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 the active coronaviral protein, the Main Protease (M^{pro}). This coronaviral protein was tested using Autodock with phytocompounds isolated from methanol extract made from *T. polium* leaves. According to the GC-MS results, the plant's methanolic extract contains 8 major natural substances (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)). As HIV-inhibitors, these phytoconstituents demonstrated good pharmacokinetics (ADMET) and drug-like characteristics (Remdesivir (-4.59 kcal/mol, 431.87 µM), Chloroquine (-4.68 kcal/mol, 373.83 µM), and Hydroxychloroquine (-4.22 kcal/mol, 812.81 µM)). Because they cannot cross the blood-brain barrier, these molecules are believed to have a strong safety profile, great absorption through the gastrointestinal system, and low central side effects. 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 for coronaviral protein has been investigated in order to determine their use at the active sites of the receptor, $3CL^{pro}$ (PDB ID: 6LU7). The major compounds showed greater affinity to $3CL^{pro}$ than the supporting control drugs. Furthermore, when the interactions of these components with the amino acids for $3CL^{pro}$ 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. These compounds could be proven as potential corona viral 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, has prompted researchers to look for alternative treatment options for a variety of diseases [1]. Several phytochemicals have recently been described as having 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]. Overall, natural substances are more chemically varied than biopharmaceutical compounds, 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, *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]. The ongoing industrialization causes uprooting, low yields, less cultivation, and destruction of *T. polium* [13]. Preservation as well as conservation of the *T. polium's* existing flora and fauna are required. Phytochemical analysis is required not only for the discovery of analytical markers, but also for the development of a valid and reliable, precise, appropriate, as well as efficient technique for the 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 the separation process [14].

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 compared with recent clinical studies and literature reviews on drugs like Remdesivir, Chloroquine, Hydroxychloroquine [15]. This study demonstrates GC-MS-based phytochemical constituents and identifies a significant bioactive compound involved in *T. polium's*. 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). 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* leaves

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

Extraction Process

10 g of Jordanian *T. polium* leaves were restored with 10 mL of methanol as well as stirred at room temperature for five days. For 10 minutes, the supernatant was 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 an HP-5MS capillary column coated with a 5% phenylmethylpoly-siloxane film (30 m 0.25 mm, 0.25 m film thickness). The agile number

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].

Pharmacokinetics prediction by Swiss ADME

Swiss ADME (http:/www.swissadme.ch/index.php) is a free online server that predicts the pharmacokinetic characteristics (Absorption, Distribution, Metabolism, and Excretion) and drug likeness of small compounds, which are vital to know before proceeding to clinical trials [21]. The compounds' 2D structures (SDF format) were uploaded and converted into a smile format before the algorithm was conducted.

Prediction of toxicity of compounds by pkCSM tool

8 identified *T. polium* species compounds and three HIV-inhibitors (Remdesivir, Chloroquine, and Hydroxychloroquine) were obtained in SDF format from the PubChem database and transferred to smile format. The toxicity properties were then predicted using the pkCSM online tool (http://biosig.unimelb.edu.au/pkcsm/) [22], where the smiling format was sent to the server and the results were processed.

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 displays detailed information for 25 peaks; 1 component has an area percent of 22.66%, 3 components have an area percent between 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 an area percent of 1.09% and 1.73%, and 18 components have an area percent of less than 1%.

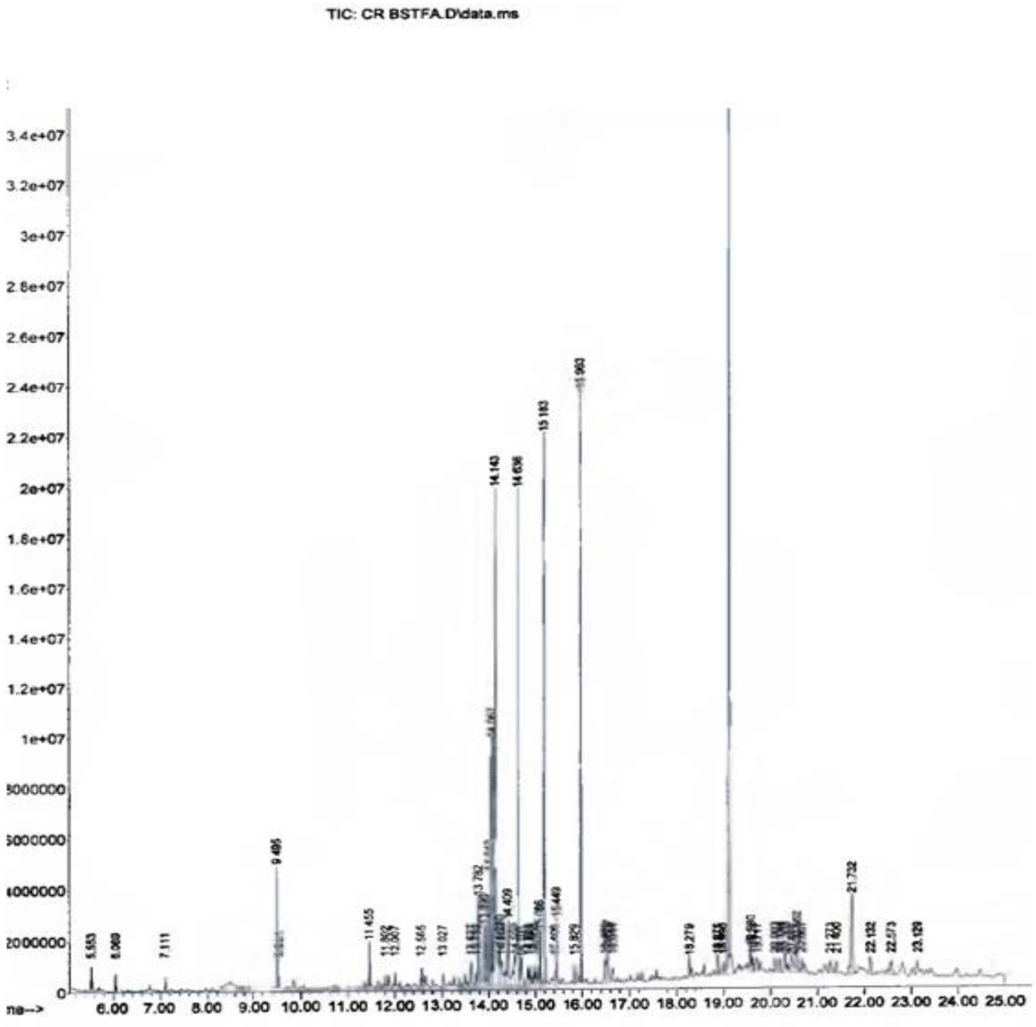


Figure 1: GC-MS chromatogram of the T. polium methanolic extract

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

T. polium contains a variety of natural phytochemical compounds. These natural phytochemical compounds have been studied in silico technique to examine the alleged anti-corona behavior. The utilized AutoDock algorithm can reveal the potential binding interactions of the 8 major known compounds found in the methanolic extract of *T. polium*. Docking simulation employs a grid-based energy evaluation method in which pre-calculated interaction energies are used as lookup tables to allow rapid assessment of ligand-protein interactions. 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 [23,24]. 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 8 *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]

Predictions of ADMET-pharmacokinetics in silico

An antagonistic reaction to protein or enzyme inhibitors in vivo has been proven to be inconclusive in terms of the inhibitor's suitability as a potential medication [25]. As a result, in drug development, the pharmacokinetic profile of the inhibitor (absorption, distribution, metabolism, and excretion), including drug-likeness assessments, is critical in determining whether the inhibitor can be supplied to a biological system [25, 26]. Furthermore, the poor pharmacokinetic features of potential inhibitors with very toxic effects on cells are the primary cause for the discontinuation of clinical trials [27]. Drug-likeness criteria are critical characteristics of filtration that aid in the production of efficient pharmacological inhibitors that are well absorbed and distributed in the system at the required time [28]. Lipinski's rule, which predicts the ability of an inhibitor to be orally active in the human system, is one of the most often used drug-likeness criteria [29]. According to the criterion, if the molecule has a molecular weight (Mw) of 500 g/mol, a partition coefficient (LOGP) $\leq$ 5, and hydrogen-bonding donors/acceptors ≤ 5 and 10, it might be orally absorbed/active [29]. Another significant drug-likeness criteria is the Ghose filter, which is used to explain the physicochemical properties of small molecules as well as the influence of existing functional groups and substructures. The molecular weight (160-480 g/mol), partition coefficient (-0.4 to 5.6), molar refractivity (MR) between 40-130, and number of atoms (20-70) including heteroatoms should be within the permitted range for the medicine to follow this criterion [30, 31]. The Egan rule is a computer model of the human intestinal membrane that aids in the prediction of active transport processes and the absorption of small substances. If the LOG $P <$ 5.88 and the topological polar surface area (TPSA) was smaller than 131.6 Å, the molecule was classified as drug-like [32].

Lastly, the Muegge filter (Bayer filter) determines if a chemical is drug-like or not based on the pharmacophore fragment matching. This model predicted that the accepted drug-likeness molecule would contain M.W (200-600 g/mol), less than 15 rotatable bonds, 150 TPSA, 5 H-bond donors, and 10 acceptors and LOG P (-2 to 5) [33]. Because there are so many algorithms, evaluating bioavailability in silico is difficult and complicated, and the aiming is to come as close to an in vivo experiment as feasible. The Abbott bioavailability score is one of the algorithms that has demonstrated its efficacy in estimating the probability of a substance having at least 10% oral bioavailability in a rat model at physiologic pH [34].

In silico pharmacokinetics methods, along with drug-likeness prediction, offer a wide array of chances to speed the discovery of novel candidate compounds with expected biological activity. Table 3 shows the ADME properties of *T. polium* bioactive compounds and three FDA-approved HIV-inhibitors to demonstrate and identify the good pharmacokinetic features of the prospective *T. polium* candidates. Furthermore, hepatotoxicity, AMES toxicity, hERG inhibition, and skin sensitization have been predicted in order to discover the inhibitors' toxic effects. These criteria have been measured and verified to ensure that they are within their typical ranges. In addition, the drug-likeness features of bioactive compounds, as well as their bioavailability scores, have been explored in Table 3 to determine which of these molecules have properties similar to those of recognized HIV-inhibitors.

Table 3: Pharmacokinetic parameters of the 3-Hydroxytetradecanoic acid bioactive compound of *T. polium* and three approved FDA-HIV inhibitors (Remdesivir, Chloroquine, and Hydroxychloroquine)

parameter		**3-Hydroxy-tetradecanoic acid**	**Remdesivir**	**Chloroquine**	**Hydroxychloroquine**
Absorption	Water solubility	Soluble	M. Soluble	M. Soluble	Soluble
	GI absorption	High	Low	High	High
	P-gp substrate	No	Yes	No	No
Distribution	BBB permeation	Yes	No	Yes	Yes
Metabolism	CYP1A2 inhibitor	No	No	Yes	Yes
	CYP2C19 inhibitor	No	No	No	No
	CYP2C9 inhibitor	No	No	No	No
	CYP2D6 inhibitor	Yes	No	Yes	Yes
	CYP3A4 inhibitor	No	Yes	Yes	No
Excretion	Renal OCT2 substrate	No	No	No	No
Toxicity	AMES Toxicity	No	No	Yes	Yes
	Max. tolerated dose (human)	-0.402	0.377	0.28	-0.045
	hEGR I inhibitor	No	No	No	No
	hEGR II inhibitor	No	Yes	Yes	Yes
	Oral Rat Acute Toxicity (LD50)	1.386	2.133	2.779	2.653
	Oral Rat Chronic Toxicity (LOAEL)	2.834	2.062	0.737	0.696
	Hepatotoxicity	No	Yes	Yes	Yes
	Skin sensitization	Yes	No	No	No
	T. Pyriformis toxicity	0.502	0.285	1.533	1.02

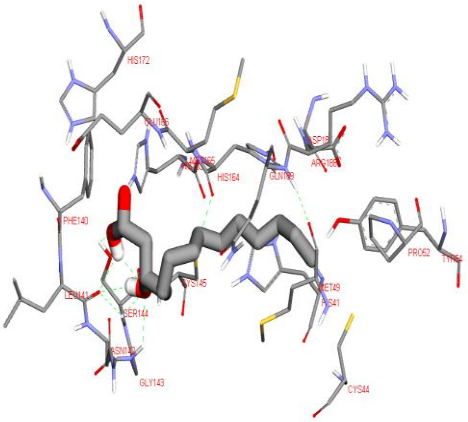
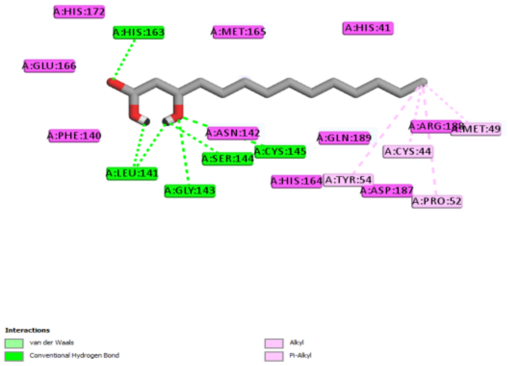
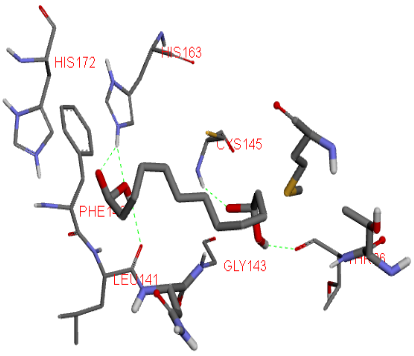
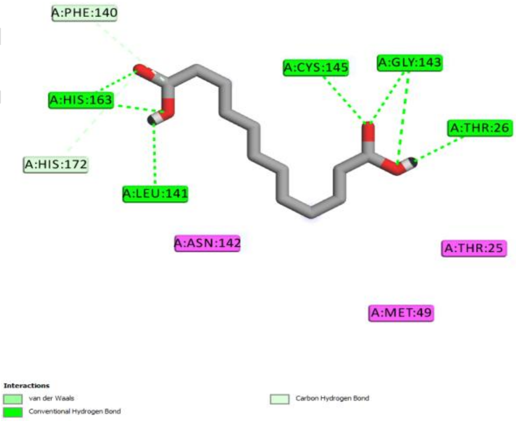
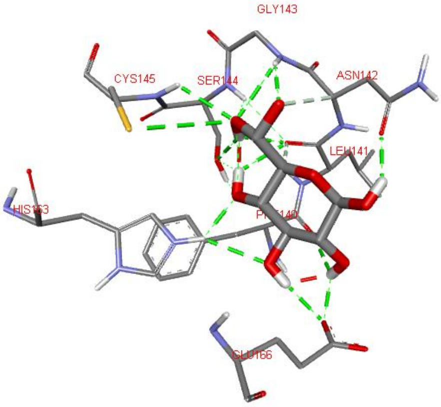
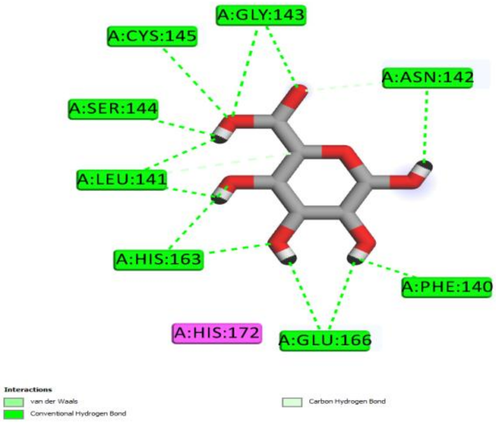
	Minnow Toxicity	-0.382	0.27	0.696	1.846
Drug-likeness	M.Wt g/mol	244.37	602.58	319.87	335.87
	R. bonds	12	14	8	9
	A.H-bond	3	12	2	3
	D.H-bond	2	4	1	2
	MR	72	150	97	99
	TPSA	57.53	213.36	28.16	48.39
	LOGP	2.81	0.18	3.20	2.35
	Lipinski # violations	0	2	0	0
	Ghose # violations	1	3	1	1
	Egan # violations	1	1	1	1
	Muegge # violations	1	3	1	1
	Bioavailability Score	0.85	0.17	0.55	0.55

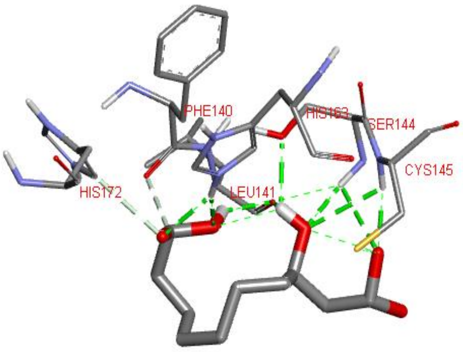
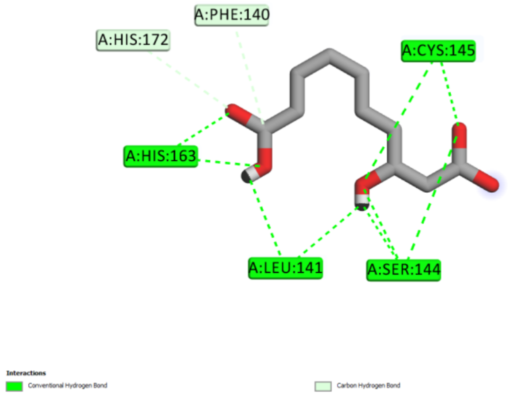
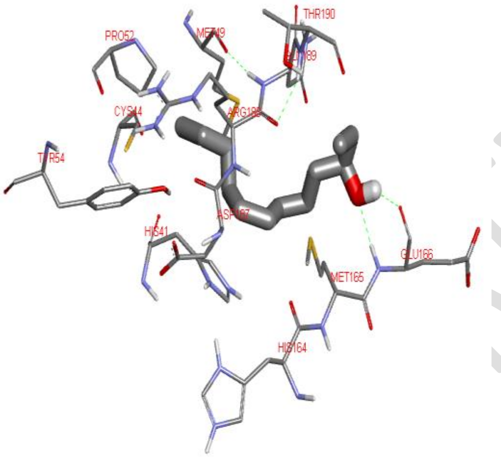
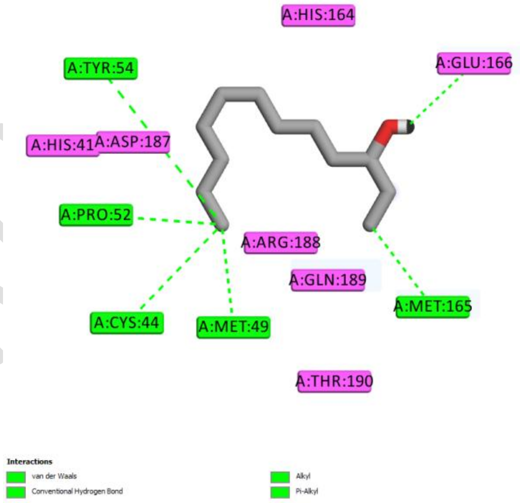
* M. Soluble: Moderate soluble, P. Soluble: Poor soluble, GI absorption: Gastro-Intestinal absorption, P-gp substrate: P-Glycoprotein substrate, BBB permeation: Blood Brain Barrier permeation. M.W: Molecular weight, R. bonds: Rotatable bonds, A.H-bond: Acceptor hydrogen bond, D.H-bond: Donor hydrogen bond, MR: Molar refractivity, TPSA: Topological polar surface area

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). According to the analysis, 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%, 8 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 2 shows the results of 5 potential inhibitor that have an area percent greater than 2% in GC-MS and LBE and Ki results for these potential compounds were found to be better than FDA approved HIV inhibitors. All known compounds (Table 4) have indeed been docked to the pocket of 3CLpro (6LU7). Interestingly, as shown in Table 2, 3-Hydroxytetradecanoic acid, 3-Dodecenedioic acid, D-Glucuronic, 3-Hydroxysebacic acid, and Dodecen-3-ol exceed the other phytoconstituent compounds as well as the control compounds by forming a strong interaction with the target protease enzyme 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}. 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 4: 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		

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 which is 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 the 6LU7 pocket are indeed the best and have the lowest binding energy.

Table 3 shows that the phytoconstituent of *T. polium* (3-hydroxytetradecanoic acid) exhibit strong pharmacological qualities as HIV-inhibitors based on ADMET outputs and drug-like features (Remdesivir, Chloroquine, and Hydroxychloroquine). This molecule has a high water solubility and gastrointestinal absorption, and it may cross the BBB and be excreted without binding to the renal OCT2 substrate. Furthermore, the toxicity profiles of this phytoconstituent demonstrated that there are no unwanted properties, and it looks to be the best when compared to the inhibitors (controls).

The bioavailability radar for 3-hydroxytetradecanoic acid and HIV-inhibitors is represented in Figure 2. The predicted values for size, lipophilicity, and structural flexibility of 3-hydroxytetradecanoic acid in the radar acceptance region (pink spider map) were much lower than those Remdesivir, Chloroquine, and Hydroxychloroquine.

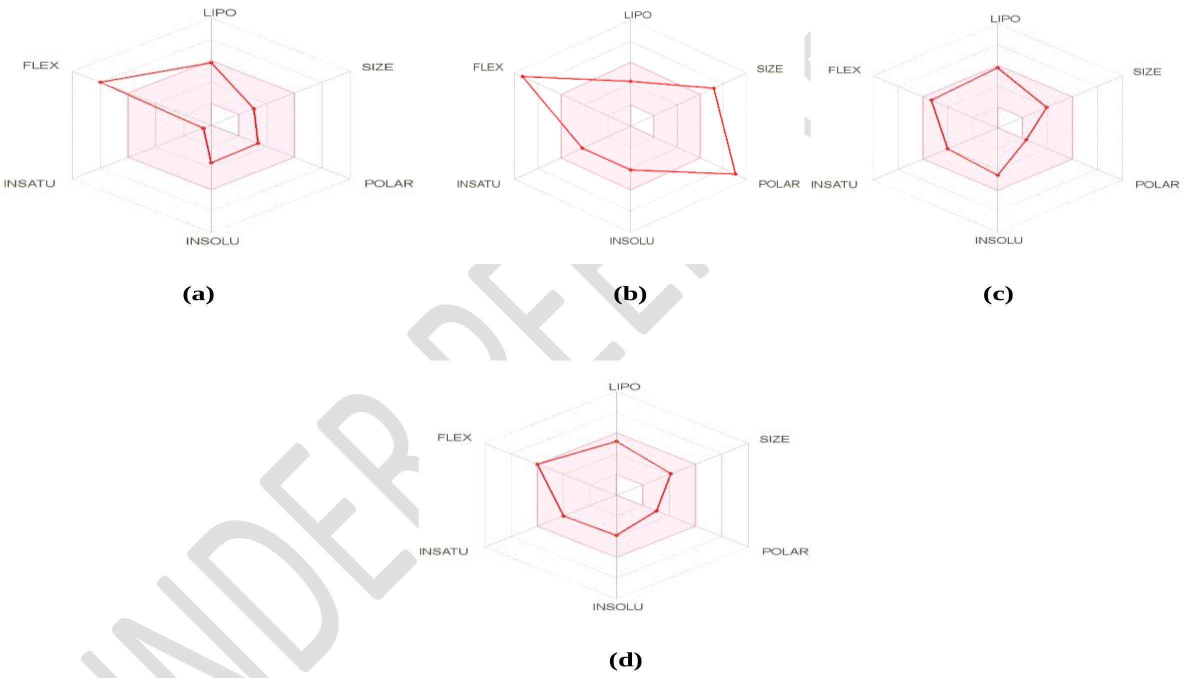


Figure 2: Bioavailability radar of the best phytoconstituents of *T. polium* (3-hydroxytetradecanoic acid) (a) and positive controls (Remdesivir (b), Chloroquine (c), and Hydroxychloroquine (d))

CONCLUSION

The methanolic extract derived from the leaves of *T. polium* were identified and evaluated using a GC-MS instrument and docking method on one of the most active coronavirus proteins ($3CL^{pro}$). According to GC-MS outcomes, the plant's methanolic extract contained 25 major natural phytochemicals, according to GC-MS outcomes. 8 of the 25 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 the lowest LBE scores and Ki values for 3CLpro than supportive control drugs. The phytoconstituents of *T. polium* (3-Hydroxytetradecanoic acid) exhibit outstanding qualities according to ADMET and drug-like properties and are predicted to be safe, orally consumed, readily absorbed in the gastrointestinal system, and have a high bioavailability score. Further in vitro and in vivo research is needed to corroborate the computational findings of this work.

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