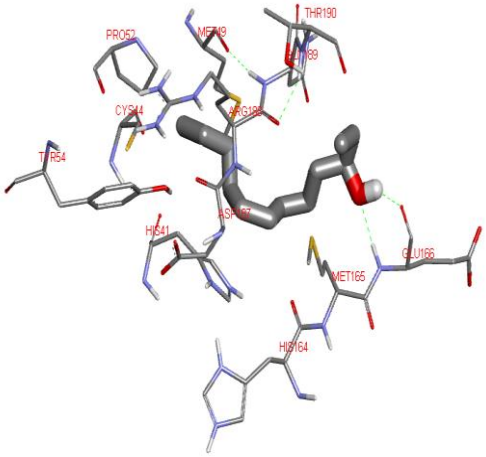
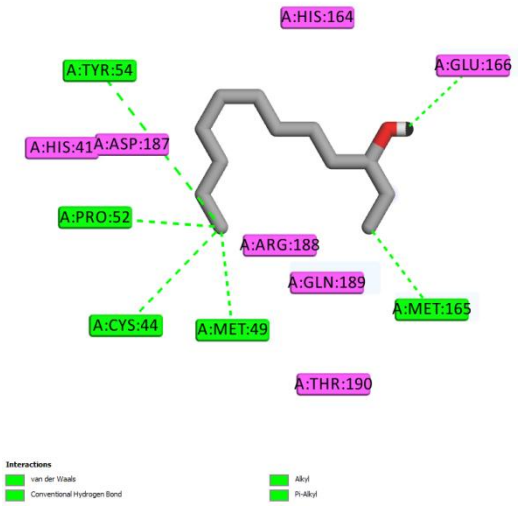
DISCUSSION

The GC-MS chromatogram (Figure 1) demonstrates that the methanolic crude extract of *T. polium* contains 25 peaks with retention times ranging from 5.55 to 24.88 min (Table 1). The extract is made up of oxygenated hydrocarbons, carboxylic acids, fatty acids (both unsaturated and saturated), and aromatic compounds, according to the analysis. With a percentage of more than 1%, eight major identified compounds were discovered in the methanolic crude extract of *T. polium*. These phytoconstituents are as follows: α-D-Glucopyranose (22.66%), 3-Dodecenedioic acid (9.78%), 3-OH-Tetradecenedioic Acid (9.70%), 3-Hydroxysebacic acid (9.59%), Dodecen-3-ol (6.74%), Glucuronic (5.69%), L-Ascorbic acid (2.70%), 1,3-Propanediol (1.73%), and Glucuronide (1.09%). Table 3 shows the results of five potential inhibitor that have area percent more than 2% and LBE and Ki results better than FDA approved HIV inhibitors as a potential inhibitor. All known compounds have indeed been docked to the pocket of 3CLpro (6LU7). The phytoconstituent compounds have different scores when compared to the enclosed amino acids. Interestingly, as shown in Table 3, 3-Hydroxytetradecanoic acid, 3-Dodecenedioic acid, D-Glucuronic, 3-Hydroxysebacic acid, and Dodecen-3-ol exceed the other phytoconstituent compounds as well as the optimistic controls by forming a strong association with destination enzyme's protease as confirmed by the LBE and Ki values. Depending on LBE and Ki values, the scoring values of the five major inhibitors increase in the following manner: 3-Hydroxytetradecanoic acid (-5.40 kcal/mol, 110.65 μM) > 3-Dodecenedioic acid (-5.38 kcal/mol, 380.36 μM) > D-Glucuronic (-5.20 kcal/mol, 153.64 μM) > 3-Hydroxysebacic acid (-5.12 kcal/mol, 175.66 μM) > Dodecen-3-ol (-4.92 kcal/mol, 247.68 μM). Definitely, 3-Hydroxytetradecanoic acid

has the top score (lowest LBE score and lowest Ki values) compared to the control drugs as well as all compounds selected with $3CL^{pro}$. Those certain potential inhibitors in *T. polium* methanolic extract could inhibit the protein of interest, but only to a lesser extent than 3-Hydroxytetradecanoic acid.

Table 3: 3D and 2D interaction results of the best *T. polium* phytocompounds with 3CLpro (6LU7)

Compound	3D Interaction results	2D Interaction results
3-Hydroxytetradecanoic acid		
3-Dodecenedioic acid		

D-Glucuronic		
3-Hydroxysebacic acid		
Dodecen-3-ol		

Surprisingly, in contrast to the normal regulation of this enzyme, the most potent natural compounds with LBE scores and Ki values are discovered to be in the following order: 3-Hydroxytetradecanoic acid > 3-Dodecenedioic acid > D-Glucuronic > 3-Hydroxysebacic acid > Dodecen-3-ol. This finding implies that these compounds could be useful medications for

preventing coronavirus viral replication by blocking the action of one the basic proteins. Remarkably, the putative pocket active site in 6LU7 has a higher affinity for 3-Hydroxytetradecanoic acid than Remdesivir, Chloroquine, and Hydroxychloroquine attributed to the prevalence of seven hydrogen bonds between 3-Hydroxytetradecanoic acid and HIS163, LEU141 (two H-bonding), GLY143, SER144, and CYS145 amino acids. Moreover, when compared to benchmark medicines, the docking scores of 3-Hydroxytetradecanoic acid with 6LU7 pocket are indeed the best and have the lowest binding energy.

CONCLUSION

The methanolic extract derived from the leaves of *T. polium* was investigated, and for the first time, its bioactive constituents were identified and evaluated using a GC-MS instrument and docking method on one of the most active coronavirus proteins ($3CL^{pro}$). The plant's methanolic extract contained 25 major natural phytochemicals, according to GC-MS outcomes. Eight of the twenty-five compounds have a composition proportion greater than 1%. Docking results indicate that 3-Hydroxytetradecanoic acid, 3-Dodecenedioic acid, D-Glucuronic, 3-Hydroxysebacic acid, Dodecen-3-ol have lowest LBE scores and Ki values for 3CLpro than supportive control drugs.

Besides that, the study of such compounds' interactions with the amino acids in $3CL^{pro}$ indicated that 3-Hydroxytetradecanoic seems to have the best LBE score and Ki value compared to approved drugs and all the other studied compounds, attributed to its ability to create tight hydrogen bonds with protease. As a result, these compounds that could be novel coronavirus inhibitors. Such computational results necessitate additional *in vitro* and *in vivo* research.

REFERENCES

1. Salman HA, Yaakop AS, Al-Mustafa A, Tarawneh K, Aladaileh S, Al-Rimawi F, Alakhras F, Abu-Lafi S, Zarzour RA, Wahab H. The dual impact of Jordanian *Ephedra alte* for inhibiting pepsin and treating microbial infections. *Saudi J. Biol. Sci.* 2021; 28(11):6245-6253. DOI: 10.1016/j.sjbs.2021.06.090.

2. Al-Mazaideh GM, Al-Swailmi F, Parrey MUR. Molecular Docking Evaluation of the Desert Truffles as Potent Antifungal Inhibitors. *J. Pharm. Res. Inter.* 2021; 33(5):36-48.

3. Krishnaiah D, Sarbatly R, Nithyanandam R. A review of the antioxidant potential of medicinal plant species. *Food Bioprod. Proc*. 2011;89(3):217-33.

4. Martins S, Amorim ELC, Peixoto Sobrinho TJS, Saraiva AM, Pisciottano MNC, Aguilar CN, Teixeira JA, Mussatto SI. Antibacterial activity of crude methanolic extract and fractions obtained from Larrea tridentata leaves. *Ind. Crop. Prod*. 2013; 41: 306-11.

5. Haddad MA, Dmour H, M. Al-Khazaleh JM, Obeidat M, Al-Abbadi A, Al-Shadaideh AN,
Al-mazra'awi MS, Shatnawi MA, Iommi C. Herbs and Medicinal Plants in Jordan. *J. AOAC* Inter. 2020; 103(4): 925-929.

6. Suleiman MS, Abdul-Ghani AS, Al-Khali S, Amin R. Effect of Teucrium polium boiled leaf extract on intestinal motility and blood pressure. *J. Ethnopharmacol*. 1998; 22: 111-116.

7. Tariq M, Ageel AM, Al-Yahia MA, Mossa JS, Al-Said MS. Anti-inflammatory activity of *Teucrium polium*. *Int. J. Tissue React*. 1989; 11: 185-188.

8. Mansouri S. Inhibition of Staphylococcus aureus mediated by extracts of Iranian plants, *Pharmac. Biol*. 1999; 37: 375-377.

9. Esmaeili MA, Zohari1 F, Sadeghi H. Antioxidant and protective effects of major flavonoids from *Teucrium polium* on b-cell destruction in a model of streptozotocin-induced diabetes. *Planta Med*. 2009; 75: 1418-1420.

10. Abdollahi M, Karimpour H, Monsef-Esfehani HR. 2003. Antinociceptive effects of *Teucrium polium L.* total extract and essential oil in mouse writhing test. *Pharm. Res. 2003;* 48: 31-35.

11. Esmaeili MA, Yazdanparast R. Hypoglycaemic effect of *Teucrium polium*: studies with rat pancreatic islets. *J. Ethnopharmacol*. 2004; 95: 27-30.

12. Tariq M, Ageel AM, al-Yahya MA, Mossa JS, al-Said MS. Anti-inflammatory activity of *Teucrium polium*. *Inter. J. Tissue Reac.*1989;11(4):185-188.

13. Bedir E, Tasdemir D, Çalis I, Zerbe O. Neo-clerodane diterpenoids from *Teucrium polium*. *Phytochem*. 1999;51(7):921–925.

14. Al-Eisawi DM. Field Guide to Wild Flowers of Jordan and Neighbouring Countries, Press-Foundation Al-Rai Jordan, 1998.

15. Abu-Reidah IM, Ali-Shtayeh MS, Jamous RM, Arráez-Román D, Segura-Carretero A. Comprehensive metabolite profiling of Arum palaestinum (Araceae) leaves by using liquid chromatography–tandem mass spectrometry, *Food Res. Inter*. 2015; 70: 74-86.

16. Krafczyk N, Glomb MA. Characterization of phenolic compounds in rooibos tea, *J. Agric. Food Chem.* 2008; 56: 3368-3376.

17. Forli S, Huey R, Pique ME, Sanner MF, Goodsell DS, Olson AJ. Computational protein-ligand docking and virtual drug screening with the AutoDock suite. *Nat. Protoc*.2016;11(5):905-19. DOI: 10.1038/nprot.2016.051.

18. Al-Mazaideh GM, Shalayel MH, Al-Swailmi FK, Aladaileh SH. Vitamin D is a New Promising Inhibitor to the Main Protease (Mpro) of COVID-19 by Molecular Docking. J. Pharm. Res. Inter. 2021; 33(29B), 186-191. https://doi.org/10.9734/jpri/2021/v33i29B31603.

19. Shalayel MH, Al-Mazaideh GM, Aladaileh SH, Al-Swailmi FK, Al-Thiabat MG. Vitamin D is a potential inhibitor of COVID-19: In silico molecular docking to the binding site of SARS-CoV-2 endoribonuclease Nsp15., Pak. J. Pharm. Sci. 2020; 33(5): 2179-21860. Doi.org/10.36721/PJPS.2020.33.5.REG.2179-2186.1.

20. Rizvi SMD, Shakil S, Haneef M. A simple click by click protocol to perform docking: AutoDock 4.2 made easy for non-bioinformaticians. *EXCLI J*. 2013; 12:831-857.

21. Pastick KA, Okafor EC, Wang F, Lofgren SM, Skipper CP, Nicol MR, Pullen MF, Rajasingham R, McDonald EG, Lee TC, Schwartz IS, Kelly LE, Lother SA, Mitja O, Letang E, Abassi M, Boulware DR. Review: Hydroxychloroquine and Chloroquine for Treatment of SARS-CoV-2 (COVID-19), Open Forum Infect. Dis. 2020; 7: ofaa130.

22. Hanwell MD, Curtis DE, Lonie DC, Vandermeersch T, Zurek E, Hutchison GR. Avogadro: an advanced semantic chemical editor, visualization, and analysis platform, J. Cheminform. 2012; 4:17.