Abstract: Two active coronaviral proteins (3CL\textsuperscript{pro} and Nsp15) have been studied using both the GC-MS and docking methods. These coronaviral proteins have been examined with the methanol extract generated from leaves of the \textit{Arum palaestinum}. According to the GC-MS findings, 19 major natural compounds are present in the plant’s methanolic extract. The lowest Binding Energy (LBE) and the inhibition constant ($K_i$) have been used to identify and classify the potential of these lead drugs with their pharmacological properties. The affinity of these compounds with coronaviral proteins has been evaluated to reveal the usage of these compounds at the active sites of the receptors, 3CL\textsuperscript{pro} (PDB ID: 6LU7) and Nsp15 (PDB ID: 6VWW). The results of β-Sitosterol, Androstan-3-one, Phenobarbital, Maltose, and α-Tocopherol show more affinity to Nsp15 and 3CL\textsuperscript{pro} than to the supporting control drugs. Furthermore, an evaluation of the interactions of these components with the amino acids of 3CL\textsuperscript{pro} and Nsp15 revealed that β-Sitosterol has the best LBE score and $K_i$ value as compared with those of the approved medication and all other compounds under investigation. Consequently, these potential compounds may be modern inhibitors of coronavirus. Further in vitro and in vivo studies are needed for such computational findings.

Keywords: \textit{Arum palaestinum}, GC-MS, Computer-Aided Drug Design, Nsp15, 3CL\textsuperscript{pro}, β-Sitosterol

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The economic burden and reported serious side effects of conventional medicines cause the researchers to seek alternative treatment sources against various diseases (1). For instance, several phytochemicals have been recently reported to have a powerful antiviral efficacy, which allows them to inhibit the high rate of coronavirus replication (2-5). Those phytochemicals give a ray of hope for the detection of new COVID-19 inhibitors in nature (3-6). In general, natural substances are highly chemically diverse, have lower production costs than biopharmaceutical compounds, and have either milder or non-existent adverse consequences as opposed to synthetic compounds (7).

In Jordanian folk medicine, herbal drugs are widely fashioned in the treatment of various ailments because they are rich sources of bioactive constituents (8). For instance, the A. palaestinum Boiss. A. palaestinum is an invaluable herb because it is commonly prescribed by numerous traditional Arabic herbalists for the treatment of bones diseases, jaundice, diabetes, renal infections, skin problems, syphilis, rheumatoid arthritis, cancer, and worms’ infection (9). To date, the available analytical data have elaborated the acceptance of the A. palaestinum Boiss. A. palaestinum due to the presence of herbal constituents, such as viz. isovanillin, linoleic acid, β-sitosterol, isovitexin, and vitexin (10). These herbal constituents are prevalent in a crude methanolic extract of the A. palaestinum (11). The embedded phytoconstituents, chemical exploration, and therapeutic activity of the A. palaestinum have been reported (12).

The day-to-day rise in industrialization leads to uprooting, low production, less cultivation, and destruction (13). Consequently, preservation and conservation of the remaining flora and fauna of the A. palaestinum are required. Therefore, phytochemical analysis is needed not only for the uncovering of the analytical markers but also for the development of a validated, precise, appropriate, and efficient technique needed for the characterization of the biography of the A. palaestinum. Detailed chemoprofiling of the A. palaestinum leaves by using liquid chromatography requires a long run time, tedious analytical procedure, and compulsory high volume of organic solvents for sample preparation (14).

The aim of this study was to assess the phytochemical architecture by GC-MS analysis and to investigate a mechanism-based study, which may be accountable for the pharmacological activity of the A. palaestinum. This work demonstrates a GCMS-based phytochemical analysis and uncovers a significant bioactive mechanism that is responsible for the positive attitude of the A. palaestinum. In addition, the current research attempts to screen and assess the probable inhibitory activity of A. palaestinum-derived compounds against SARS-CoV-2 (3CLpro and Nsp15), possibly presenting new recognized compounds against the novel pandemic coronavirus disease (COVID-19). Moreover, our proposed method is simple, short, precise, accurate, economical, and requires a low volume of organic solvent for sample preparation.

MATERIALS AND METHODS

Collection of plant materials

Wild-growing leaves of fresh Jordanian A. palaestinum plant were obtained from the Karak governorate of Jordan at the start of the spring term of 2019. The method of obtaining these leaves was described by Al-Eisawi (13). The plant’s leaves were dried in the shade, isolated at room temperature to a consistent weight, crushed, and stored in a dark area.

Extraction process

One gram of the cleaned and dried Jordanian A. palaestinum leaves was recovered with 10 mL of methanol and stirred at room temperature for 5 days. The supernatant was centrifuged at 4500 rpm for 10 minutes. At room temperature, 3.0 mL of the supernatant was transferred to a 10 mL test tube then was evaporated. The residues were reformed in 100 μL of N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) solution before injection into the GC-MS.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS investigation was conducted out using an Agilent technology model 6890 GC that was fitted with a Split-splitless injector and HP-5MS capillary column type that coated with a film of 5 percent phenylmethylpolysiloxane (30 m × 0.25 mm, 0.25 μm film thickness). The agile amount 6890 GC was fitted with 5973C mass spectrometer style with Inert MSD (Mass Spectrometer with mass Selective Detector and GC-MS). The temperature applied on the column oven was programmed as follows: The starting temperature was 60°C, then was raised up to 300°C with a ramp of 15°C/min, and finally was remained at 300°C for 7 min before all elution was done. The split valves were opened after 15 seconds to purge the injector for 3 minutes. Both injections (1 μL) were produced using a syringe of

Detailed
Molecular modeling process

The structures of the targeted protein receptor included pp1ab from SARS-CoV-2 (chain A of PDB ID: 6LU7 (16)) and (chain B of PDB ID: 6VWW (17)). UCSF Chimera 1.15 (18) was used for modeling and analysis processes. Ligands and solvent molecules were removed from the protein structures. Hydrogen atoms were added, Dunbrack 2010 rotamer library (19) was used for building incomplete side chains, and the AMBER14SB force field (20) was used for standard residue parameterization. The default AMBER force field parametrization is to fulfill the physiological pH condition (i.e., pH = 7.4) in each receptor. That is, Asp and Glu residues were deprotonated, Arg and Lys residues were protonated, and His residues kept neutral. Receptor file format (pdb) was generated using UCSF Chimera 1.15 (18), whereas receptor file format (pdbqt) was generated using AutoDockTools (21, 22) in MGLTools 1.5.7.

An in-house ligand library of potential binding compounds was generated from the PubChem database (23) or built using Avogadro molecule editor (24). Missing hydrogen atoms were added, and the Gasteiger charges using Antechamber (26). Ligand mol2 file format was generated using UCSF Chimera 1.15 (18), whereas ligand pdbqt file format was generated using open babel 2.4.1 (27).

Molecular docking process

Molecular docking was performed using Autodock Vina 1.1.2 (28) in a virtual screening study. Binding sites for each receptor were predicted using P2Rank 2.2 (29) and were searched in a grid box of 30 × 30 × 30 Å³ with exhaustiveness 24 to ensure comprehensive sampling.

Molecular docking analysis

The formation of the ligand-receptor complex is represented by the following basic thermodynamic equilibrium equation, where $K_{eq}$ is the equilibrium constant.

$$K_{eq} = \frac{[\text{Ligand} \cdot \text{Receptor}]}{[\text{Ligand}] \cdot [\text{Receptor}]}$$

The Gibbs free energy ($\Delta G$), which is also called the binding affinity, is related to the equilibrium constant by $\Delta G = -RT \ln K_{eq}$, where $R$ is the gas constant in a unit of kcal/(mol·K) and $T$ is the temperature in a unit of kelvin. The inhibition constant ($K_i$) is related to the equilibrium constant by $K_i = 1/K_{eq}$; therefore, the free energy of binding in terms of $K_i$ can be written as $\Delta G = RT \ln K_i$. The 2D ligand–protein interaction diagrams were generated using LigPlot+ 2.2.4 (30).

RESULTS

GC-MS Analysis of the Extract

The extracted constituents of the A. palaestinum have been investigated using GC–MS. Each ingredient in the methanolic extract has been measured and identified by comparing mass fragmentation patterns to standards such as Wiley 9 library spectral data and NIST standards (Figure 1). The detailed information of 19 peaks is shown in Table 1; one component has an area percent of 18.4%, 2 components have area percent of around 5.6%, 11 components have area percent between 1 and 5%, and 5 components have area percent less than 1%.

![Figure 1. GC-MS chromatogram of the A. palaestinum methanolic extract.](image)
silico technique to examine the alleged anti-corona behavior. The utilized AutoDock Vina algorithm can reveal the potential binding associations of 19 known compounds found in the methanolic extract of the *A. palaestinum*. 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. The grid box parameters for the most probable binding pockets in the proteins and their residues are shown in Table 2.

The association of 19 *A. palaestinum* phytoconstituents with the two target SARS-CoV-2 proteins has been evaluated using molecular docking

| Components                        | Retention Time (min) | Composition Percentage (%) |
|-----------------------------------|----------------------|-----------------------------|
| Ethylamine                        | 5.53                 | 0.17                        |
| Cyanuric acid                     | 6.06                 | 0.03                        |
| 1,3-Propanediol                   | 9.49                 | 1.15                        |
| Androstan-3-one                   | 11.45                | 0.03                        |
| Malic acid                        | 11.46                | 2.01                        |
| N-Butylglycine                    | 12.55                | 0.08                        |
| 3-Hydroxydodecanoic acid          | 14.14                | 18.40                       |
| L-Ascorbic acid                   | 14.27                | 5.61                        |
| 3-Hydroxytetradecanedioic acid    | 14.64                | 4.48                        |
| 3-Hydroxysebacic acid             | 14.64                | 4.48                        |
| Undecanedioic acid                | 14.65                | 3.19                        |
| Palmitic acid                     | 15.45                | 1.35                        |
| Glucuronic acid                   | 15.95                | 2.49                        |
| Isobutyric acid                   | 16.53                | 1.51                        |
| Phenobarbital                     | 18.89                | 1.53                        |
| α-D-Glucopyranose                 | 19.03                | 4.80                        |
| Maltose                           | 20.92                | 0.02                        |
| α-Tocopherol                      | 22.26                | 5.63                        |
| β-Sitosterol                      | 24.67                | 2.66                        |

| Receptor | PDB ID       | Pocket No | Grid Center | List of Adjacent Residues |
|----------|--------------|-----------|-------------|---------------------------|
| pplab from SARS-CoV-2 | 6LU7 (3CL₃⁰) | 1 | -11.0778 | 16.9853 | 67.6274 | Thr3288, Thr3289, Leu3290, His3304, Met3312, Phe3403, Leu3404, Asn3405, Gly3406, Ser3407, Cys3408, His3426, His3427, Met3428, Glu3429, Asp3450, Arg3451, Gln3452 |
| pplab from SARS-CoV-2 | 6VWW (Nsp15) | 1 | -46.0066 | 32.7991 | 27.9534 | His6686, Gin6696, Leu6697, Gly6698, His6701, Lys6741, Val6743, Ser6745, Met6782, Trp6784, Thr6792, Tyr6794, Leu6797 |
|                      |               | 2 | -64.3941 | 31.1839 | 27.5128 | Lys6522, Lys6541, Ser6649, Arg6650, Asn6651, Leu6652, Leu6703, Leu6717, Asp6719, Met6723, Asp6724, Ser6725, Thr6726, Lys6728, Tyr6730, Val6746, Ile6747, Asp6748 |
The efficiency of some active ingredients…

on 3CL\textsuperscript{exo} and Nsp15 proteins. The best binding ability of the interacting molecules to 3CL\textsuperscript{exo} and Nsp15 active site residues has been determined. As a positive control, the analysis has included FDA-approved HIV inhibitors, namely, hydroxychloroquine and chloroquine (31). Table 3 displays the Lowest Binding Energy (LBE) scores and \( K_i \) values for 19 A. palaestinum phytoconstituents and two FDA approved HIV inhibitors (Hydroxychloroquine and Chloroquine) with 3CL\textsuperscript{exo} (6LU7) and Nsp15 (6VWW).

### DISCUSSION

GC-MS chromatogram (Figure 1) shows the methanolic crude extract of A. palaestinum has 19 peaks with retention times between 5.5 and 24.6 min (Table 1). The analysis has revealed that the extract is composed of oxygenated hydrocarbons, carboxylic acids, fatty acids (saturated and unsaturated), and aromatic compounds. Ten major identified compounds have been found in the methanolic crude extract of the A. palaestina with an area percent more than 2%. These phytoconstituents are 3-Hydroxydodecanoic acid (18.4%), \( \alpha \)-Tocopherol (5.63%), L-Ascorbic acid (5.61%), 3-Hydroxysebacic acid (4.48%), Undecanedioic acid (3.19%), \( \beta \)-Sitosterol (2.66%), D-Glucuronic acid (2.49%), and Malic acid (2.01%). Figure 2 shows the structure of the main compounds that have area percent more than 2% and Phenobarbital as a potential inhibitor based on LBE and \( K_i \) results.

All the known compounds have been docked to 3CL\textsuperscript{exo} (6LU7) pocket as well as to the first and second pockets of Nsp15 (6VWW). The phytoconstituent

| Group | No. | Compounds                        | 6LU7 (3CL\textsuperscript{exo}) | 6VWW (Nsp15) |
|-------|-----|----------------------------------|---------------------------------|--------------|
|       |     |                                  | LBE (kcal/mol) \( K_i \) (μM)  | LBE (kcal/mol) \( K_i \) (μM)  |
|       |     |                                  | 1st pocket                      | 2nd pocket   |
|       |     |                                  | LBE (kcal/mol) \( K_i \) (μM)  | LBE (kcal/mol) \( K_i \) (μM)  |
|       | 1   | 1,3-Propanediol                  | –3.4 3217.55                    | –3.5 2717.8  |
|       | 2   | 3-Hydroxydodecanoic acid         | –4.9 255.803                    | –4.6 424.45  |
|       | 3   | 3-Hydroxysebacic acid            | –5.4 109.993                    | –4.9 255.80  |
|       | 4   | 3-Hydroxytetradecanedioic acid   | –5.2 154.164                    | –4.6 424.45  |
|       | 5   | \( \alpha \)-D-Glucopyranose     | –4.8 302.840                    | –4.4 594.90  |
|       | 6   | \( \alpha \)-Tocopherol          | –6.5 20.337                     | –5.7 66.289  |
|       | 7   | \( \beta \)-Sitosterol           | –6.7 12.256                     | –7.8 1.914   |
|       | 8   | Androstan-3-one                  | –6.4 20.337                     | –7.1 6.239   |
|       | 9   | Cyanuric acid                    | –5.1 182.511                    | –4.1 987.12  |
|       | 10  | D-Glucuronic acid                | –5.3 130.219                    | –4.6 424.45  |
|       | 11  | Ethylamine                       | –2.4 17402.3                    | –2.7 10487   |
|       | 12  | Isobutyric acid                  | –3.7 1939.11                    | –3.7 1939.1  |
|       | 13  | L-Ascorbic acid                  | –5.1 182.511                    | –4.7 358.52  |
|       | 14  | Malic acid                       | –4.8 302.840                    | –4.8 302.84  |
|       | 15  | Maltose                          | –6.2 28.504                     | –6.0 39.950  |
|       | 16  | N-Butylglycine                   | –4.1 987.121                    | –4.3 704.29  |
|       | 17  | Palmitic acid                    | –4.5 502.502                    | –5.0 216.07  |
|       | 18  | Phenobarbital                    | –6.6 14.510                     | –5.7 66.289  |
|       | 19  | Undecanedioic acid               | –5.1 182.511                    | –5.1 182.51  |
| Positive Controls | 1 | Chloroquine                      | –5.9 47.296                     | –5.5 92.909  |
|       | 2   | Hydroxychloroquine               | –6.3 24.076                     | –5.9 47.296  |
compounds have varying scores with the enclosed amino acids (Table 3). Surprisingly, β-Sitosterol, Phenobarbital, Maltose, and α-Tocopherol outperform the other phytoconstituent compounds and the optimistic controls as shown in Table 3 by creating a powerful association with the protease of both enzymes as revealed by the LBE and $K_i$ values. Clearly, β-Sitosterol has a lower LBE score and lower $K_i$ value than that of the control drugs and all chosen compounds with 3CLpro and both pockets of Nsp15. Phenobarbital could inhibit both proteins despite its area percent is less than 2% in the methanolic extract of A. palaestinum. Figure 3 and Figure 4 show the analysis of the best score of these potential inhibitors by LigPlot+.

Even though Phenobarbital and Androstane-3-one have small composition percentages in the extract, specifically, 1.53% and 0.03%, respectively, they are expected to have significant

Figure 2. Structure of the major identified compounds in methanolic extract of A. palaestinum.

Figure 3. 2D interaction diagram of β-Sitosterol with 3CLpro (6LU7).

[Chemical structures and diagrams of 3-Hydroxylstearic acid, 3-Hydroxysadecanoic acid, Malic acid, Undecanedioic acid, β-Sitosterol, D-Glucuronic acid, a-Tocopherol, L-Ascorbic acid, a-D-Glucopyranos, and Phenobarbital are shown.]
The efficiency of some active ingredients on the coronavirus. In Table 3, four compounds are found to inhibit 3CL\textsuperscript{pro} (6LU7) with LBE scores and $K_i$ values less than that of the positive controls, Hydroxychloroquine (-6.3 kcal/mol, 24.076 μM) and Chloroquine (-5.9 kcal/mol, 47.296 μM). These potential inhibitors are arranged in the following order: β-Sitosterol (-6.7 kcal/mol, 12.256 μM) > Phenobarbital (-6.6 kcal/mol, 14.510 μM) > α-Tocopherol (-6.5 kcal/mol, 20.337 μM) > Androstan-3-one (-6.4 kcal/mol, 20.337 μM).

Furthermore, three potential inhibitors are found to inhibit first pocket of Nsp15 (6VWW) with LBE scores and $K_i$ values less than that of the positive controls, Hydroxychloroquine (-5.9 kcal/mol, 47.296 μM) and Chloroquine (-5.5 kcal/mol, 92.909 μM). These potential inhibitors are arranged in the following order: β-Sitosterol (-7.8 kcal/mol, 1.914 μM) > Androstan-3-one (-7.1 kcal/mol, 6.239 μM) > Maltose (-6.0 kcal/mol, 39.950 μM). In addition, five potential inhibitors are found to inhibit second pocket of Nsp15 (6VWW) with LBE scores and $K_i$ values less than that of the positive controls, Hydroxychloroquine (-6.5 kcal/mol, 17.178 μM) and Chloroquine (-6.2 kcal/mol, 28.504 μM). These potential inhibitors are arranged in the following order: β-Sitosterol (-8.5 kcal/mol, 587 μM) > Phenobarbital (-7.7 kcal/mol, 2.266 μM) > Androstan-3-one (-7.6 kcal/mol, 2.683 μM) > Maltose (-7.5 kcal/mol, 3.176 μM) > α-Tocopherol (-7.4 kcal/mol, 3.760 μM).

Interestingly, the most potent natural compounds with LBE scores and $K_i$ values in contrast to the normal regulation for both enzymes are discovered to be in the following order: β-Sitosterol > Androstan-3-one > Phenobarbital > Maltose > α-Tocopherol. This finding suggests that these compounds may be effective medications for inhibiting coronavirus viral replication by inhibiting the action of the two basic proteins.

Surprisingly, the putative pocket protein binding site in 6LU7 has shown a greater binding affinity to β-Sitosterol than to Chloroquine and Hydroxychloroquine due to the presence of two hydrogen bonding between β-Sitosterol and Leu3404 and Gly3406 amino acids. Furthermore, the binding of β-Sitosterol to the first binding pocket of 6VWW demonstrates two hydrogen bonding with Ser6745 and Val6743. In addition, the second binding pocket of 6VWW shows three hydrogen bonding between β-Sitosterol and Lys6522,
Ser6725, and Thr6726. According to our findings, the docking scores of the β-Sitosterol with all three pockets are the best and have the lowest binding energy. When compared to reference medicines, these results show that β-Sitosterol has a high affinity to all pockets.

CONCLUSIONS

The methanolic extract extracted from the leaves of the *A. palaestinum* has been examined, addressed for the first time for its bioactive constituents, and analyzed using a GC-MS instrument and docking method on two active coronavirus proteins. According to the results of GC-MS, the plant’s methanolic extract contains 19 main natural compounds. Ten of the 19 compounds have a composition percentage greater than 2%. Docking results have shown that β-Sitosterol, Androstan-3-one, Phenobarbital, Maltose, and α-Tocopherol have higher LBE scores and $K_i$ values than supportive control drugs for Nsp15 and 3CLpro. Furthermore, the study of the interactions of these compounds with the amino acids in 3CLpro and Nsp15 has revealed that β-Sitosterol has the best LBE score and $K_i$ value than the licensed drugs and all other studied compounds, owing to its ability to form tight hydrogen bonds with proteins. Consequently, we have possible compounds that may be novel coronavirus inhibitors. Such computational results need further *in vitro* and *in vivo* research.

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Conflict of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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