# *Chapter 4*

# *A Computational Effort to Deciphering Putative COVID-19 3C-like Protease Binders in the Selected Recipes of Kurdish Ethnomedicine: An Approach to Find an Antiviral Functional Tea*

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**Key words:** Functional Tea, COVID-19, Protease Binders, Ethnomedicine, SARS-CoV-2 Protease

## INTRODUCTION

Paleoanthropological expedition of the Shanidar cave, Zagros Mountains of Kurdistan, Iraq *shed* the *light* of truth and *knowledge* upon applications of herbal medicine through millennia [1, 2]. Ancient Kurdish used herbs for alleviating or suppressing fever. Some of the herbs used for thermoregulation in the Kurdish area are discussed here (*vide infra*).

In 2020, the World Health Organization (WHO) states that the SARS-CoV-2 coronavirus of the coronaviridae family causes novel coronavirus type 2 induced disease (COVID-19) and should be considered a pandemic disease. Since there was not an available vaccine based on results from clinical trials against this novel disease in the market, clinicians tried implementing the usual protocol of acute respiratory distress syndrome (ARDS) and, through risky drug repurposing and using an array of antiviral drugs, they want to limit virus proliferation and its shedding. Parallel to these therapeutic strategies, some computational bioscientists tried to blind screen available databases of known chemicals to discover new agents and some of these groups were lucky to be submitted to experimental and clinical trials regarding their discovered chemicals or repurposed drugs. Seminal papers [3, 4] have categorized protein targets for possible antiviral drugs or binders, amongst these papers the main SARS-CoV-2 protease (3CLpro, also known as 3-chymotrypsin-like protease) would be a canonical enzyme target and plays cardinal functions in the self-build process of coronavirus [5]. The 3CLpro known as Nsp5 (nonstructural protein 5) is initially self-cleaved from structural viral polyproteins to produce a bunch of intermediate enzymes and is finally released as Nsp4–Nsp16 for virus proliferation. Therefore, 3CLpro has been appreciated as a striking target for anti-SARS-CoV-2 drugs. In this essence, small molecules and therapeutic recombinant peptides are major compounds targeted

at druggable SARS-CoV 3CLpro until now. The 3CLpro monomer has domain I (residues 8–101), domain II (residues 102–184), domain III (residues 201–306 in α-helices) and a long loop (residues 185–200) which joins domain II and III. The torus of domain I and II primarily consists of β-barrels constructs at the active site of 3CLpro presented as uncharged Cys-His catalytic dyad (Cys145 and His41) [5, 6].

In parallel to many scientists in the world, we hypothesized that ARDS might have occurred in the history of Kurdish people settled down in Zagros mountains during millennia. We searched for Kurdish ethnomedicine books but, unfortunately, this ancient and valuable heritage has been rotted or looted due to an array of reasons not suitable to discuss here. In addition, since fever is a cardinal sign of ARDS, we hypothesized that antipyretic and anti-flu remedies used in traditional Kurdish ethnomedicine might possess antiviral effects in orthodox medicine. Based on these hypotheses, our team interviewed all traditional healers that are currently known in Erbil, Iraq (36.2$^{o}$N, 44.0$^{o}$E and 420 MASL) we recorded data and, finally, selected some plants for computational drug discovery (*vide infra*).

## Antipyretic and Anti-flu Remedies Used in Kurdish Ethnomedicine

**Fenugreek** (*Trigonella foenum-graecum*) is one of the most famous herbal medicines that has been traditionally used in medicine and the food industry [7]. Fenugreek is a multi-effective herb known for its anticancer, antifungal, antibacterial and antiviral effects [8]. Fenugreek is the oldest medicinally used plant originating from India, north of Africa, the Kurdistan region of Iraq and Iran [9, 10]. It has been acknowledged as an antioxidative, antibacterial and antiviral remedy and can even be used for gynecological problems [11]. Fenugreek is an impressive source for the production of raw materials for the pharmaceutical industry like steroid hormones, antipyretics, and antibacterial and antiviral agents [12-14]. Likewise, it has been utilized in making sifting face covers. The current manufacture is to forestall or diminish the transmission of microbial (zoonotic) pathogens by the means of different entrances like salivation, nasal liquid or inward breath. Since the virus or microorganism inflicting such infections is often found in aerosolized media, such as excreta ejected throughout sneezing or respiratory or coughing, wearing a mask over the mouth and/or nose can be an effective approach for preventing or decreasing the transmission of sickness inflicting pathogens or viruses. However, the pores in a mask may be larger than the virus or microorganism leading to a confined utility as well as the opportunity of transmission open, despite providing a crude shielding barrier [15, 16]. Phytochemicals reported in fenugreek include flavonoids, diosgenin, alkaloids, steroids, amino acids, polyphenol compounds (e.g., rhaponticin and isovitexin), vitamin C, vitamin A, and minerals (zinc, iron, and phosphorus) [10, 11, 17, 18; Figure 1, Table 1].

**Chamomile** (*Matricaria chamomilla* L.), or Asteraceae, is one of the most popular herbal medicine that utilized by Kurdish people through millennia. It has been utilized by both Kurdish and Iranian people since it is a spice local to southern and eastern Europe. Chamomile blossoms has been applied to treat fever and contaminations for many years [19, 20]. Phytochemicals found in chamomile flowers include sesquiterpenes, *β*-farnesene, coumarins, flavonoids, phenolic acid, and various glucosides. The dried flowers and essential oils extracted from chamomile have been considered as therapeutics, functional ingredients or herbal teas [20-22; Figure 1, Table 1]. The antiviral effects of various formulations of chamomile against viruses that attack the human respiratory system such as the common cold and the influenza virus has been reported [20, 22, 23]. The formulations of chamomile which are affluent with *β*-farnesene, flavonoids, bisabolol oxid, matricin, chamazulene, umbelliferone, and chlorogenic acid can potentiate the immune system against viruses, especially respiratory viruses [20, 22, 23].

*Salvia officinalis,* or Labiatae/Lamiaceae, is usually known as **sage**, kitchen sage, Dalmatian sage, golden sage, garden sage, and maramia in the east. Nowadays, *Salvia* has been adopted globally, although it is a native plant to the Mediterranean area. *Saliva* species have been employed traditionally for a set of common problems including pain, fever, oxidative stress, angiogenesis, and inflammation while it also possesses antibacterial and antiviral effects [24, 25]. *Salvia* is popularly used to treat infection, cough, and

mouth and throat inflammations. The *Salvia* oil remedy is a source of anticancer and antioxidative phytocompounds that prove to be useful against diseases of the respiratory system and the oil can also control proliferation of human cells [26-28].

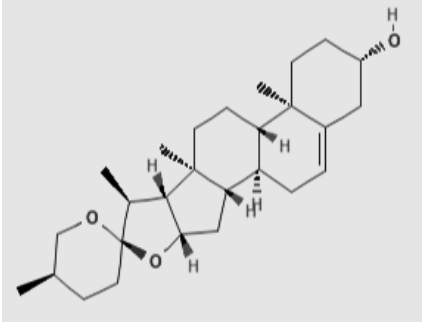
Famous phytocompounds of *salvia* include phenolics, polyphenols, terpenes, and flavonoids [29]. In this essence, ursolic acid as a pentacyclic triterpenoid has strong anti-inflammatory properties, while hydroalcoholic extracts derived from salvia contain polyphenols, terpenes, and flavonoids that inhibit bacteria and viruses [25, 30; Figure 1, Table 1]. The pharmacological properties of salvia-based formulations as anticancer, antiviral, antibacterial, antiseptic, antioxidative and anti-inflammatory agents have been supported by both experimental and clinical research [29, 31, 32].

**Ginger** (*Zingiber officinale* Roscoe), also called Zingiberaceae, is dubbed for its application as a pungent, fragrant spice in the culinary system. This spice is constituted of the chipped or ground rhizome (underground stem) of the plant. Ginger is a natural pharmacy of antispasmodic, antistomachic, vasodilator, expectorant, bronchodilator, analgesic, and antitussive functional formulations in the hands of traditional healers for treating pulmonary diseases. Ginger modulates the inflammatory cytokines to counterbalance pro-inflammatory and anti-inflammatory ones [33] and, more specifically, ginger consists of very effective anti-inflammatory compounds known as gingerols [34, 35; Figure 1, Table 1]. Ginger is a rich wellspring of nutrients such as vitamins (C, E, B3, B5, and B6), *β*-sesquiphellandrene, flavonoid, camphene, sabinene, pinocarvone, and borneol. Ginger can diminish chemotherapy-induced nausea and can provide some protection towards cancer cells. In essence, ginger is dubbed as the golden phytomedicine prescribed in cancer therapy [28, 36-38]. Ginger is highly effective against the respiratory syncytial virus, the influenza A virus subtype H1N1, and the common influenza virus and is a treasure to be deciphered for its antiviral agents to treat ARDS [36, 39, 40].

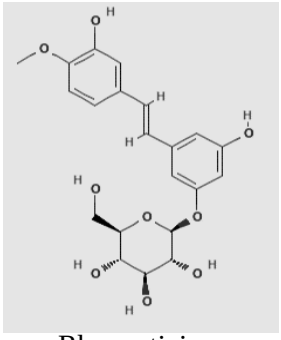

**Figure 1**. Putative anti-COVID-19 plants selected from Kurdish ethnomedicine (Erbil, Iraq); a: Fenugreek (*Trigonella foenum-graecum*); b: Chamomile (*Matricaria chamomilla* L.); c: Sage (*Salvia officinalis*); d: Ginger (*Zingiber Officinale*)

**Table 1**. Selected therapeutic phytocompounds of putative anti-COVID-19 plants used in Kurdish ethno-medicine (Erbil, Iraq)

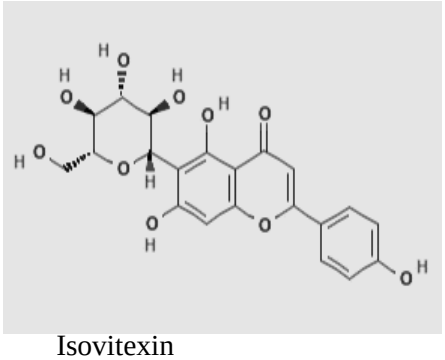
**A**. Fenugreek *(Trigonella foenum-graecum*)



Diosgenin

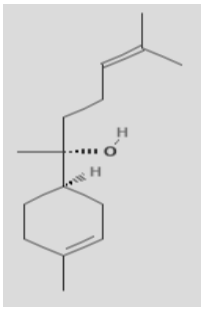


Rhaponticin

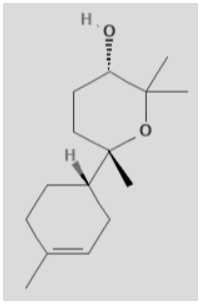


Isovitexin

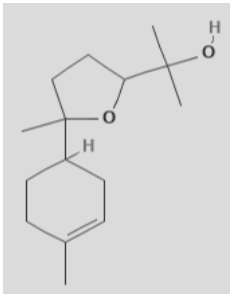
**B**. Chamomile (*Matricaria chamomilla* L.)



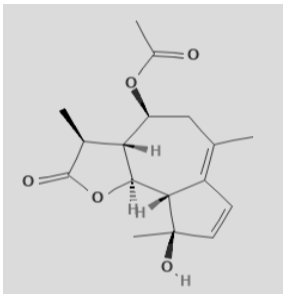
(-)-α-bisabolol



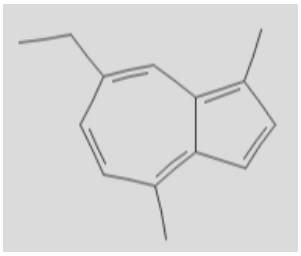
Bisabolol oxide



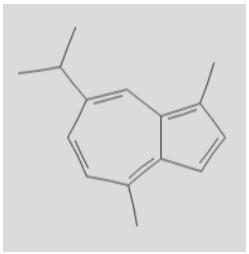
Bisabolol oxide B



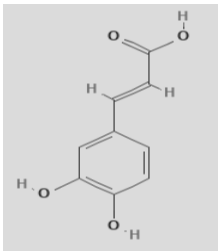
Matricin



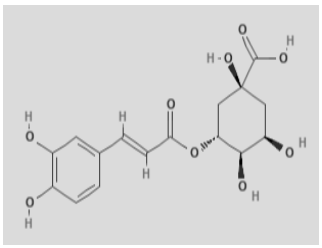
Chamazulene



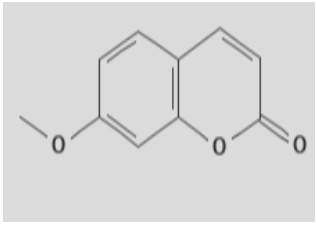
Guaiazulene



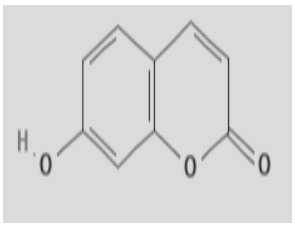
Caffeic acid



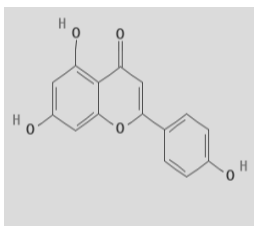
Chlorogenic acid



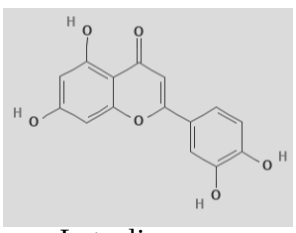
Herniarin



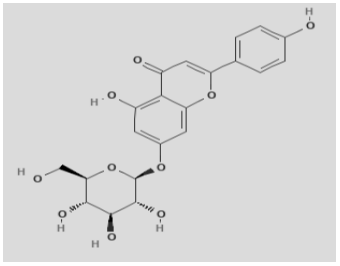
Umbelliferone



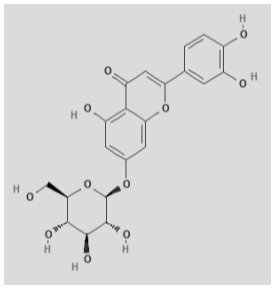
Apigenin



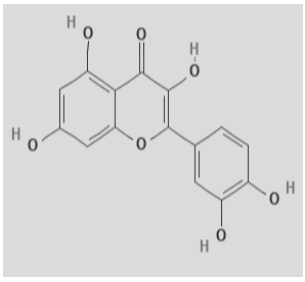
Luteolin



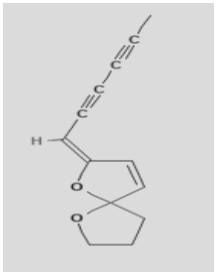
Apigenin-7-*O*-glucoside



Luteolin-7-*O*-glucoside

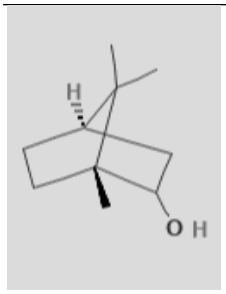


Quercetin

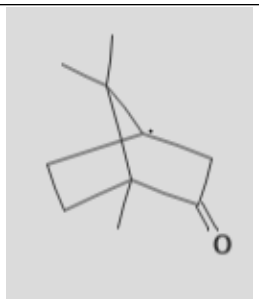


Enyne dicycloether

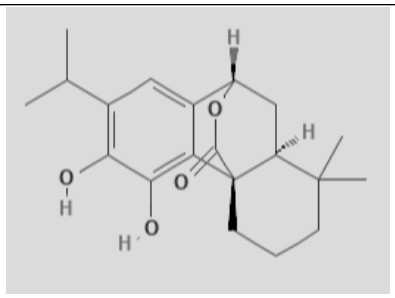
**C**. Sage (*Salvia officinalis*)



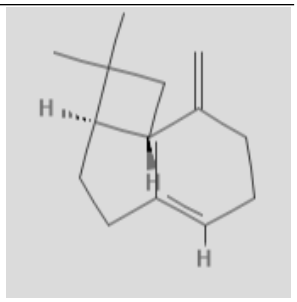
Borneol



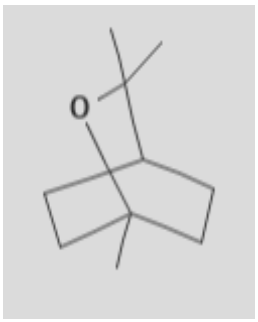
Camphor



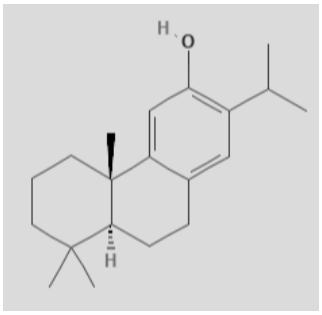
Carnosol



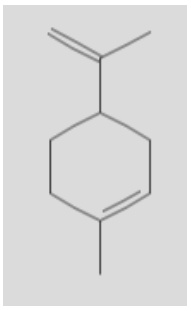
Caryophyllene



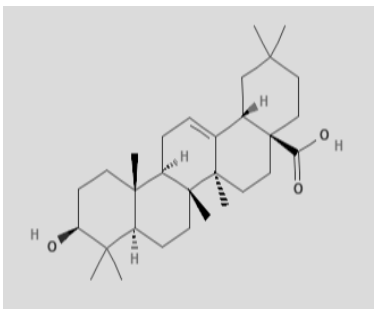
Cineole



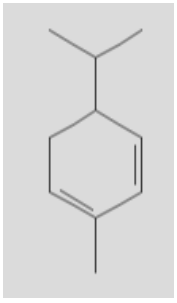
Ferruginol



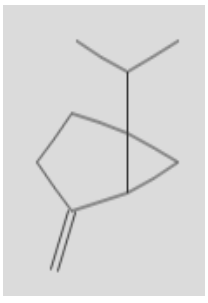
Limonene



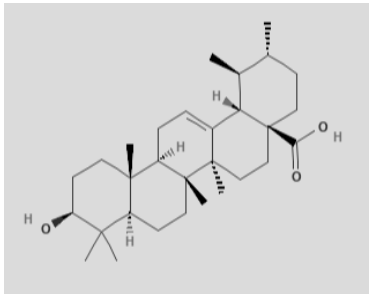
Oleanolic acid



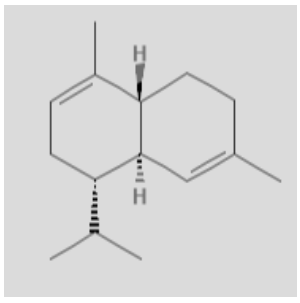
Phellandrene



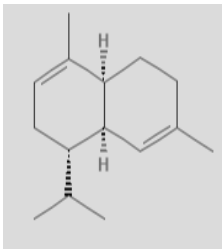
Sabinene

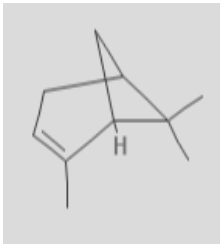


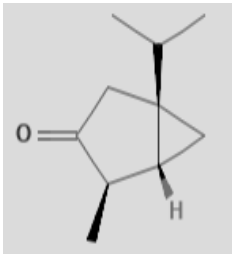
Ursolic acid

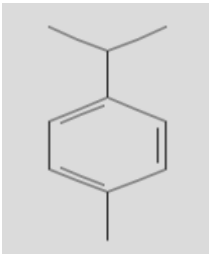


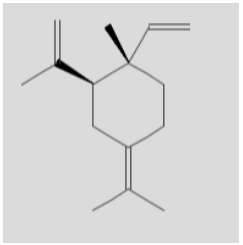
*Alpha*-cadinene


*Alpha*-muurolene

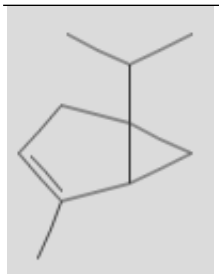

*Alpha*-pinene

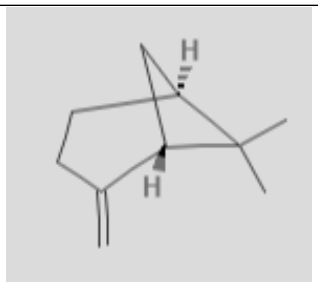

*Alpha*-thujone

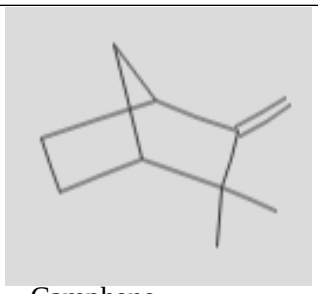

p-Cymene

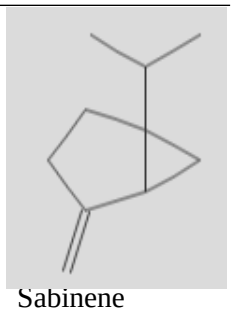

*Gama*-elemen

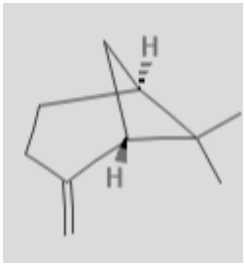
**D**. Ginger (*Zingiber Officinale*)

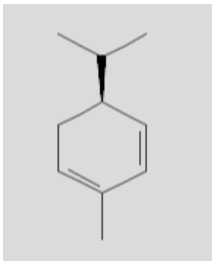

*α*-Thujene


*α*-Pinene


Camphene


Sabinene


*ß*-Pinene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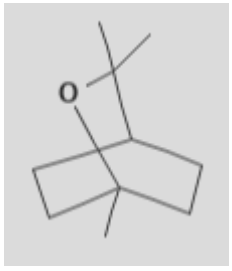
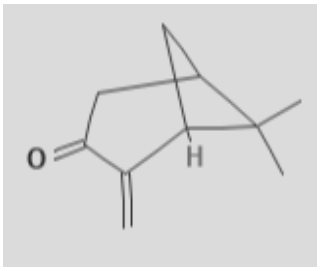
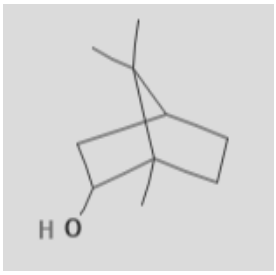

1,8-Cineole


Pinocarvone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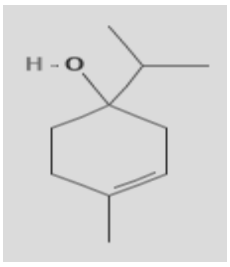
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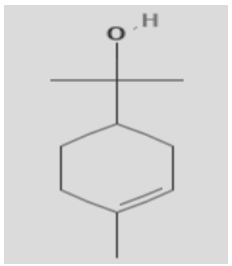
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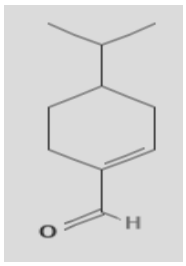
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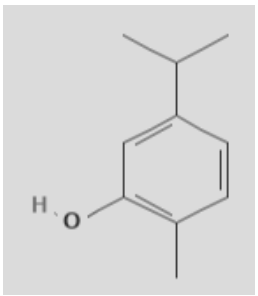
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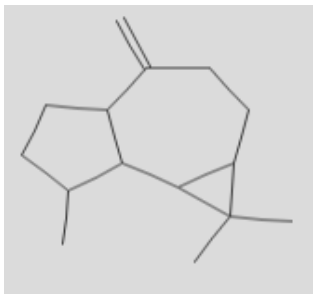
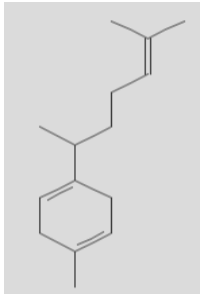

Aromadendrene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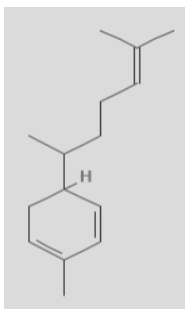
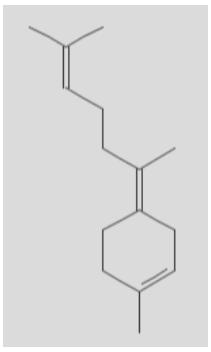
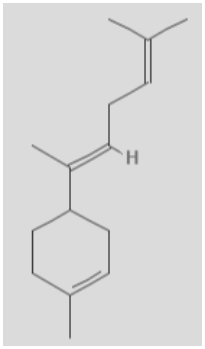
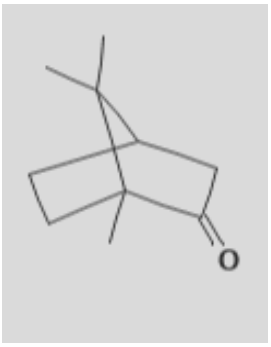

*α*-Zingiberene



(E)-$\gamma$-Bisabolene




(E)-$\alpha$-Bisabolene




Camphor

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**Computational Antiviral Assay**

Computational molecular docking of the aforementioned phytocompounds (*vide supra*) and the druggable protein 6Y84 was accomplished by VINA WIZARD module introduced on PyRx programming adaptation 0.8 [41]. In this sense, the PDB format of 6Y84 was recovered from the Research Collaboratory for Structural Bioinformatics (http://www.RCSB.org) and was cut, advanced, and prepared utilizing Molegro Virtual Docker [42] and Chimera 1.8.1 (http://www.rbvi.ucsf.edu/chimera) programming projects before attempting PyRx programming. The structures of the major phytocompounds were reaped from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The results of sub-atomic docking have been expressed as a binding affinity (BA; kcal/mol) of a set of molecular poses. The best posture of ligand, phytocompound, in counter to the target protein, prompts the most negative BA and is revealed here (*vide infra*). The best pose and 6Y84 were combined using Chimera 1.8.1 (http://www.rbvi.ucsf.edu/chimera) or Molegro Virtual Docker [42] and their graphical interface were inspected with LigPlot $^{+}$ programming to recognize amino acid deposits that engaged with bindings [43]. Conventionally, the binders, phytocompounds, which demonstrated BA lesser than -7.0 kcal/mol were discussed here.

**Pharmaco- and Toxico-Kinetic Parameters**

Physico-chemical properties and the computational aspects of absorption, distribution, metabolism, excretion, and toxicity (ADMET) were completed utilizing Swiss ADME programming recreation [SwissADME;http://www.swissadme.ch/] and online ADMET indicator instrument [http://biosig.unimelb.edu.au/pkcsm/prediction]. ADMET Predictor is a product instrument that rapidly and precisely predicts more than 140 properties including dissolvability, log P, pKa, locales of cytochrome P450 (CYP) digestion, and Ames mutagenicity. The program has an instinctive UI that permits one to handily control and picture information for selected compounds. In light of ADMET results, carnosol fits consummately inside the characterized boundaries for non-infringement of Lipinski's standard. A particle's log P is comprised of the expansion of its molecules. The impact of hydrogen holding onto the log P is viewed when there is a chance of shaping a six-membered ring between proper contributor and acceptor particles [44]. The molecules have log P esteems running from 0.05 to 5.18 which infers that these can successfully have reasonable cell membrane penetrability. Moreover, certain boundaries including blood-brain barrier

(BBB) infiltration, P-glycoprotein hindrance, human gastrointestinal tract assimilation, volume appropriation, subcellular limitation, CYP substrate or inhibitor, and human *ether-a-go-go-related gene* (HERG) inhibition reflect the fitness of any compound to be categorized as lead- or drug-like [45].

## DISCUSSION

**Fenugreek** is referred to as Alhulba in Mesopotamia, Shemlia in Kurdish and Shanbalila in Persian and is used in Kurdish ethnomedicine. The Kurdish recipe for fenugreek is comprised of one big spoon of fenugreek seed boiled in hot water. It has been prescribed to drink fenugreek tea twice per day or to add a small spoon of fenugreek powder to a tablespoon of honey. Three phytocompounds of fenugreek showed acceptable BA with 3CLpro (PDB:6Y84). In this context, **diosgenin** interacted hydrophobically with a bunch of amino acid residues of all domains of 3CLpro and also used hydrogen bonds between diosgenin and Asp295 residues of 3CLpro (Figure 2). Diosgenin, a phytosteroid sapogenin, is spirostan found in Dioscorea (wild yam) species with the potential to be considered as the starting point for the commercial synthesis of a number of steroids. It plays roles as an apoptosis inducer, an antiviral agent, an antineoplastic agent and a metabolite [46]. A pivotal review [47] emphasized the pharmacology (e.g., antiviral effect) of diosgenin [48]. Based on ADMET criteria, it will not be considered as a drug-like compound (see supplementary file).

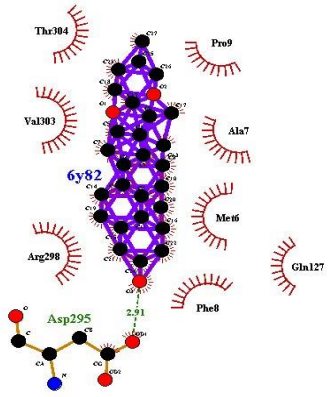
**Rhaponticin** displayed an array of hydrophobic interactions with 3CLpro and also employed hydrogen bonds with Met6, Val303 and Tyr154 residues of domain II and III of 3CLpro. *Rhaponticin* is a stilbenoid glucoside compound and its aglycone is called rhapontigenin [46]. A seminal review was written for the pharmacological effects of rhaponticin [49] with special appreciation to its anti-inflammatory compound. Based on the ADMET results, rhaponticin has a low water solubility which limits its pharmacological applications in pristine form (see supplementary file).

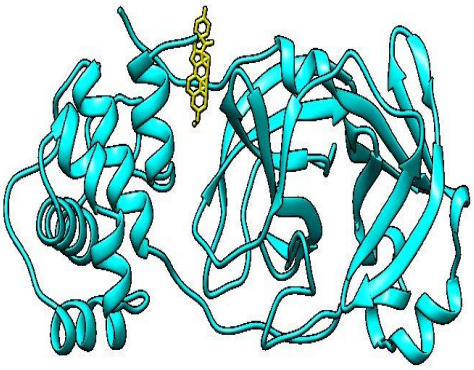
**Isovitexin** hydrophobically interacted with a bunch of amino acid residues of 3CLpro and also employed hydrogen bonds with the Ala7 residues of 3CLpro. Isovitexin (6-*C*-glucosylapigenin) is an alpha-glucosidase (EC 3.2.1.20) inhibitor [46]. Isovitexin was considered to donate antiviral and anti-inflammatory effects via the inhibition of cyclooxygenase-2 mRNA expression [50].

**Table 2**. Computational binding affinities of major phytochemicals of *Trigonella foenum-graecum* with protease (PDB code 6Y84) of coronavirus

| Ligand of PubChem; ID) | Binding affinity (Kcal/mol) | RMSD/UB | RMSD/LB |
|---|---|---|---|
| Diosgenin 99474 | -8.9 | 2.77 | 1.045 |
| Rhaponticin 637213 | -8.3 | 2.442 | 1.474 |
| Isovitexin 162350 | -7.6 | 3.75 | 2.615 |

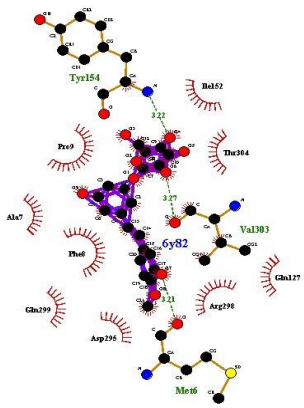
Note: RMSD: root mean-square deviation is the normal separation between the particles. UB: upper bound; LB: lower bound.

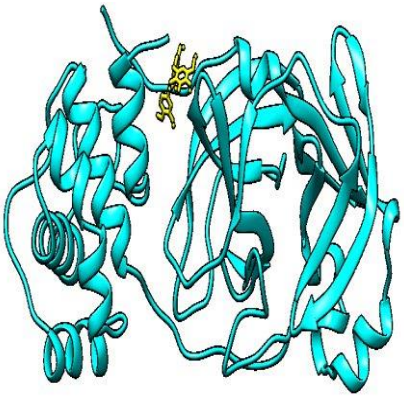


**6y84**

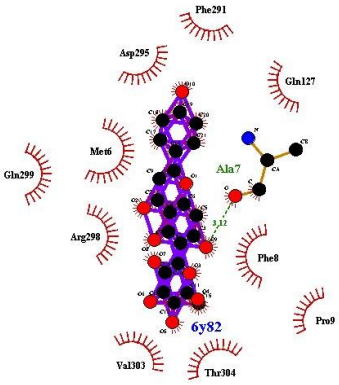
**6Y84-Diosgenin**

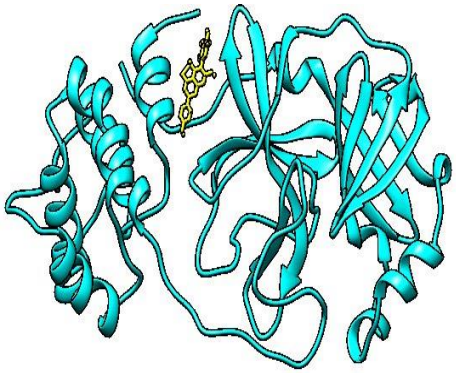


**6y84**

**6Y84-Rhaponticin**



**6y84**

**6Y84-Isovitexin**

**Figure 2**. Molecular docking of phytocompounds of *Trigonella foenum-graecum* (in yellow shading) with protease (PDB code 6Y84) of *coronavirus*; in cyan shading). Hydrogen bonds are highlighted by dashed lines, while hydrophobic interactions are represented by an arc.

**Chamomile** is known as Albabunaj in Mesopotamia, Chawa Peshila in Kurdish and Babunah in Persian. A hot drink of chamomile is one of the oldest known folk remedies because of its many health promoting properties. Chamomile is prescribed as a suspension of one tablespoon of orange juice to a cup of hot chamomile tea, or as a mixture of small spoon of ginger powder and a cup of chamomile hot tea. Based on the *in-silico* findings, phytocompounds reported in chamomile showed reliable BAs with 3CLpro with this order: chlorogenic acid < luteolin-7-*O*-glucoside < apigenin-7-*O*-glucoside < quercetin < luteolin < matricin < caffeic acid (Table 3). In this continuum, luteolin-7-*O*-glucoside, cynaroside flavone, and chlorogenic acid with the best BAs amongst chamomile derived phytocompounds interacted hydrophobically with 3CLpro and also employed hydrogen bonds with 3CLpro (Figure 3). Luteolin 7-glucoside is found in various plants including *Capsicum annuum* (red pepper), *Ferula varia*, *F. foetida*, dandelion coffee, *Campanula persicifolia*, Campanula *rotundifolia*, and *Cynara scolymus* (artichoke) [46]. More specifically, **luteolin-7-*O*-glucoside** was recognized as a functional antiviral and antioxidative constituent of the lettuce (*Lactuca sativa* L.) extracts [51]. The poor water solubility of luteolin-7-*O*-glucoside is a principal factor in restricting further investigations on its pharmacological activities and new innovation of medication conveyance is mentioned to expand its bioavailability, however, it is not the substrate or inhibitor of CYP isoenzymes which mediate xenobiotic metabolism (see supplementary file). **Luteolin**, a flavonoid, is named as a fundamental phytomedicine of the human eating routine [46] and is used in hydrophobic interactions and hydrogen bonds with Asp295, Met6 and Gln299 residues of 3CLpro with considerable looser BA in comparison to its conjugated form, luteolin-7-*O*-glucoside (Figure 3). It has been reported that luteolin is a potent antiviral bioflavonoid utilized against Japanese encephalitis virus replication [52]. Based on ADMET results, lutein showed better results in comparison to its glycosidic conjugate, luteolin-7-*O*-glucoside, however, it interacts with CYP isoenzymes (see supplementary file).

**Apigenin-7-*O*-glucoside**, a glycosyloxyflavone, showed hydrophobic interactions and hydrogen bonds with the loop of 3CLpro (Figure 3). This conjugated flavonoid compound, like its other congeners, possesses various antiviral effects (see a review [53]). Apigenin-7-*O*-glucoside demonstrated low water solubility, suitable metabolism, and three violations against Lipinski's rule (see supplementary file).

**Chlorogenic acid**, an ester of quinic acid and caffeic acid [46], hydrophobically interacted with an array of amino acid residues in domain I and III of 3CLpro. It also employed a set of hydrogen bonds to interact with loop and domains of 3CLpro (Figure 3). Chlorogenic acid is known as the major polyphenolic compound in coffee and is usually isolated from dicotyledonous plants [46]. This caffeoylquinic acid moiety, known as an antioxidative and cardioprotective component, may affect COVID-19-induced cardiovascular disorder. *Lonicera japonica* Thunb, is a rich wellspring of chlorogenic acid endorsed in customary Chinese medication to treat upper respiratory tract infections like the flu, parainfluenza, and respiratory syncytial infection. Additionally, it is also known as a neuraminidase blocker of influenza A virus [54]. Chlorogenic acid showed two violations against Lipinski's rule due to low water solubility and the number of hydrogen donor atoms and new portal like liposome that is needed to reach the cytoplasm (see supplementary file).

**Matricin**, a sesquiterpene lactone, has interacted hydrophobically with domain I and III and via hydrogen bonds with Asp295 residue of the loop of 3CLpro (Figure 3). This natural profen has various pharmacological effects like anti-flu activities and has been considered to be a prodrug [55]. Matricin showed suitable ADMET results, however, it has low water solubility and volume distribution in addition to some kinds of toxicity (see supplementary file).

**Caffeic acid**, a catechol of hydroxycinnamic acid derivative and polyphenol, hydrophobically interacted with domain II and III of 3CLpro and also employed hydrogen bonds with Asp295 residue in domain III of 3CLpro (Figure 3). Caffeic acid possesses antioxidative, anti-inflammatory, enzyme inhibitory, and

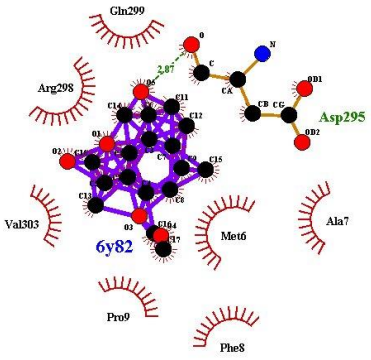
antineoplastic activities [46]. The antiviral potential of caffeic acid has been accounted for in flu [56] and severe fever with thrombocytopenia syndrome virus [57]. Caffeic acid has been shown to have good intestinal absorption as well as suitable ADMET results (see supplementary file).

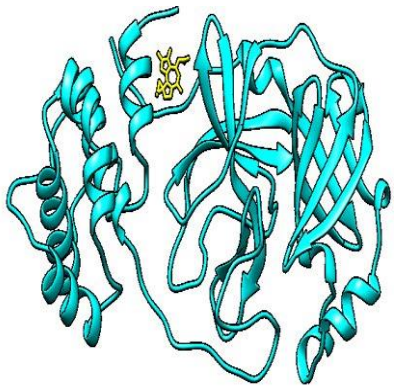
**Quercetin** has interacted with all domains of 3CLpro through hydrophobic interactions and hydrogen bonding with the Asp295 residue of domain III (Figure 3). Quercetin is a glycan polyphenolic flavonoid found ubiquitously in fruits and vegetables with special immunomodulatory [58] and antiviral activity against influenza A virus **[**59]. The number of hydrogen donor atoms of quercetin exceed 5 and, thus, violates Lipinski's rule (see supplementary file). The water solubility and intestinal absorption of quercetin also are not suitable for consideration it as a drug-like compound, however, quercetin may interfere with virus entry to cells **[**59].

**Table 3**. Computational binding affinities of major phytochemicals of *Matricaria chamomilla* L. with protease (PDB code 6Y84) of coronavirus

| Ligand of PubChem; ID) | Binding affinity (Kcal/mol) | RMSD/UB | RMSD/LB |
|---|---|---|---|
| Matricin 92265 | -7.1 | 3.903 | 2.024 |
| Caffeic acid 689043 | -7.0 | 3.078 | 2.445 |
| Chlorogenic acid 1794427 | -9.2 | 2.067 | 1.380 |
| Luteolin 5280445 | -7.9 | 30.743 | 27.341 |
| Apigenin-7-*O*-glucoside 5280704 | -8.9 | 2.58 | 1.269 |
| Luteolin-7-*O*-glucoside 5280637 | -9.1 | 29.959 | 25.778 |
| Quercetin 5280343 | -8.1 | 1.887 | 1.364 |

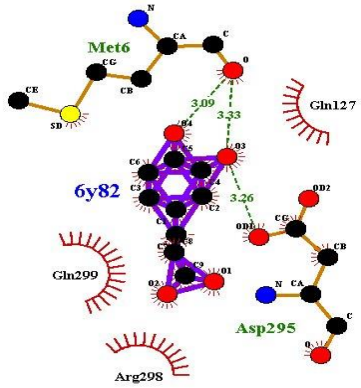
Note: RMSD: root mean-square deviation is the average distance between the atoms. UB: upper bound; LB: lower bound.

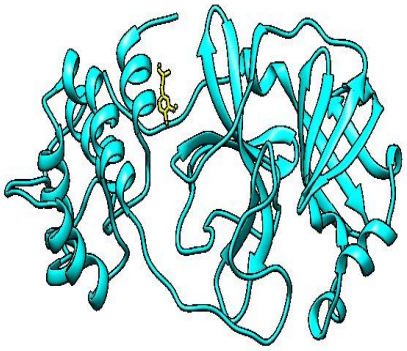


**6y84**

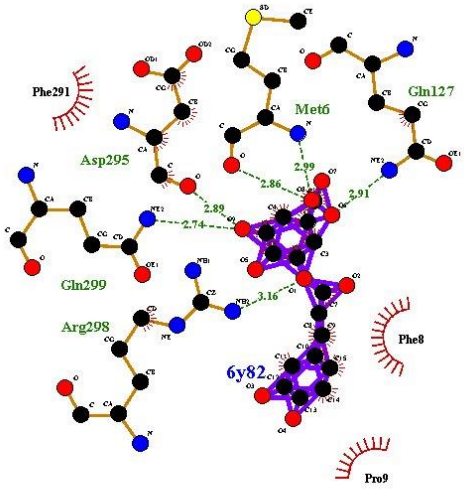
**6Y84-Matricin**

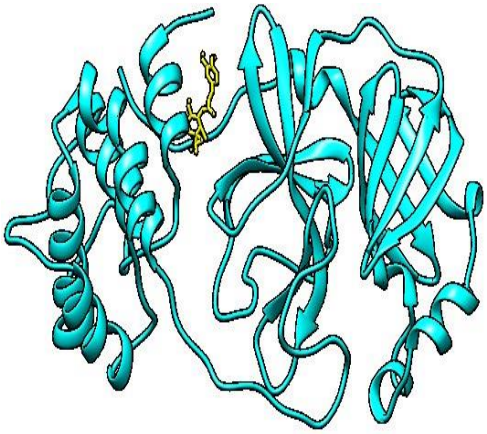


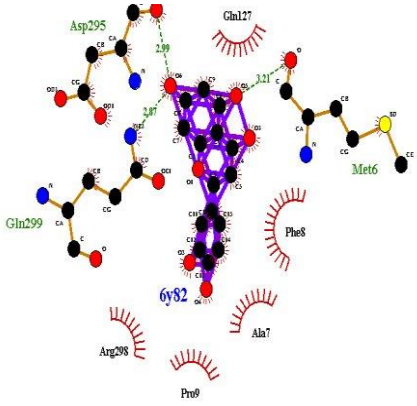
**6y84**

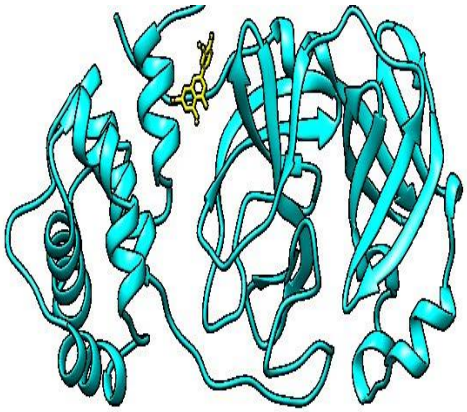
## 6Y84-Caffeic acid



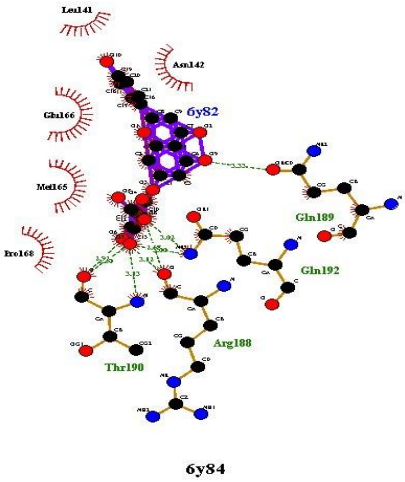
**6y84**



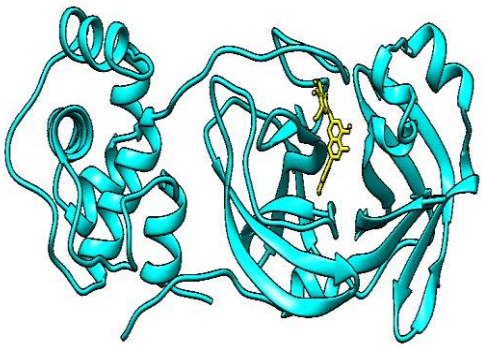



**6y84**

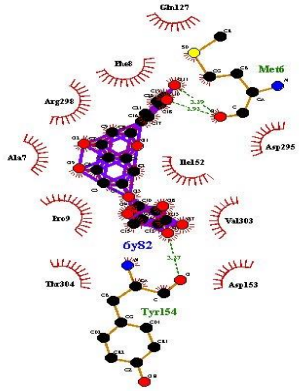
## 6Y84-Luteolin



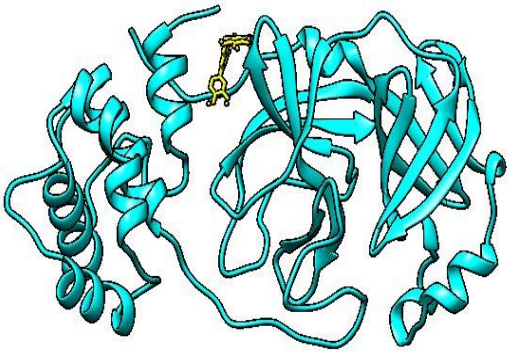

6y84



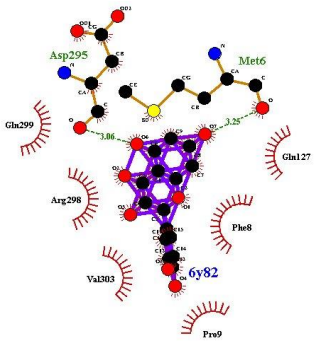
**6Y84-Apigenin-7-*O*-glucoside**



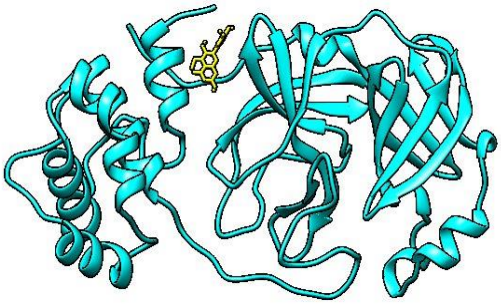

6y84



**6Y84-Luteolin-7-*O*-glucoside**




6y84



**6Y84-Quercetin**

**Figure 3**. Molecular docking of phytochemicals of *Matricaria chamomilla* L. (in yellow shading) against protease (PDB code 6Y84) of *coronavirus*; in cyan shading). Hydrogen bonds are highlighted by dashed lines, while hydrophobic interactions are represented by an arc.

**Salvia** is known as Murimia in Arabian and Kurdish and MaryCale in Persian literature. A Kurdish culinary recipe includes salvia as a functional tea, a tablespoon of salvia in a cup of hot water. Additionally, salvia has been used as an inhalator to treat respiratory system problems in Kurdish ethnomedicine. Based on our *in-silico* findings, phytocompounds reported in salvia showed more reliable BAs with 3CLpro in comparison to those of other plants reported in this order: oleanolic acid < *gamma*-elemene < ursolic acid < carnosol < ferruginol (Table 4).

**Oleanolic acid,** a pentacyclic triterpene, is found in the non-glyceride portion of olive pomace oil [46]. Pentacyclic triterpenes are natural ubiquitous phytocompounds that possess anti-inflammatory and antioxidative properties [46]. Oleanolic acid, glycyrrhizic acid, ursolic acid, and nomilin exhibited immunomodulatory effects [60]. Oleanolic acid, also known as caryophyllin, astrantiagenin C, giganteumgenin C, and virgaureagenin B, showed the best BA with 3CLpro in the present study and employed both hydrophobic interactions with all domains and hydrogen bonds with Gln299 and Asp295 residues of domain III of 3CLpro (Figure 4). In an influential review, antiviral effects of oleanolic acid and its derivatives against viral diseases such as influenza, hepatitis, human immune deficiency virus (HIV), and herpes viruses showed promising information based on *in vivo* and *in vitro* studies [61]. The drug-likeness of oleanolic acid is unacceptable because it violates Lipinski's rule of five due to high lipophilicity (≠ hydrophilicity), low hydrogen acceptivity, along with unsuitable pharmacokinetics parameters including low volume of distribution, zero unbound fraction of plasma protein, CYP3A4 substrate, and low total clearance (see supplementary file).

There was no report regarding the antiviral activity of **gamma-elemene**, a sesquiterpene [46]; however, it interacted hydrophobically with an array of amino acid residues of domain III of 3CLpro (Figure 4). Among all the compounds reported, gamma-elemene has the second and a reliable position in the BA with 3CLpro and which encourged us to dig deeper into its antiviral activity in future experimental studies. Indirect insecticidal [62] and larvicidal [63] activities of gamma-elemene were reported. Based on ADMET results, gamma-elemene follows the Lipinski's rule of five and hsd suitable pharmacokinetic parameters to be considered as a drug-like compound (see supplementary file).

**Ursolic acid**, a pentacyclic triterpenoid, is urs-12-*en*-28-oic acid substituted by a beta-hydroxyl moiety at position 3 and derives from a hydride of an ursane [46]. Various aspects of the antiviral potential of ursolic acid has been displayed in the rotavirus infection [64] and human papillomavirus-associated cervical cancer cells [65]. The various pharmacological properties of this dexamethasone-like structure has also been acknowledged in a ground-breaking patent review [66]. There was no report regarding the ant-SARS activity of ursolic acid, however it interacted hydrophobically with an array of amino acid residues of domain II and III of 3CLpro (Figure 4). Low hydrogen acceptivity, high lipophilicity, and the high topological polar surface area of ursolic acid violate the Lipinski's rule of five; however, it has very good intestinal absorption and zero unbound fraction of plasma protein. It has acceptable clearance in comparison to similar to its congener, oleanolic acid (see supplementary file).

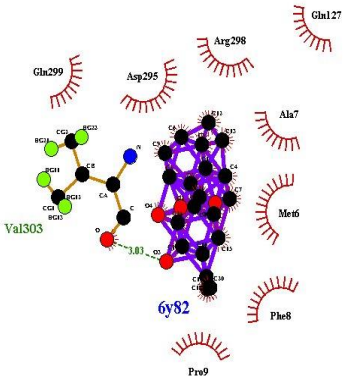
**Table 4**. Computational binding affinities of major phytochemicals of *Salvia officinalis* with protease (PDB code 6Y84) of coronavirus

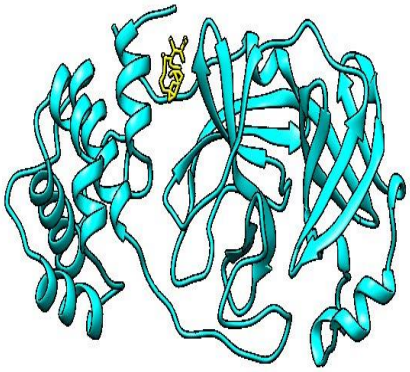
| Ligand of PubChem; ID) | Binding affinity (Kcal/mol) | RMSD/UB | RMSD/LB |
|---|---|---|---|
| Carnosol 442009 | -7.2 | 25.971 | 22.036 |
| Ferruginol 442027 | -7.1 | 47.766 | 45.165 |
| Oleanolic acid 10494 | -12.7 | 31.401 | 28.403 |
| Ursolic acid 64945 | -8.9 | 31.707 | 26.404 |
| *Gamma*-elemene 6432312 | -9.1 | 0.309 | 0.309 |

Note: RMSD: root mean-square deviation is the average distance between the atoms. UB: upper bound; LB: lower bound.

**Carnosol** is a diterpenoid compound which hydrophobically interacted with domain II and III of 3CLpro and also employed hydrogen bonds with Val303 residues of 3CLpro (Figure 4). Recently, reliable and BA -8.2 Kcal/mol of carnosol with active site of SARS-CoV-2 main protease has been reported in a high throughput *in silico* study [67]. Carnosol is naturally occurring in rosemary (*Rosemarinus officinalis*, Labiatae) and other the labiate herbs like sage. Its antioxidative activity has been reported [46]. Based on ADMET results, carnosol follows Lipinski's rule of five, but some of its pharmaco-kinetic parameters are not acceptable (see supplementary file).

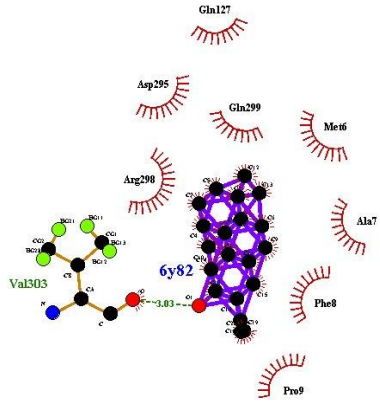
**Ferruginol** is an abietane diterpenoid that is abieta-8,11,13-triene substituted by a hydroxy group at positions 12 [46]. Roa-Linares and coworkers [68] reported the antiviral effects of ferruginol analogues against human herpesvirus type 2, human herpesvirus type 1, and Dengue virus type 2. Ferruginol hydrophobically interacted by 3CLpro through a set of amino acid residues of domain II and III and also employed hydrogen bonds with Val303 residues of 3CLpro (Figure 4). Based on ADMET results, ferruginol also follows Lipinski's rule of five, but it would be considered as a HERG II inhibitor and potentially a cardiotoxic compound (see supplementary file).

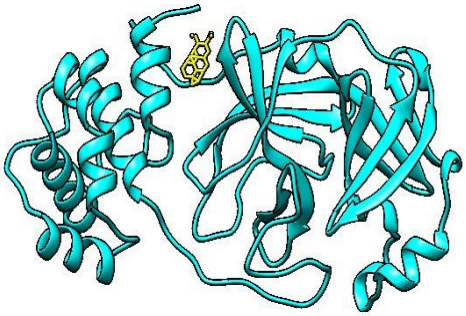


6y84

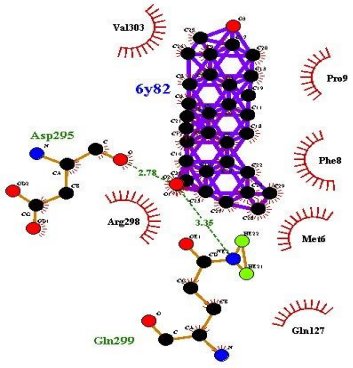
**6Y84-Carnosol**

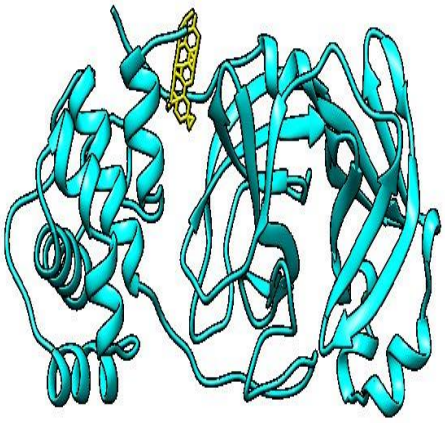


6y84

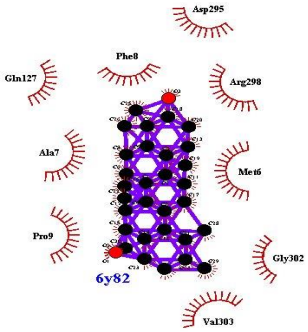
**6Y84-Ferruginol**

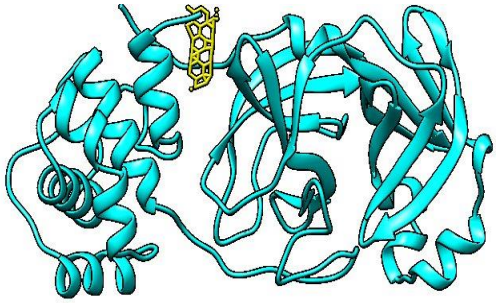


**6y84**

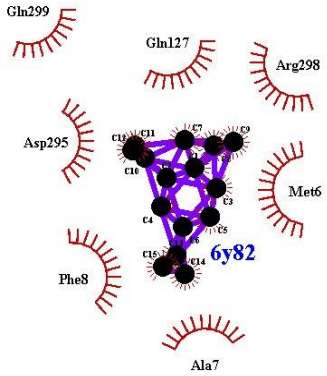
**6Y84-Oleanolic acid**

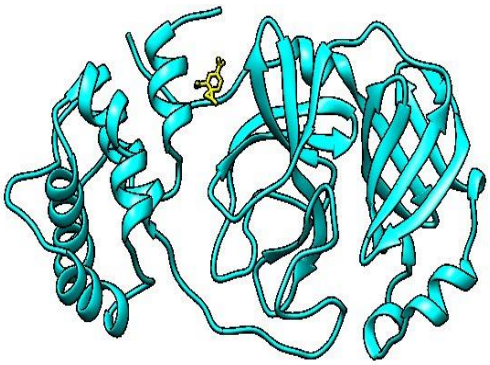


**6y84**

**6Y84-Ursolic acid**



**6y84**

**6Y84-*Gamma*-elemene**

**Figure 4**. Molecular docking of phytocompounds of *Salvia officinalis* (in yellow shading against protease (PDB code 6Y84) of *coronavirus*; in cyan shading). Hydrogen bonds are highlighted by dashed lines, while hydrophobic interactions are represented by an arc.

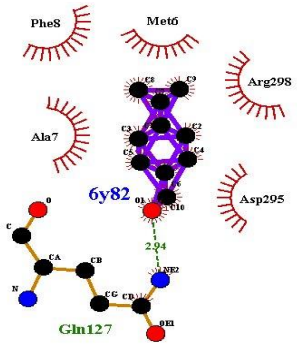
**Ginger**, also called Alzanjabil in Mesopotamia, Zanjafel in Kurdish, and Zyngbir in Persian traditional medicine, is usually used in the form of pieces or powder. Ginger is used as a tea, food additive, or a safe food by U.S. Food and Drug Administration which has invigorated people to use it more frequently. Among the phytochemicals of ginger, (E)-γ-Bisabolene has the strongest BA with 3CLpro through a set of amino acid residues of domain II and III (Figure 5; Table 5). This sesquiterpene compound is found in anise [46] and ginger and is known as a flavoring agent in the market. As far as we could possibly know, no trustful antiviral action of this bisabolene has been accounted for, but its antitumor effect has been researched [69]. Based on ADMET results, (E)-γ-Bisabolene is lipophilic and can cross the lipid membrane easily, however its low water solubility may interfere with its volume distribution (see supplementary file).

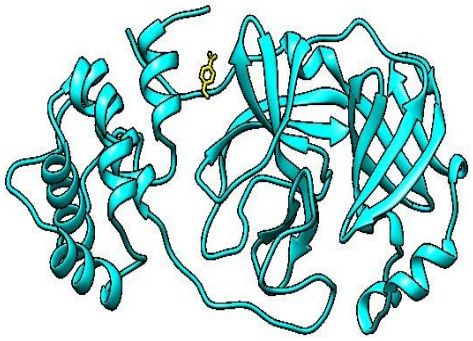
**Beta-curcumene**, a sesquiterpene [46], hydrophobically interacted with domain II and III of 3CLpro (Figure 5) and its pharmacological properties remain unknown. In addition to its presence in ginger, **(S)-Phellandral** is a constituent of *Anethum sowa* (Indian dill) [46]. The (S)-Phellandral hydrophobically interacted with 3CLpro and also bound to 3CLpro through hydrogen bonds with the Gln127 residue of domain II and III of 3CLpro (Figure 5). No overt violation against Lipinski's rule of five was detected for *β*-curcumene and (S)-Phellandral and they have suitable pharmacokinetic parameters except low water solubility to be considered as lead-like compounds (see supplementary file). Similar to beta-curcumene, pharmacological properties of (S)-Phellandral remain unknown.

**Table 5**. Computational binding affinities of major phytochemicals of *Zingiber officinale* with protease (PDB code 6Y84) of coronavirus

| Ligand of PubChem; ID) | Binding affinity (Kcal/mol) | RMSD/UB | RMSD/LB |
|---|---|---|---|
| Phellandral 89488 | -7.1 | 1.921 | 1.413 |
| *β*-Curcumene 6428461 | -7.5 | 1.518 | 0.867 |
| (E)-γ-Bisabolene 5352437 | -8.0 | 2.619 | 2.067 |

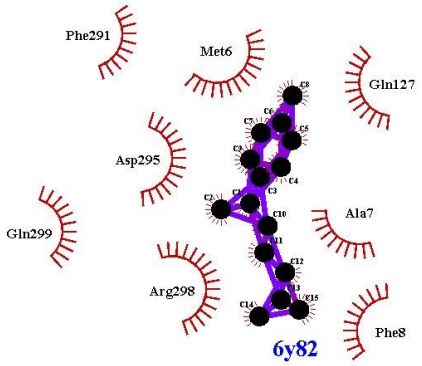
Note: RMSD: root mean-square deviation is the average distance between the atoms. UB: upper bound; LB: lower bound.

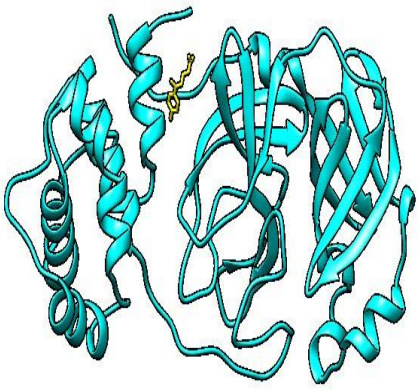


6y84

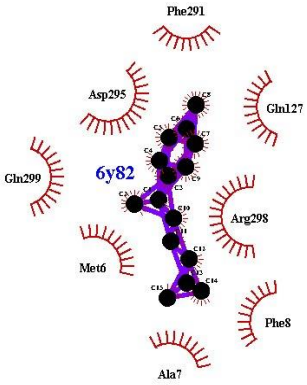
**6Y84-Phellandral**

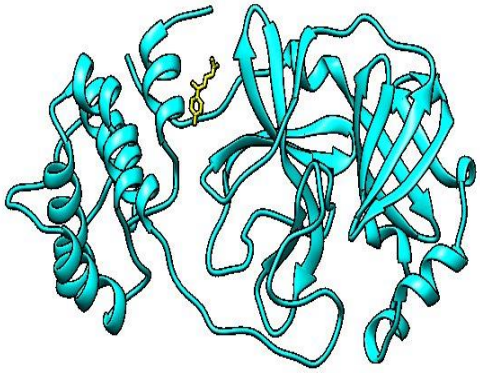


**6y84**

**6Y84-*β*-Curcumene**



**6y84**

**6Y84-(E)-γ-Bisabolene**

**Figure 5**. Molecular docking of phytocompounds of *Zingiber officinale* (in yellow shading) with protease (PDB code 6Y84) of *coronavirus*; in cyan shading). Hydrogen bonds are highlighted by dashed lines, while hydrophobic interactions are represented by an arc.

## SUMMARY

- Coronavirus disease 2019 (COVID-19) is a contagion caused by severe acute respiratory *syndrome coronavirus* 2 (SARS-CoV-2) which led to huge socioeconomic losses throughout the world. The pathogenesis of COVID-19 has yet to be cleared, while its high mutation rate led to the high mor-bidity and mortality of this (re)emergent disease which further limits the effectiveness of routine preventive and therapeutic recipes.
- Kurdish ethnomedicine developed around the Zagros mountains and Mesopotamia where their in-habitants experienced many ancient and modern pandemics throughout millennia. Therefore, we screened the botanical formulations used for preparing antipyretic (putative orthodox antiviral) rec-ipes throughout history. Hence, the objective of this chapter was to find evidence of possible anti-SARS-CoV-2 activity in Kurdish recipes by deciphering the binding affinity (BA; kcal/mol; *vide infra* in the parentheses) of screened phytochemicals to targ3CLpro (PDB:6Y84) *in silico*.

- We screened fenugreek (*Trigonella foenum-graecum*), chamomile (*Matricaria chamomilla*), sage (*Salvia officinalis*), and ginger (*Zingiber officinale*) through a rapid survey of traditional herbalists and reviews of remnant literature of the Kurdish people. In brief, an antipyretic recipe was a boiled drink prepared from fenugreek seeds which was prescribed twice per day and a small spoon of fenugreek powder mixed with a tablespoon of honey. Other recipes contained one tablespoon of squeezed orange exhausted to some hot chamomile tea and a little spoon of ginger powder blended in with some hot chamomile tea.
- A culinary recipe of salvia was its tea or a tablespoon of salvia blended with heated water. Other recipes contained chips or powder of ginger used as a tea or a food additive. More notably, salvia has been used in the form of steam inhalation to treat respiratory disorders like acute respiratory distress syndrome (ARDS) caused by COVID-19.
- The results of molecular docking showed that diosgenin (-8.9), rhaponticin (-8.3), and isovitexin (-7.6) found in fenugreek; luteolin-7-*O*-glucoside (-9.1), apigenin-7-*O*-glucoside (-8.9), quercetin (-8.1), luteolin (-7.9), chlorogenic acid (-9.2), matricin (-7.1), and caffeic acid (-7.0) found in chamomile; oleanolic acid (-12.7), *gamma*-elemene (-9.1), ursolic acid (-8.9), carnosol (-7.2), and ferruginol (-7.1) found in sage; and (E)-γ-bisabolene (-8.0), *β*-curcumene (-7.5), and phellandral (-7.1) found in ginger have reliable BA < -7.0 kcal/mol and can be considered as putative strong protease binders including loop and domain binders.
- Moreover, experimental investigations supported the previously strict antiviral activities of *β*-cur-cumene, ferruginol, carnosol, ursolic acid, oleanolic acid, caffeic acid, chlorogenic acid, luteolin, quercetin, luteolin-7-*O*-glucoside, diosgenin, and isovitexin.
- In sum, oleanolic acid, chlorogenic acid, and luteolin-7-*O*-glucoside can be considered as *hit* molecules of this computational effort which should be submitted to quantitative structure activity relationship (QSAR) analyses and *similarity research* against protease *in silico* and *in vitro*.
- Cautiously, sage is an ethnic gift of Kurdish ethnomedicine for the prevention and treatment of COVID-19 if prescribed by Kurdish herbalists and we encourage clinicians to prescribe it as a *functional tea* or an inhaler for patients and medical recruits.

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**TEST QUESTIONS**

1. Which plant has been used as an inhalator to treat respiratory disorders like acute respiratory distress syndrome (ARDS) in Kurdish ethnomedicine:
    a. Fenugreek
    b. Sage
    c. Chamomile
    d. Ginger
2. Which plant contains the most promising functional ingredients against COVID-19 in this study
    a. Fenugreek
    b. Chamomile
    c. Sage
    d. Ginger
3. Lipinski's rule of five [70] varies based on
    a. hydrogen bond donors < 5; hydrogen bond acceptors < 10
    b. An octanol-water partition coefficient (log P) < 5
    c. A molecular mass < 500 daltons
    d. All of the above
4. Which phytocompounds inhibited as profen in this study?
    a. Physical performance

b. Mitricin
c. Organ or system function
d. Cognitive, behavioral, and psychological function

5. Based on *in silico* effort of the present study, which of the following phytocompounds showed the best docking with 3CLpro?
    a. Oleanolic Acid
    b. *Gamma*-elemene
    c. Luteolin-7-*O*-glucoside
    d. Rhaponticin

**Answers:** 1:(B) 2:(C) 3:(D) 4:(B) 5:(A)

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