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Exploring the Antiviral Potential of Single Garlic Oil against COVID-19: A Natural Remedy for Viral Infections

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Abstract

In recent decades, the incidence of viral pandemics has risen significantly. These pandemics not only result in millions of deaths but also hinder economic growth and development. The World Health Organization (WHO) declared COVID-19 a global health crisis in 2020. The disease is caused by the β-coronavirus, severe acute respiratory syndrome-related coronavirus-2 (SARS-CoV-2). This study aimed to investigate the in-vitro anti-SARS-CoV-2 and in silico activities of volatile constituents from single clove garlic, as well as to identify these constituents using Gas Chromatography-Mass Spectrometry (GC/MS) analysis. A total of 27 compounds were identified. The major compounds detected were diallyl disulfide, allyl trisulfide, and 2-vinyl-1,3-dithi-4-ene. The in-vitro cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, and the 50% cytotoxic concentration (CC50) of the volatile constituents was established. Additionally, a geometry-based molecular docking approach (PatchDock) was employed to create docking modifications that result in favorable molecular shape complementarity.

Keywords: Single clove garlic; Volatile constituents; SARS CoV-2; IL-6; TNFα; Antiviral; Molecular docking

1. Introduction

Coronavirus is a zoonotic virus. It is a family of viruses that cause respiratory tract infections. They were first isolated in 1937 and designated coronaviruses because they have a crown-like appearance under a microscope. There are several types of coronaviruses, such as the alpha coronaviruses, the beta coronaviruses, SARS-CoV, which causes severe acute respiratory syndrome (SARS), MERS-CoV, which causes Middle East respiratory syndrome (MERS), and SARS-CoV-2 [1].

Coronavirus (2019-CoV) was first isolated from Wuhan market, China, on 7 January 2020. Coronavirus causes respiratory infection, including pneumonia, cold, sneezing and coughing [2]. Although there is yet no effective approved antiviral agent capable of inhibiting SARS-COV-2 host cell infection, like M2 inhibitors or neuraminidase inhibitors against influenza viruses, various targets to alleviate or treat the disease are currently being studied. Moreover, several natural products have been reported as potential candidates in its management; their active constituents are an excellent precursor for new antiviral drugs [3]. Many plant extracts have inhibitory activity against virus replication, such as Herpes simplex virus-2 (HSV-2), human immunodeficiency virus-1 (HIV-1), hepatitis B virus (HBV), and SARS virus [4].

Allium sativum (garlic) belongs to the family Amaryllidaceae, which is a monocotyledonous family. It comprises about 1,100 species and 75 genera that are widely distributed in tropical and subtropical areas of the world [5] . Garlic was reported to treat fever, common cold, wounds, coughs, and asthma in many Asian countries [6]. Moreover, the potent antiviral potential of multi-clove garlic against several viruses, including influenza B, human rhinovirus type 2, human cytomegalovirus (HCMV), parainfluenza virus type 3, herpes simplex type 1 and 2, vaccinia virus, and vesicular stomatitis virus was previously reported [7].

Garlic and its constituents possess antiviral activity by inhibiting replication against several pathogenic viruses [8]. Also, it was found to have a good selectivity index (SI) and to inhibit the virus penetration and proliferation in cells [9]. This activity was attributed to its content of organosulfur compounds that improve the production of neutralizing antibodies and prevent the

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adhesive interaction and fusion of leukocytes [10]. More than 30 sulfur compounds belonging to two main chemical classes, L-cysteine sulfoxides and γ-glutamyl-L-cysteine peptides, are present in garlic [11]. Alliin (S-allyl-L-cysteine sulfoxide) is the most abundant sulfur compound in fresh and dry garlic (10–30 mg/g) [12], which can be easily converted into allicin by alliinase enzymes after chopping, crushing, or chewing of fresh garlic [13].
Allicin (diallyl thiosulfinate) is the major thiol-reactive organosulfur compound in garlic plants[14] . Several phytochemicals have been reported as inhibitors of the coronavirus main protease using a molecular docking study [15]. On the other hand, allicin was reported to possess in-vivo therapeutic effect by significantly improving and speeding up the healing process within 2 weeks of treatment [16]. This study aimed at investigating the in-vitro and in silico anti-SARS-CoV-2 activity of single clove garlic and identifying its major volatile constituents responsible for this activity.

2. Materials and methods

2.1. Plant material

Single clove garlic was collected from the local market in Yemen. Dr. Mohamed El Gebaly, Senior botanist, Former researcher in the Department of Phytochemistry and Plant Systematics at the National Research Centre, identified the plant.

2.2. Extraction of volatile constituents

Single clove garlic (0.5 kg) was mixed in a blender with 2.0 L of 95% ethanol, then filtered, and 250 mL of H2O was mixed with the filtrate, whereby the filtrate was seen to be turbid, then it was partitioned with pet. Ether (40-60) according to the method described by [17]. The petroleum ether fraction was evaporated under vacuum by rotatory evaporator at 30°C to get 2 mL of single clove garlic volatile constituents [18].

2.3. Gas chromatography-mass -mass spectrometry analysis (GC/MS)

Analysis of the samples of single clove garlic was conducted by using a gas chromatography (Agilent 8890 GC System), coupled to a mass spectrometer (Agilent 5977B GC/MSD) and equipped with an HP-5MS fused silica capillary column (30 m, 0.25 mm i.d., 0.25 mm film thickness). The oven temperature was maintained initially at 50 °C, then programmed from 50 to 200°C at a rate of 5°C/min and from 200°C to 280°C at a rate of 10°C/min, then held for 7 min at 280°C. Helium was used as the carrier gas at a flow rate of 1.0 mL/ min. The volatile constituents were dissolved in diethyl ether (20 µL volatile constituents / mL diethyl ether), and then 1 µL of this solution was injected into the GC with a split ratio of 1:50. The temperature of injection was 230 °C. Mass spectra in the electron impact mode (EI) were obtained at 70 eV and scan m/z range from 39 to 500 amu. The isolated peaks were identified by matching them with data from the library of mass spectra (National Institute of Standards and Technology, NIST).

2.4.Anti-SARS-CoV-2 activity (Cells and Virus)

Vero-E6 cells were cultured in Dulbecco modified Eagle's medium (DMEM) (Lonza, Basel, Switzerland) containing fetal bovine serum (10%) (Lonza), and antibiotic-antimycotic mixture (1%) (Lonza). The cells were then incubated at 37 ˚C in a humidified atmosphere containing 5% CO2. A SARS-COV-2, hCoV-19/Egypt/NRC 03/2020 (Accession Number on GSAID: EPI_ISL_430820) virus was propagated in VERO-E6 cells. The virus was titrated by using a plaque titration assay[19].

2.5.Cytotoxicity

To evaluate the in-vitro viability of single clove garlic volatile constituents, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed as mentioned before[19,20] with some modifications. Shortly, cells were seeded in 96-well plates in DMEM supplemented with 10% fetal bovine serum and 1% antibiotic antimycotic mixture.
After 24 hrs of cell preparation, cells were confluent to 90% was checked, and the growth medium was aspirated from each well then, the cells were washed with 1X phosphate buffered saline (PBS). Different concentrations of single clove garlic volatile constituents starting from 0.4 µg/ml were two-fold serially diluted in DMEM, then added to cultured cells in a 96-well plate in triplicate and incubated for 24 hrs post-treatment to determine the cytotoxic concentration 50 (CC50).

2.6. Molecular docking

2.6.1.Preparation for protein receptor

The crystal structures of two proteins human transmembrane protease serine 2 (TMPRSS2) in complex with Nafamostat [21] (PDB: 7MEQ, resolution: 1.95 Å) and the SARS-CoV-2 spike receptor-binding domain (RBD) bound to ACE2 (PDB: 6M0J, resolution: 2.45 Å) were retrieved from the Protein Data Bank (www.rcsb.org). Nafamostat, a drug used to treat acute pancreatitis and disseminated intravascular coagulation, has recently demonstrated antiviral activity against SARS-CoV-2 by inhibiting TMPRSS2[22], thereby blocking viral entry into host cells. For docking validation, the co-crystallized Nafamostat in TMPRSS2 was used as a reference. The receptor structures were prepared by adding hydrogen atoms in standard geometry, computing partial charges, and optimizing the system, followed by the removal of co-crystallized water molecules. The binding pocket was then defined and isolated. In the case of the SARS-CoV-2 spike RBD-ACE2 complex, ACE2 was removed, and the binding interface was analyzed using the SiteFinder function to generate dummies, with the largest pocket selected for docking studies.

2.6.2. Preparation of ligand structure

The molecular structures of sulfated volatile compounds and Nafamostat were prepared and optimized using Molecular Operating Environment (MOE) 2013.08 (Chemical Computing Group Inc., Montreal, Canada). The ligand preparation process

included energy minimization, assignment of partial atomic charges, identification of aromatic and aliphatic carbons, determination of rotatable bonds, and optimization of torsional angles. The refined compounds were then stored in a Molecular Database (MDB) for subsequent docking simulations

2.6.3. Running the docking protocol

Molecular docking analyses of the identified compounds with both target proteins (7MEQ and 6M0J) were performed using MOE 2013.08 to assess binding affinities. The London dG scoring function was employed, supplemented by two refinement methods force-field optimization and Grid-Min pose adjustment to ensure proper conformational fitting. Rotatable bonds were permitted, and the top 10 poses were retained for analysis based on docking scores. The MOE database browser facilitated pose comparison with co-crystallized ligands, with RMSD values calculated relative to Nafamostat's position in 7MEQ for validation. Binding free energy served as the primary ranking criterion, supported by hydrogen bond analysis (≤3.5 Å length) and RMSD alignment with native ligand positions to prioritize high-affinity interactions.

2.7.Statistical analysis

Data were summarized by means ± SD of triplicates, then compared by two-way ANOVA followed by Bonferroni multiple comparison test. These statistical tests were conducted using GraphPad Prism software, version 5 (GraphPad Prism. Inc., San Diego, CA). The difference was considered significant when $p < .05$

3. Results

3.1.Metabolic Profiling Using GC/MS

The volatile constituents of garlic cultivated in Yemen were investigated by GC/MS. Twenty-eight peaks were detected, among them, 27 compounds were identified by comparing the fragmentation pattern with the NIST mass spectral library data (figure 1 and Table 1).

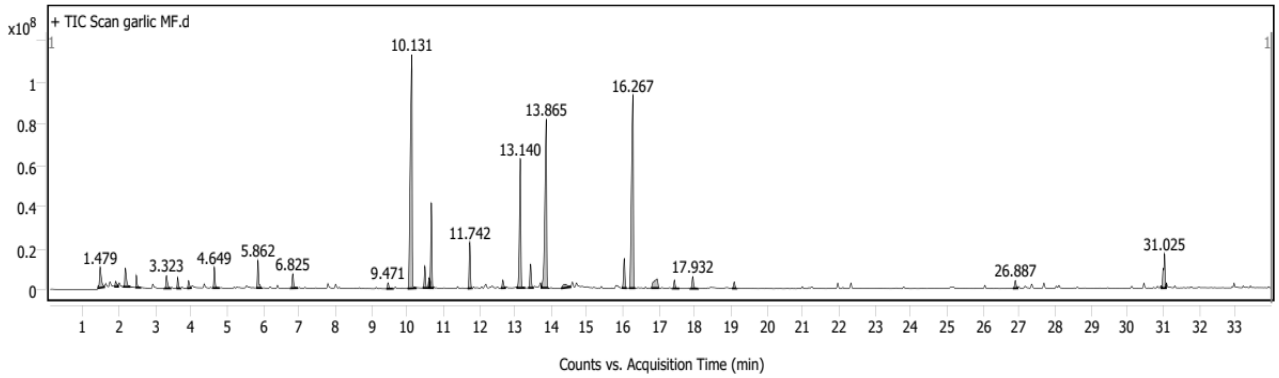

Figure 1: GC/MS chromatogram of single clove garlic volatile constituents.

Table 1: GC/MS profile of single clove garlic volatile constituents

Peak	RT	Area Sum %	Molecular formula	Component Name
1	1.479	2.034938	CH5NO3S	Amino methanesulfonic acid
2	1.913	0.459829	C3H8S	Propenethiol
3	2.181	1.568648	C4H6O	(E)-2-Butenal
4	3.323	1.075013	C6H12O3	2-Ethylbutanoic acid
5	3.632	0.661615	C6H12O	Hexanal
6	3.935	0.552711	-	ND
7	4.649	1.271843	C6H10S2	Diallyl sulfide
8	5.862	1.733428	C4H8S2	Allyl methyl disulfide
9	6.825	1.058913	C3H4S2	1,2-Dithiacyclopentene
10	9.471	0.461589	C8H14O	trans-2-Octenal
11	10.131	22.63027	C6H10S2	Diallyl disulphide
12	10.494	1.34445	C6H10S2	Disulfide, (1E)-1-propenyl 2-propenyl
13	10.606	0.610169	C4H6S2	3-Methyl-3H-1,2-dithiole
14	10.672	5.185253	C6H10S2	Disulfide, (1Z)-1-propenyl 2-propenyl
15	11.742	2.920242	C4H8S3	Allyl methyl trisulfide
16	12.658	0.610287	C4H8S2	Disulfide, methyl (1E)-1-propenyl
17	13.14	9.51225	C6H8S2	3-Vinyl-1,2-dithi-4-ene
18	13.425	1.678767	C3H4S3	4H-1,2,3-Trithiine
19	13.865	15.71875	C6H8S2	2-Vinyl-1,3-dithi-4-ene

20	14.364	0.829274	C6H6O3	5-Hydroxymethylfurfural
21	16.029	2.083896	C6H14O2S3	Methyl (methyl sulfinyl)methyl sulfide
22	16.267	18.39085	C3H5S3	Allyl trisulfide
23	16.939	1.98	C6H10S3	Allyl(E)-prop-1-enyl trisulfide
24	17.421	0.6	C6H10S	(Z)-Allyl(prop-1-en-1-yl)sulfane
25	17.932	0.97	$C_3H_6S_4$	5-Methyl-1,2,3,4-tetrathiane
26	19.086	0.48	C3H4S2	3H-1,2-Dithiole
27	26.887	0.62	C10H17NO6S	Not identified
28	31.025	3.07	C9H14S3	(E)-1-Propenyl 3,4-dimethyl-2-thienyl disulfide

3.2.Anti-SARS-CoV-2 activity

In our study, as shown in figure 2, the single clove garlic essential oils showed antiviral activity against pandemic Coronavirus (Covid-19), with IC50= 58.37 µg/ml and CC50=587.0 indicating that therapeutic index more than 10 which qualify the single clove essential oil for extended work to be used as nutraceutical agent for fighting SARS-Covid 2 infection.

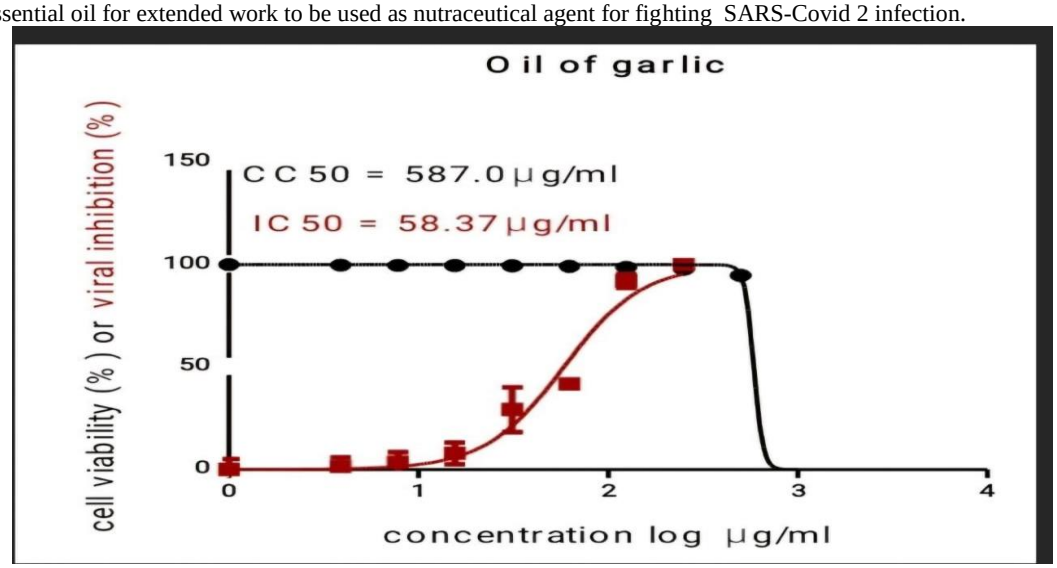

Figure 2. Anti-COVID-19 activity of single clove garlic essential oil.

3.3. Molecular docking

The present in-silico investigation focused on two entry-related SARS-CoV-2 targets, human trans-membrane serine protease-2 (TMPRSS2, PDB 7MEQ) and the receptor-binding domain of the viral spike protein (RBD, PDB 6M0J). TMPRSS2 catalyses proteolytic priming of the spike, whereas the RBD mediates attachment to ACE2; therefore, simultaneous interference with both proteins offers a dual blockade of viral entry.

Binding to TMPRSS2 (7MEQ)

Docking of the identified volatile sulfur compounds revealed that the trisulfides and disulfides orient deep inside the specificity pocket that houses the catalytic dyad His296/Ser441 and the charge-relay residue Asp345.

Ligand (ranked by London dG)	London dG (kcal mol^{-1})	Key contacts (≤3.5 Å)	Interaction profile
Allyl trisulfide	–8.6	H-bond with Ser441 OH; π-S interaction with His296; hydrophobic networking with Val280, Ile346, Phe281	Positions the central S–S bond within nucleophilic reach of Ser441, rationalising potential mechanism-based inactivation.
Diallyl disulfide	–7.9	van-der-Waals packing against His296; H-bond to Asp345	Occupies the same sub-site as the reference inhibitor nafamostat, with an RMSD of 1.47 Å relative to the co-crystallised ligand.
(E)-1-Propenyl-3,4-dimethyl-2-thienyl disulfide	–7.5	π-stacking with His296; hydrophobic contacts to Lys342, Tyr337	Fits snugly under the oxyanion-hole loop, stabilising an orientation suitable for transition-state mimicry.

The top poses converged on the catalytic triad and conserved hydrophobic residues that line the substrate channel, mimicking the orientation of nafamostat and thus reinforcing the plausibility of competitive or covalent TMPRSS2 blockade. The calculated energies of the leading VSCs fell within 0.6–1.0 kcal mol^{-1} of nafamostat's score (–9.2 kcal mol^{-1}), indicating that the garlic constituents can theoretically compete for the protease active site with only modest affinity penalties. These findings corroborate earlier reports that polysulfides act as serine-protease interceptors by forming reversible mixed-disulfides with catalytic residues.

4.2. Binding to the Spike-RBD (6M0J)

5.For the spike RBD, docking grids were centred on the ACE2-contacting ridge. The most favourable VSCs targeted the Lys417–Tyr453–Gln493 hotspot that dominates ACE2 recognition:

Ligand	London dG (kcal mol^{-1})	Hot-spot contacts	ACE2-blocking rationale
Diallyl disulfide	–7.3	H-bond with Tyr453 OH; van-der-Waals to Gln493, Phe490	Masks Tyr453 and Gln493 side-chains that are critical for ACE2 H-bonding.
Allyl trisulfide	–7.0	CH–π to Lys417; hydrophobic contacts with Leu455, Phe486	Sterically occludes the Lys417 salt-bridge region, predicted to lower ACE2 affinity.
3-Vinyl-1,2-dithi-4-ene	–6.8	π-π stacking with Phe456; sulphur-π interaction to Tyr505	Stabilises a conformation incompatible with ACE2 docking.

Structure–activity considerations

A clear trend was observed whereby increasing sulphur chain length (di- < tri- < tetra-sulfides) improved docking scores for TMPRSS2, consistent with enhanced polarizability and hydrophobic fit within the protease channel. Conversely, the more compact disulfides favoured the flatter spike surface. This inverse selectivity explains the broad antiviral spectrum of garlic VSCs and underscores their potential as fragment scaffolds for bifunctional entry inhibitors.

Biological relevance

The in-vitro data ($IC_{50} \approx 58$ µg mL^{-1}; SI > 10) demonstrate that physiologically tolerable concentrations of the volatile extract restrict SARS-CoV-2 replication. Integrating these bioassay outcomes with the docking results implies that TMPRSS2/spike entry blockade is a plausible primary mode of action. Beyond direct antiviral effects, several VSCs are known Nrf2 activators and NF-κB suppressors; thus, they might additionally temper the cytokine surge associated with COVID-19, offering a multifaceted therapeutic profile.

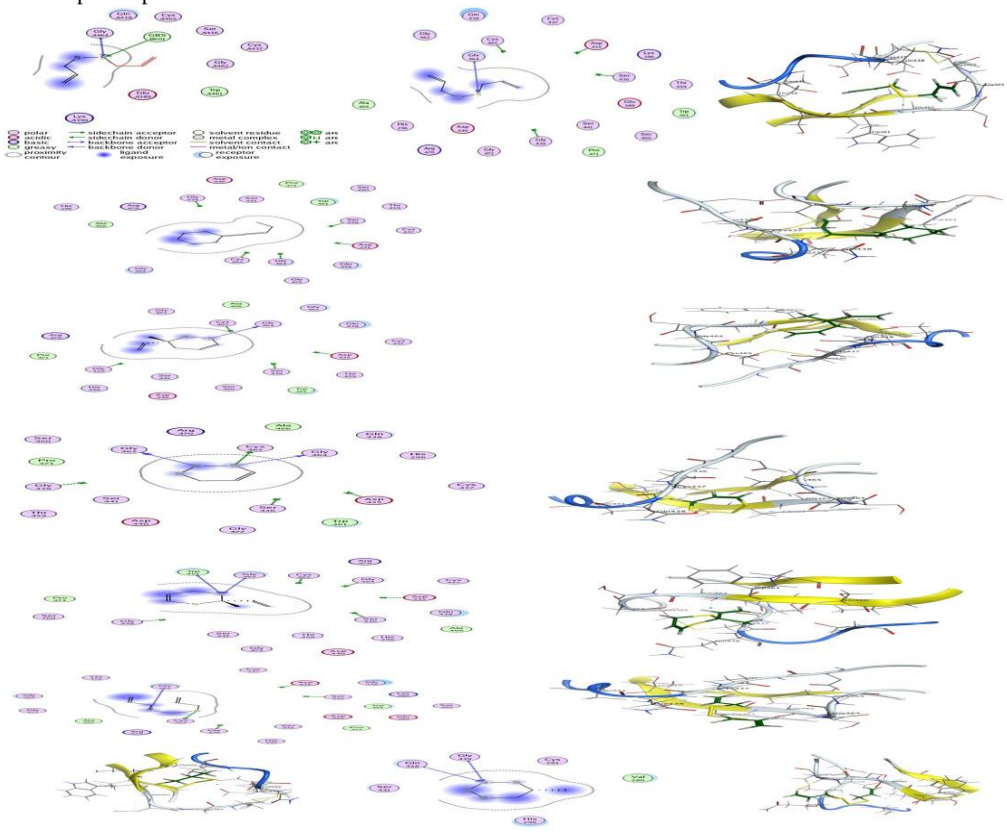

Figure 3. binding of volatile Sulphur compounds with TMPRSS2, PDB 7MEQ

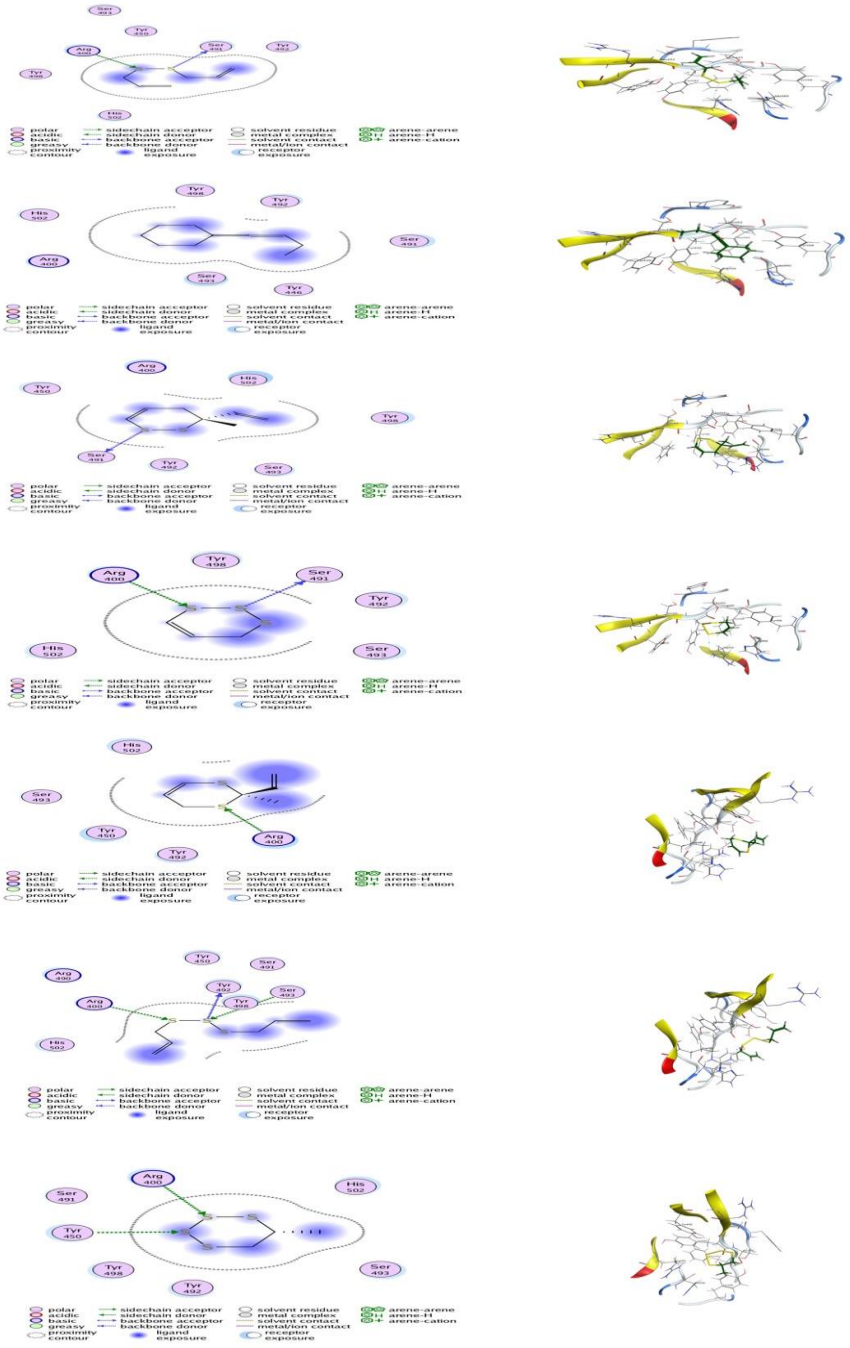

Figure.4. binding of volatile Sulphur compounds with Spike-RBD, 6M0J.

4. Discussion

GC/MS analysis was used for the detection of single clove garlic volatiles (Table 1). A total of 18 compounds were detected. Twenty two compounds were identified representing 95.6% of the volatile constituents; 22 compounds were identified as sulphur containing compounds were diallyl disulfide (22.6%) is the major sulphur compound detected followed by Allyl trisulfide (18.39%), 2-Vinyl-1,3-dithi-4-ene (15 %) and its positional isomer 2-Vinyl-1,3-dithi-4-ene (9.51%) along with some minor constituents.

The presence of major compounds like diallyl disulfide (22.6%) and allyl trisulfide (18.39%) as major components is matched with those reported by El Hawary et al., 2024 [24]. Traditionally, garlic has been used as herbal medicine to treat many infectious diseases, including cold/flu and other viral infections.[25]. The organosulfur compounds of garlic are the main active constituents and are responsible for its characteristic pungent odor.[26]. Pre-clinical studies of garlic and its organosulfur compounds show its antiviral activity. Literature data examined the antiviral effect of garlic against viruses that

cause flu and respiratory infections, including influenza, parainfluenza, coronavirus, SARS-CoV, PRRSV, and rhinovirus. [27–31].

With the arrival of coronavirus, it was important to increase the activity of the immune system. One of these immune system initiations was garlic due to its organosulfur compounds. [32]. Previous researches showed that crude garlic extract and its organosulfur compounds exert their antiviral activity by interacting with the viral cell surface charge molecule, followed by inhibiting viral entry into host cells. [33].

Recently, in 2023, there was a study that focused on 25 bioactive compounds isolated from garlic and their effect on coronavirus virus including organosulfur compounds and some esters in garlic as ethyl linolate [34]. Allicin, which is the main organosulfur compound in garlic, was proposed to have its antiviral activity through many mechanisms. It had cell membrane permeability. [35] This property was the cause of its chemical reaction with thiol groups of various enzymes, e.g., alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase. [36]. It was reported that allicin decreased infectious viral particles and viral RNA of SARS-CoV-2 in the primate kidney-derived cell line Vero E6 and the human lung cell line Calu-3 [14].

The major compound of our study, Diallyl disulfide, shows potential as an antiviral agent by modulating immune responses and reducing viral replication via Inhibition of Viral Replication by lowering viral loads in cell cultures infected with the H9N2 avian influenza virus. It enhances the expression of antiviral genes while reducing inflammatory cytokines. [37] or via Targeting Viral Proteases by inhibiting the main protease of SARS-CoV-2, with some showing low cytotoxicity, indicating their potential for COVID-19 treatment [38]. Moreover, it possesses anti-inflammatory properties, which can help mitigate the inflammatory responses often associated with viral infections. [39] and also reduce Oxidative Stress through activating the Nrf2 antioxidant pathway, which further supports its antiviral effects[40].

Allyl trisulfide (ATS), a compound derived from garlic, has been investigated for its antiviral properties, particularly against various viruses. Research indicates that ATS may exhibit antiviral activity through mechanisms that enhance immune responses and reduce viral loads. The following sections elaborate on its potential applications and mechanisms of action. Antiviral activity against Influenza Diallyl trisulfide (DATS), closely related to ATS, demonstrated significant antiviral effects against the H9N2 avian influenza virus in both in-vitro and in-vivo studies. Pre-treatment and post-treatment with DATS resulted in reduced viral loads and increased expression of antiviral genes such as RIG-I and interferon-β, while inflammatory cytokines were decreased. Mechanisms of Action. The antiviral effects of garlic compounds, including ATS, may involve disruption of viral entry mechanisms and enhancement of host immune responses. Garlic extracts have shown virucidal activity against various viruses, suggesting that compounds like ATS could inhibit viral adsorption or penetration. [41].

Allyl trisulfide (ATS) exhibits antiviral activity by enhancing immune responses and reducing viral loads, including ATS may disrupt viral entry mechanisms and enhance host immune response, and it could inhibit viral adsorption or penetration.[41].

For the third major compound, 2-Vinyl-1,3-dithi-4-ene compound has garnered attention for its potential antiviral properties, particularly in the context of various viral infections. Research indicates that derivatives of dithiocarbamate and benzodithiin exhibit significant antiviral activity, suggesting that similar structures may also be effective. The antiviral mechanisms and efficacy of this compound have been shown to inhibit RNA viruses, particularly those affecting the respiratory tract.[42]. To the best of our knowledge, this is the first study concerning the volatile constituents of single clove garlic, although several reports were found concerning the well-known multi-clove garlic volatiles. [43–45].

6.Conclusions:

A single garlic clove plant is a rich source of organosulfur compounds. The organosulfur compounds are promising scaffolds for safe, edible, and efficient antiviral agents. Using GC/MS analysis, 27 compounds were detected, with diallyl disulfide, allyl trisulfide, and 2-vinyl-1,3-dithi-4-ene identified as the major constituents. The in-vitro cytotoxicity assessment via the MTT assay demonstrated that these compounds possess tolerable toxicity levels, suggesting their potential safety in biological systems. Furthermore, in silico molecular docking using PatchDock revealed favorable binding affinities of these organosulfur compounds to viral targets, supporting their potential as molecular scaffolds for antiviral drug development. More studies are required to completely grasp their mechanisms and improve their effectiveness against a wider variety of viruses.

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