Molecular Docking Analysis for Evaluating the Efficacy of Active Components of Essential Oils against Water-Borne Multi-Virulent Bacteria and Virus

Kshama Balapure*, Vinay M. Bhandari
Chemical Engineering and Process Development Division
CSIR-National Chemical Laboratory, Pune-411008, India
*Email: kshama.balapure@gmail.com

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Abstract

Traditional Indian knowledge of Ayurveda mentions a large number of essential oils that contain many active compounds such as thymol, eugenol and cinnamaldehyde etc. which contemplate nontoxic for human being and have been used mostly in the field of medicine. An application of essential oils comprising antimicrobial activities was recently described in the SWASTIIK technology (Safe Water and Sustainable Technology Initiative from Indian Knowledgebase) in conjunction with cavitation/ intense mixing for drinking water treatment. Further, it is intended to demonstrate the efficacy of SWASTIIK technology to combat multi-virulent bacteria and emerging viruses such as *Escherichia coli* and SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2) respectively with the help of *in silico* computational tools. Docking study indicated eugenol and thymol were the most effective ligands against the microorganisms, although SARS-CoV-2 performed better with a low binding affinity (-7.2 kcal/mol) than multi-virulent *E. coli* (-4.5 kcal/mol). SWASTIIK technology for eliminating harmful microorganisms is, therefore, expected to be more effective in combating viruses, including SARS-CoV-2. Further, favorable ADMET results suggest suitability of the active components for further clinical trials study or for possible health benefits of water treated using SWASTIIK technology. In view of lack of human studies, to evaluate the effectiveness and safety of essential oils for direct intake, clinical trials with more well-designed protocols are needed.

Key Words: SWASTIIK, Molecular Docking, Water Disinfection, Essential oil, SARS-CoV-2

1. Introduction:

Safe and pure water is important for preventing many waterborne diseases and for saving millions of lives[1]. Drinking water contaminated with coliform bacteria, especially *Escherichia coli (E. coli)*, may cause dysentery, vomiting, abdominal pain, loss of appetite, headaches, fever, and sometimes creates life threatening complications[2]. Waterborne diseases cause millions of deaths worldwide each year because of severe bacterial infections[3,4]. Nowadays, antimicrobial resistance is also one of the world's biggest concerns, affecting millions of lives. A multidrug-resistant *E. coli* is the one of main culprits in this regard due to its capability to produce a wide variety of virulence factors, especially cell membrane and adhesion proteins[5]. Furthermore, a number of emerging infectious diseases have also been identified in recent years, such as COVID-19, which is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), declared as a pandemic by the World Health Organization. The spread of it was rapid, infecting 71,351,695 people and causing 1, 612, 372 deaths globally as of 15 December 2020[6]. The traces of the SARS-CoV-2 virus in water samples were also detected[7]. A reliable and sustainable water management is imperative in these types of situations. It is necessary to implement new technologies as possible alternatives to existing chemical methods (e.g. chlorination) which have serious disadvantages, as well as to enhance immunity in the COVID-19 scenario.

Safe Water and Sustainable Technology Initiative from Indian Knowledgebase (SWASTIIK) is recently reported as one of the effective and techno-economically feasible water disinfection technology[8-11]. In contrast to most of the physico-chemical methods of treatment, the SWASTIIK technology demonstrated promising application of different essential oils such as ajwain oil, peppermint oil for the disinfection of pathogenic bacteria from water by just simple mixing or in conjunction with hydrodynamic cavitation. The phenomenon of hydrodynamic cavitation involves formation, growth and collapse of cavities in a liquid creating extremely high temperatures (up to 10000 K) and pressures (up to 5000 atm) at the implosion point, consequently producing oxidizing agents and, subsequently creating an environment favorable for disinfection[12,13] . Hydrodynamic cavitation using essential oils achieves complete disinfection in less than a minute by combining the effects of antimicrobial activity of the essential oils and cavitation. It is cost-effective, sustainable, and offers a greener way to treat water containing variety of disease-causing bacteria. Essential oils can contain large number of chemical compounds such as thymol, carvacrol,

cinnamaldehyde etc. which are considered safe for mankind and have also been accepted by the Food and Drug Administration (FDA)[14]. The efficiency of the SWASTIIK technology is already proven at pilot plant scale for disinfecting different harmful microorganisms such as gram-negative (*E. coli*) and gram-positive (*S. aureus*) bacteria, antimicrobial-resistant bacteria (methicillin-resistant *Staphylococcus aureus*) and *Pseudomonas aeruginosa,* within less than 10 min. The basis for the selection of essential oils requires evaluating active components like thymol, menthol, cinnamaldehyde, eugenol, and terpinen-4-ol, which play a significant role in disinfecting water. The SWASTIIK process can give an alternative to the chlorination as well as other water treatment processes such as UV, membrane, ozone etc. However, the technology needs to be evaluated further against multivirulent pathogenic bacteria and viruses in order to determine its broad effectiveness. The requirement of a specialized laboratory for working on multivirulent bacteria and viruses limits the experimentation *in vitro*. Though, hundreds of essential oils are listed having antimicrobial properties, it does not necessarily infer their direct use in water disinfection, and selecting appropriate oils against specific organism is crucial to maximize its usefulness for effective disinfection and for additional health benefits.

Computer- aided drug design (CADD) is a useful tool to check the efficacy of the active ingredients of essential oils against harmful pathogens and for the selection of appropriate oil, to save the time/ laborious experimentations and resources. CADD techniques such as *in silico* molecular docking can virtually analyze binding efficacy of the ligand molecule (active components of essential oil) against target compound (protein compound of an organism[15,16]. The molecular docking provides insights into the binding conformation of antimicrobial compounds with pathogenic bacteria and viruses, as well as to evaluate the mode of action, protein-ligand interactions. Various essential oils exhibit varied binding activities towards different organisms. It is possible to predict efficacy of essential oils against various organisms by analyzing its binding affinity scores. These predictions will assist in designing and conducting specific experiments for water disinfection under laboratory conditions[17,18].

The objectives of this research include evaluation of the efficacy of essential oils used in SWASTIIK technology against two proteins, one from *E. coli* and second from SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2) and to determine potential therapeutic benefits associated with essential oils. The present investigations on docking interactions, ADME (absorption, distribution, metabolism, excretion) and toxicity profiles of various active components found in essential oils such as thymol, menthol, eugenol, cinnamaldehyde,

and terpinen 4-ol can shed light on suitability of these compounds against multivirulent *E. coli* and SARS-CoV-2.

2. Experimental:

2.1 SWASTIIK technology

SWASTIIK technology is selected for the present investigation as it seems to be a sustainable methodology and assures safe drinking water [8-11]. The process contemplates the use of essential oils and intense mixing, or hydrodynamic cavitation under ambient conditions for the disinfection of bacteria. It is effective for low to very high concentration of bacteria (~10^5 CFU/mL) and a small concentration of essential oils, 0.05 to 0.1% (v/v) (500-1000 µg/ml) is required. Different kinds of essential oils were examined namely Ajwain oil (*Trachyspermum ammi*), Peppermint oil (*Mentha x piperita*), Tea tree oil (*Melaleuca alternifolia*) Harsingar oil (*Nyctanthes arbor-tristis*), Cinnamon leaf oil (*Cinnamomum zeylanicum*) etc for disinfection of gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*), antimicrobial-resistant (methicillin-resistant *Staphylococcus aureus*) and *Pseudomonas aeruginosa*.

2.2 In silico analysis

2.2.1 Protein structure preparation

Molecular docking studies were performed using two different proteins: the outer membrane protein A (OmpA) from *E. coli* (PDB ID: 1BXW) and S1 subunits of the receptor binding domain (RBD) from SARS-CoV-2 (PDB ID: 6M0J). Protein structures were downloaded from Protein Data bank[5,19.] In the case of *E. coli*, outer membrane proteins (OMPs) were selected for study because they can express numerous virulence factors, such as fimH and kpsM, which are important for adhesion and survival. Similarly, in case of SARS-CoV-2 the glycoprotein (S) was examined. The protein consists of two parts: S1 – a receptor binding domain that binds to Angiotensin converting enzyme 2 (ACE2), and S2, which is responsible for fusion of cellular and viral membranes. To prepare protein for docking experiment, first co-crystallized ligands, water molecules, and cofactors were removed, and then target protein files were prepared using Auto Dock 4.2 (MGL tools 5.6). Protein active sites were recognized by literature scrutiny and structure visualization feature on PDB. In *E. coli* the major region of interest is C8E region, whereas in SARS-CoV-2 the region of interest is S1 subunits of the receptor binding domain (RBD). Further, to define the search space within the protein, AutoDock Vina needs a grid box encompassing the region of interest (active site)

within the macromolecule[20]. The grid box size (A°) for outer membrane protein A (OmpA) of *E. coli* was set at 86.51, 54.52, 26.2 for x, y and z respectively, and the grid center was set to, -19.80, 0.167 and 9.66 for the coordinates x, y and z respectively. The grid box size (A°) for S1 subunits of the receptor binding domain (RBD) from SARS-CoV-2 was set to 104, 114 and 60 for x, y and z respectively, and grid center was set to, -21.02, -14.4 and 0.19 for the coordinates x, y and z respectively. Molecular visualization of the best docked pose was carried out by using in built docking algorithm of Auto Dock Vina. Further, the favorable protein-ligand conformations with a least potential binding energy were selected and consequently examined by Discovery studio visualizer.

2.2.2 Selection and preparation of ligands

The active components of essential oils used in SWASTIIK technology such as Ajwain oil-(thymol), Peppermint oil (menthol), Tea tree oil (terpinen 4-ol), Cinnamon leaf oil (cinnamaldehyde) was selected as ligands to check its suitability against multivirulent *E. coli* and SARS-CoV-2. The structure of ligands thymol, menthol, eugenol, cinnamaldehyde and terpinen 4-ol was downloaded from the PubChem NCBI database. The PubChem ID, 2D, 3D structures and molecular properties of all the compounds are shown in **Table 1**. The docking experiment was performed by creating 3D structures of ligands using SMILES format and saving them into MDL-MOL files, followed by creating PDB files via Open Babel[21].

2.2.3 PreADMET analysis of active compounds

The pharmacokinetic and toxicological characteristic of all active compounds of essential oils i.e. thymol, menthol, eugenol, cinnamaldehyde, terpinen 4-ol were predicted using online PreADMET server (https://preadmet.webservice.bmdrc.org-). A number of pharmacokinetic properties were predicted using ADME property prediction, including Caco-2 values for *in vitro* parameters of cell permeability (Caco-2 cell model), intestinal absorption (HIA value), plasma protein binding (PPB value), blood–brain barrier (BBB) penetration, and P-glycoprotein (Pgp) binding parameters. Furthermore, the active compounds were virtually scrutinized to analyze its toxicological attributes such as carcinogenicity and mutagenicity using toxicity prediction tools. The results of ADMET analysis were predicted using existing literature of drug discovery studies. However, for the first time PreADMET analysis has been used to assess the effectiveness of essential oils in SWASTIIK technology.

2.2.4 Prediction of plausible health benefits and/or potential clinical application of active compounds of essential oil

The plausible health effects/application of active compounds were identified by searching the scientific literature via two different platforms PubMed and Clinicaltrials.gov. Reported data

of clinical trials on living beings for the effect of thymol, menthol, eugenol, cinnamaldehyde, terpinen-4-ol were considered.

3. Results and Discussion:

3.1 SWASTIIK technology

SWASTIIK technology reported the application of essential oils such as ajwain oil, peppermint oil, clove oil, cinnamon oil and tea tree oil, etc. in combination with hydrodynamic cavitation and/or by simple mixing for removal of harmful bacteria from water. A complete disinfection was observed using just 0.1% oil concentrations within minutes[8-11]. Further, it can efficiently destroy different types of bacteria such as *E. coli*, *S. aureus*, antimicrobial resistant bacteria (AMR), and difficult, opportunistic pathogen *P. aeruginosa,* making it suitable for potential commercial applications of drinking water treatment as well as in rural use. An effective disinfection of pathogenic microorganisms could be attributed to the antibacterial activity of active components of essential oils, such as thymol in ajwain oil, and menthol, cinnamaldehyde, eugenol, and terpinen-4-ol in peppermint oil, cinnamon oil, clove oil, and tea tree oil, respectively, each of which plays significant role in disinfecting water. Essential oils are biological in nature and decontaminate water with high rate of degradation through biochemical reactions within living cells[22]. A number of essential oils exhibit antibacterial properties, but SWASTIIK technology is the first to demonstrate that these properties could be applied to drinking water treatment in the real life with satisfactory rates of disinfection. Thus, it is instructive to examine the utility of the technology with multi-virulent bacteria and emerging viruses such as *E. coli* and SARS-CoV-2 by *in-silico* analysis of active compounds (e.g. thymol, menthol, cinnamaldehyde, eugenol and terpinen-4-ol) present in essential oils against multivirulent *E. coli* and SARS-CoV-2.*in silico* study.

3.2 Molecular Docking

A Molecular docking technique helps to identify potential inhibitors of a specific protein. Molecular interactions of thymol, menthol, eugenol, cinnamaldehyde and terpinen4-ol against multivirulent *E. coli* and SARS-CoV-2 are shown in Table 2. Molecular docking of multivirulent *E. coli* indicated that eugenol (-4.5 kcal/mol) and thymol (-3.9 kcal/mol) have the highest binding affinity and docking scores as compared to other ligands. Among all various conformations produced during docking, the conformations with the least RMSD (Root mean square deviation) were chosen. The docking poses of each ligand against multivirulent *E. coli* is shown in Figs 1-5. Eugenol formed one or more hydrogen bonds with

residues ASN 33, Thr 30, His 31, while thymol formed hydrogen bonds with Tyr 63 with bond length 1.8 A° and Thr 30 with bond length 2.1 A°. Thymol also forms Pi alkyl interactions with Lys 64 (Table 2). Cinnamaldehyde also formed hydrogen bond with His 31 and Thr 30 with bond length 2.2 and 2.5 A°. Terpinen-4-ol, and menthol also showed good binding affinity, but none of them formed hydrogen bonds with proteins. The formation of intermolecular hydrogen bonds between the active components of essential oil and amino acid residues is anticipated to exert a stronger influence on the formation of a stable protein-ligand complex, resulting more precise docking results[23]. The docking results of all the ligands with SARS-CoV-2 indicated that thymol had the strongest bonding interaction with -7.2 kcal/mol but lacks hydrogen bonding interactions, whereas eugenol and cinnamaldehyde exhibited -6.5 kcal/mol affinity and did not form any hydrogen bonding interactions. The binding of thymol involves Pi alkyl interactions with Pro 415, while eugenol interacts with Lys 541, His 540, and Pro 415. Cinnamaldehyde, on the other hand, forms Pi alkyl interactions specifically with Pro 415. Additionally, menthol forms a hydrogen bond interaction with Ser 420, with a bond length of 3.2 Å, although it exhibits a lower binding affinity in case of SARS-CoV-2. The protein-ligand interactions and residue information are summarized in Table 2. Kulkarni et al.[19] also confirmed the absence of hydrogen bond interactions with thymol, eugenol, for COVID protein. However, in the present study, an amino acid interacted differently than reported by Kulkarni et al.[19], and, the reason for this discrepancy remains unclear. Jani et al. [24] found that the interacting residues on the surface of the RBD domain of the SARS-CoV-2 spike protein were Tyr505, Gly502, Asn501, Thr500, Gln498, Gln493, Tyr489, Asn487, Phe486, Ala475, Phe456, Leu455, Tyr453, Tyr449, Gly446 and Lys417. Further, Li et al.[25] determined that important amino acids of RBD S-protein of SARS-CoV-2 were found between Gly354, Lys353, Asn330, Glu329, Gln325, Asn90, Tyr83, Met82, Leu79, Leu45, Gln42, Tyr41, Asp38, Glu37, His34, Lys31, Thr27, Gln24. According to Sahlan et al.[26] it might be that the different docking algorithms and scoring functions of each simulation were the most likely causes of the differences.

Figs. 6-10 clearly display the binding efficiencies of all of the ligands to SARS-CoV-2 protein along with their molecular interaction. Eugenol and thymol appear to act most effectively against both microorganisms, although SARS-CoV-2 showed better performance with a high potential energy (-7.2 kcal/mol) compared to multi-virulent *E. coli* (-4.5 kcal/mol). Accordingly, SWASTIIK technology can be considered to have potential activity at combating viruses, including SARS-CoV-2. The active compounds have already been proven to eliminate various pathogenic bacteria[8-11]. Biologically active compounds are being

increasingly reported for eliminating drug resistant bacteria and viruses. Numerous researchers have sought to identify essential oils that can be applied as safe substitutes for germicides in order to moderate or completely impede bacterial growth. Recently, Elfaky et al.[5] reported molecular docking study and mice protection assay of active ingredient of the essential oil and stated promising role of thymol, eugenol and curcumin for combating multi virulent *E. coli*. Further, Benencia et al.[27] reported eugenol, the active ingredient of clove oil has virucidal activity against human herpes virus, while Terstappen et al.[28] noted antiviral activity of clove and oregano oils against polio, coxsackie virus B1, and adenovirus type 3. Kulkarni et al.[19] carried out *in silico* analysis of the active components of plant essential oils and found that cinnamaldehyde, carvacrol, thymol and pulegone etc. have ability to hinder the covid viral spike protein. Nazzaro et al.[29] also reported that some essential oils such as rosemary, oregano etc. are effective against *E. coli*, *S. aureus*, *B. cereus* and *Salmonella* sp. but less effective against *Pseudomonas* sp. Essential oils have different binding efficiencies against different microorganisms, and it is essential to select the appropriate oil when seeking antibacterial/antiviral action or disinfection. Thus, molecular docking study can help in the screening of potential active compound against specific pathogens and to prove utility of SWASTIIK technology.

3.3 PreADMET analysis and toxicity assessment

The absorption, metabolism, distribution and toxicity parameters of all five compounds were assessed using the online PreADMET server. The calculated parameters of all active compounds i.e. thymol, menthol, eugenol, cinnamaldehyde and terpinen 4-ol are summarized in Table 3. In general, ADMET properties can be predicted during drug discovery, but in this study, PreADMET analysis was applied to evaluate the efficacy of essential oils used in SWASTIIK technology and to determine how the active components of each essential oil are processed by living organisms. The predicted absorption values for Caco-2 cell permeability were observed to be 23 to 50, henceforth all compounds considered for the study were moderately penetrable (Table 3). In addition, the predicted HIA value for all compounds were observed to be above 95 %, indicating that the compounds were well absorbed through the intestinal cells. Sahar et al.[30] stated that maximum intestinal absorption is essential for the absorption of any drug or phytochemical taken orally, since it represents the primary site of absorption for any oral medication. The predicted PPB values of all tested compounds were 100 %, indicating a strong binding, except cinnamaldehyde which had a value of 52.8%, indicating a weak binding [31]. The compounds tested were all inhibitors of P glycoprotein

(PgP), and the inhibition can increase the bioavailability of susceptible molecules. If all the tested compounds pass by all the ADMET properties, it could be said that the compounds are suitable for further clinical trials or for applicability of SWASTIIK technology for drinking water treatment. Additional *in vitro* studies may be required to confirm this. The results of the toxicity study indicated that despite all compounds testing mutagenic in the *in-silico* AMES model, thymol and cinnamaldehyde displayed non-carcinogenicity in mice and rats, while eugenol appeared to carcinogenic to the rat and mice. To ensure that purified water is fit for drinking, early characterization of its properties may help to eliminate compounds with unacceptable toxicity parameters. The toxicity data showed eugenol to be carcinogenic to rats and mice, but it exhibits proficient antibacterial and antiviral, and disinfection efficiency. Thus, to avoid the toxicity of eugenol in water, it is recommended to remove it through an appropriate separation technique after the disinfection or avoid use of essential oils containing eugenol, as a safer practice at this point of time.

3.4 Health benefits and/or potential clinical application of essential oil active compounds

PubMed and the Clinicaltrials.gov database were used to identify case studies illustrating the potential of active components of essential oils used in SWASTIIK technology and their potential health benefits/clinical applications. The outcome of the case studies and plausible effect of all the essential oils are represented in Table 4. It can be seen that almost all the essential compounds have antibacterial, antifungal, anticancer and antiviral activity, and have related clinical trials also. The active ingredients of ajwain oil and thyme oil are thymol, which has been useful to treat upper respiratory tract infections such as common cold, sinusitis, pharyngitis, bruises, and sprains, as well applied as a remedy in coughs caused by colds and also used as disinfectants in dentistry[32]. Thymol has antifungal, antioxidant, and anticancer properties as well as shows antibacterial activity on Gram-positive and Gram-negative bacteria, while showing antiviral activity on herpes simplex virus type I, human rhinoviruses and influenza viruses[33-35]. Thymol also has anti-inflammatory properties as reported by Zhou et al.[36], who examined the effects of thymol on allergic inflammation in ovalbumin-induced mice asthma (OVA) and conclude that pretreatment with thymol bring down OVA-specific IgE antibody in mice and decreased the values of interleukins (i.e. IL-4, IL-5, and IL-13) thereby recommending its application for the treatment of allergic asthma. The efficacy of thymol as a drug for treating high-fat diet (HFD)-induced obesity in rats was demonstrated by Rafiul Haque et al. [37]. The use of thymol to treat obesity has also been supported by some clinical trials (Table 4). Further, eugenol an active ingredient in clove oil,

has been reported to have multiple applications in dental procedures such as cementing temporary prostheses and restoring teeth and cavities[38]. The application of propolis, clove oil, and chlorhexidine for managing tooth caries has also been studied clinically. Hussain et al.[39] suggested that eugenol enhances the anticancer activity of drug gemcitabine and help to reduce the level of carcinogenic and inflammatory activities in human cervical cancer cells. The wide range of eugenol activities includes antimicrobial, anti-inflammatory, analgesic and antioxidant have been reported by several authors [40, 41]. Recently, Naz et al.[41] evaluated the strong interactivity of human serum albumin (HAS) and SARS-CoV-2 against eugenol for novel COVID-19 drug discovery. Eugenol is recommended as a safe for consumption, but excessive amounts of clove oil (ten to thirty milliliters) can result in coma, acidosis, respiratory depression and, in some cases, severe hypoglycemia requiring ventilation and intravenous glucose[42]. Several characteristics and activities of eugenol have not yet been discovered and should be investigated further *in vitro* and *in vivo* through comprehensive research on human trials. Furthermore, the antibacterial and antiviral activity of cinnamaldehyde and terpinen-4-ol have also been well documented in the research articles [43,44]. Cinnamaldehyde was found to be not toxic to vertebrates or invertebrates in an *in vivo* trial conducted by Alves et al. [45]. Subash Babu et al.[46] found that oral administration of cinnamaldehyde in induced diabetic rats produces a significant antihyperglycemic effect and simultaneously help to reduce total cholesterol and triglyceride levels effect. In addition, several studies have demonstrated terpinen 4-ol's usefulness as an anticancer agent [47,48]. Clinical trials on the use of terpinen 4-ol for treatment of Demodex Blepharitis eye disease were described in the previous reports. Furthermore, Freires et al.[49] stated that menthol and eugenol demonstrate antibacterial properties specific to cariogenic bacteria. According to the clinical trials database, menthol may be useful as an antipruritic, an analgesic, and an anticancer agent (Table 4).

According to the aforementioned/ discussed clinical trials and experiments, it is evident that the study can provide insight into a therapeutic relationship between active components of essential oil used in SWASTIIK and their potential benefits. It is important to note that each oil has a different application and health effect. Some of oil also showed toxicity in certain amount, hence it is essential to appropriately implement the devised methodology for separation of the oil from the treated water, where required. A suitable dosage of the oil may also be selected or with a specific oils mix, depending on its nature and requirements of the individual or environment. Formulations of different essential oils may be useful in this respect in order to meet the expectations of both disinfection and health benefits. It is

obligatory to perform detailed *in vitro* and *in vivo* long-term studies in this regard, and controlled separation of oils may be recommended till medical experts validate and consider the health benefits. It may be noted that studying Ayurveda enhances the effectiveness of the SWASTIIK technology and highlights the relevance of traditional knowledge in water treatment and human wellness. The use of natural compounds such as thymol, eugenol, cinnamaldehyde etc in water disinfection can be seen as an extension of the traditional knowledge of Ayurveda, utilizing the inherent properties of essential oil for practical applications in promoting health and well-being.

Conclusions

Molecular docking study indicated that eugenol and thymol exhibited the highest efficacy and demonstrated potential activity against SARS-CoV-2 and multivirulent *E. coli*. However, SARS-CoV-2 displayed superior performance with a high binding affinity value of -7.2 kcal/mol, surpassing multi-virulent *E. coli*, which had a potential energy of -4.5 kcal/mol. The ADMET studies showed that all active compounds exhibited good absorption properties. *In silico* techniques can be advantageous in the selection of innovative essential oils, suitable for drinking water treatment, through virtual screening. The present study provides a useful and innovative strategy to save time and resources and for speedy classical microbiological studies especially used in water disinfection.

Acknowledgement

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Figures:

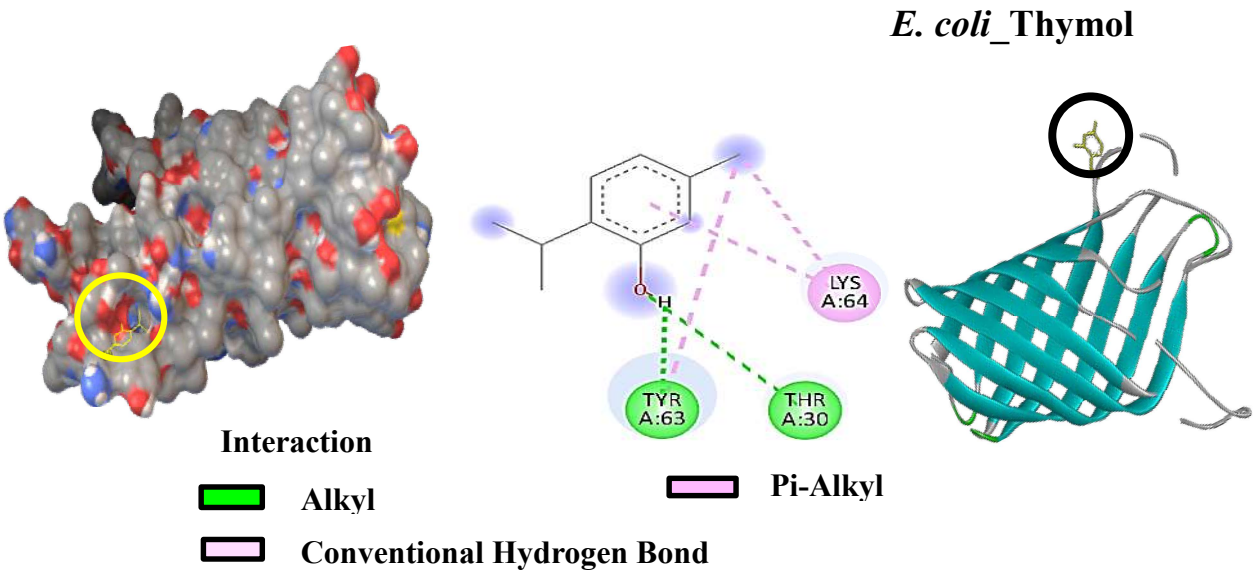


Fig. 1 Molecular interactions of ligand thymol with multi virulent *E. coli*

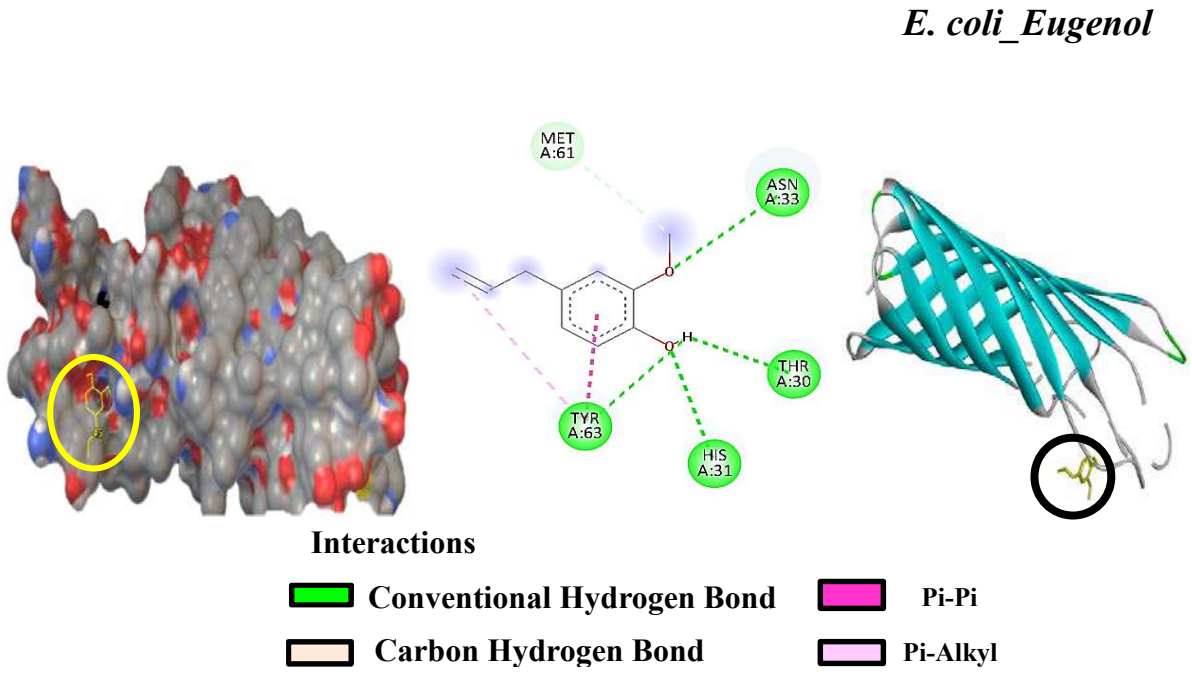


Fig. 2 Molecular interactions of ligand eugenol with multi virulent *E. coli*

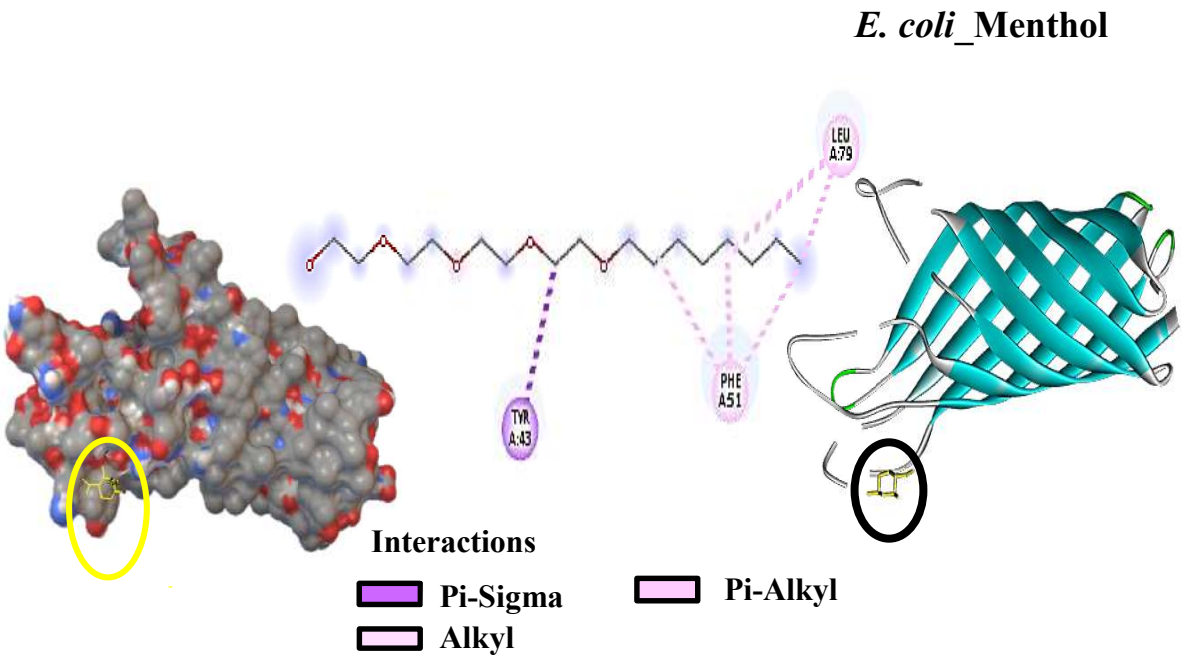


Fig. 3 Molecular interactions of ligand menthol with multi virulent *E. coli*

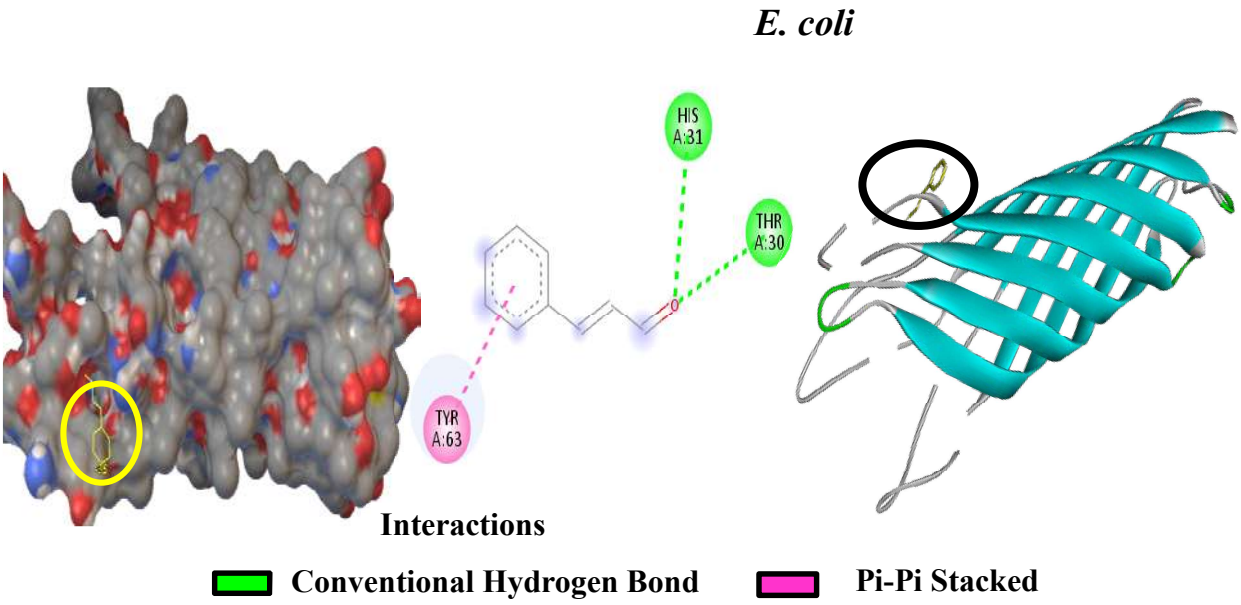


Fig. 4 Molecular interactions of ligand cinnamaldehyde with multi virulent *E. coli*

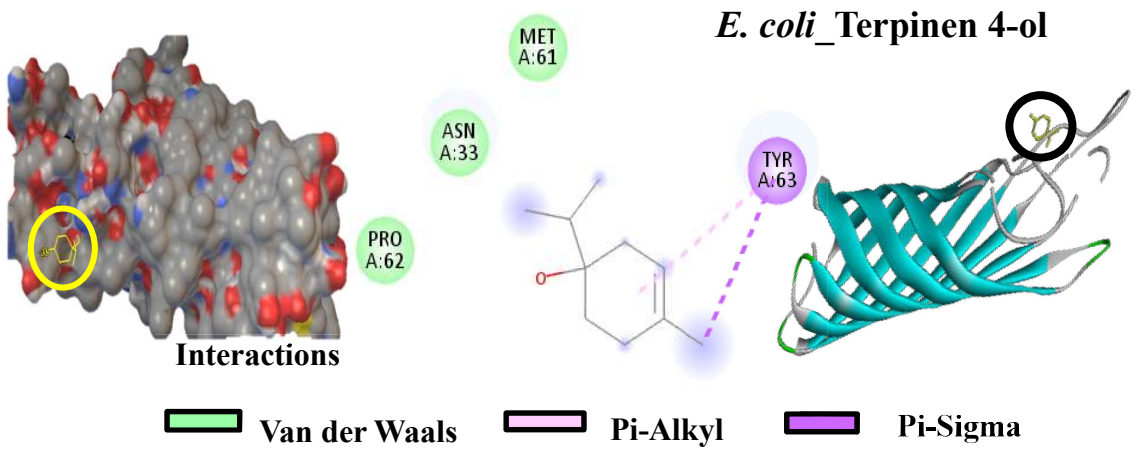


Fig. 5 Molecular interactions of ligand terpinen 4-ol with multi virulent *E*

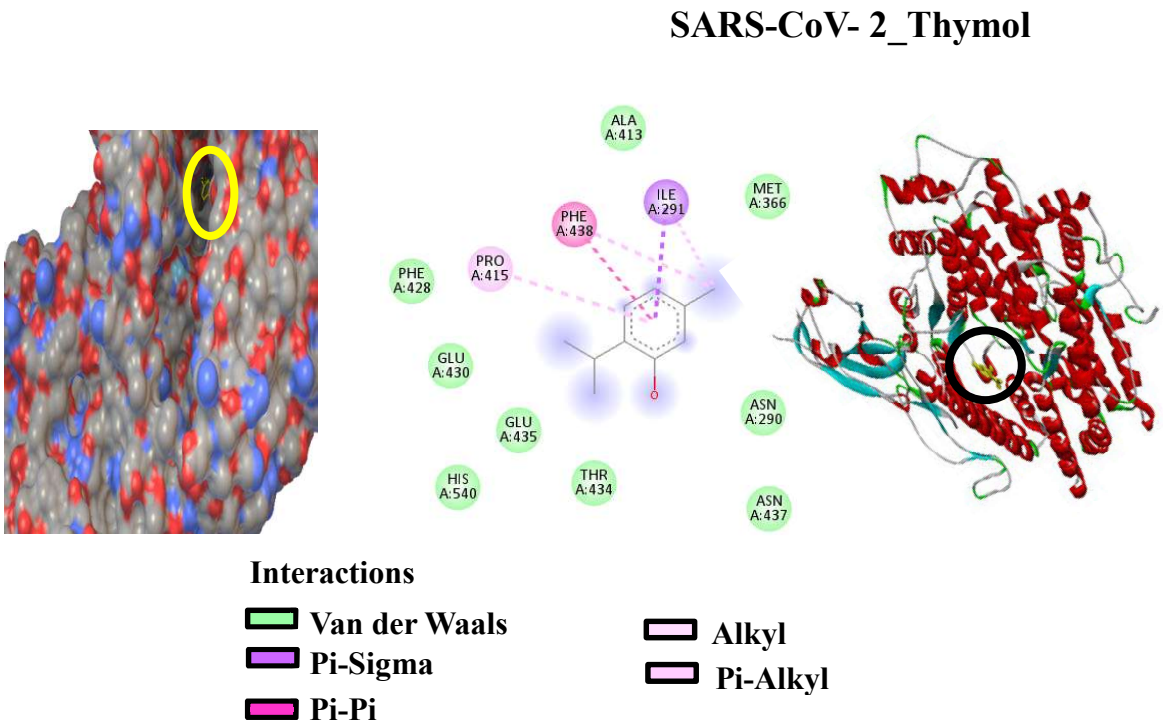


Fig. 6 Molecular interactions of ligand thymol with SARS-CoV-2

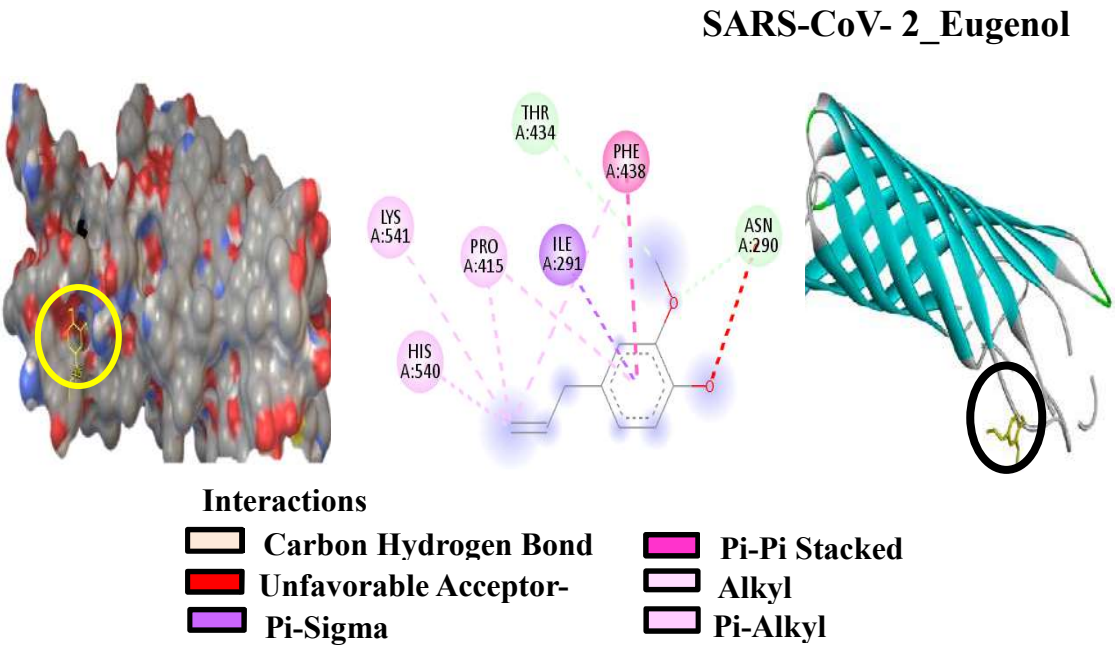


Fig. 7 Molecular interactions of ligand eugenol with SARS-CoV-2

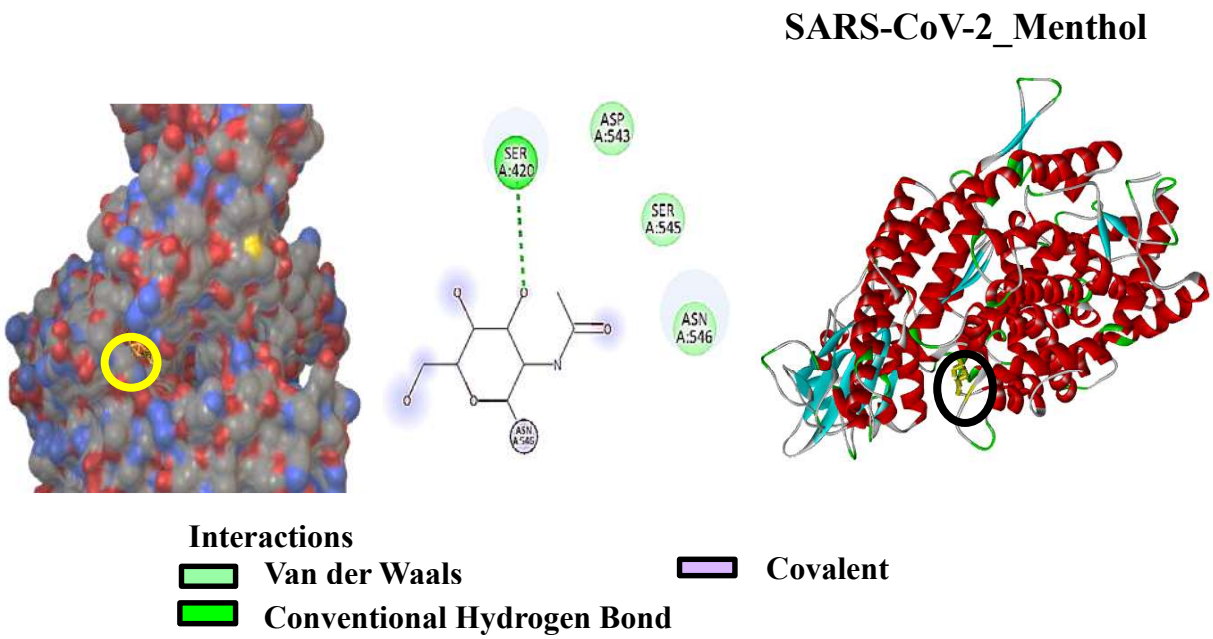


Fig. 8 Molecular interactions of ligand menthol with SARS-CoV-2

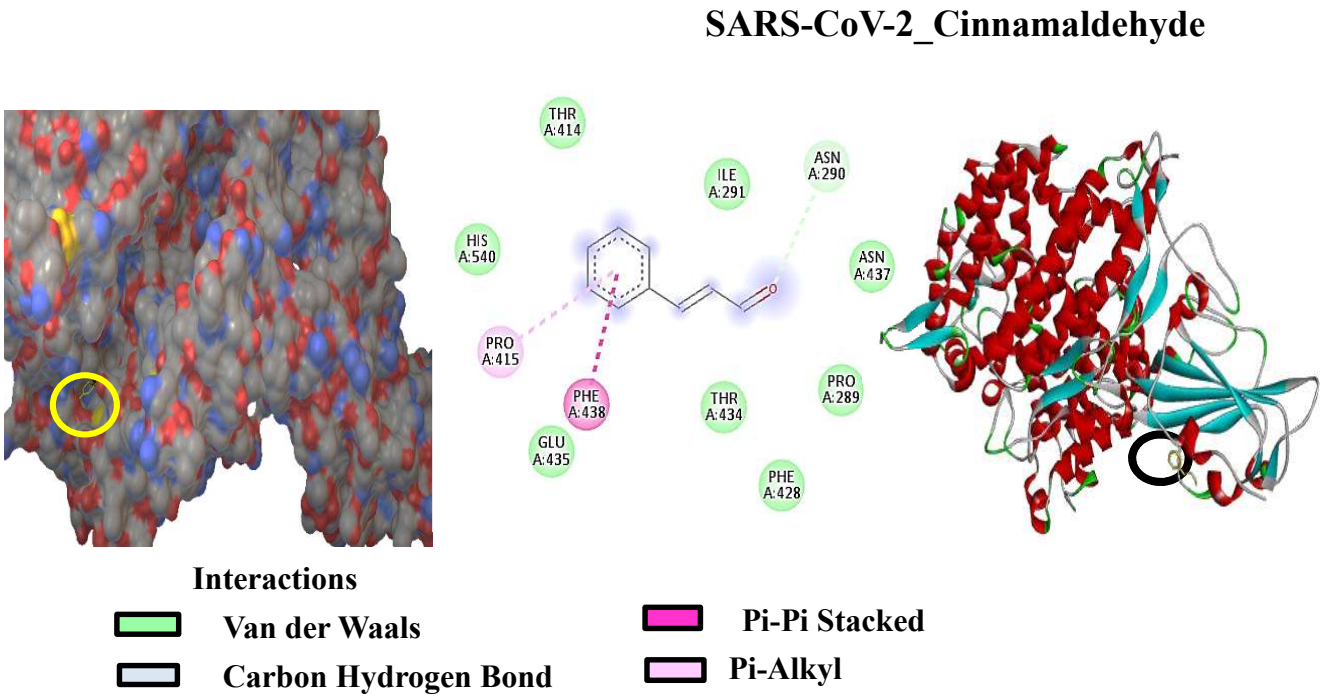


Fig. 9 Molecular interactions of ligand cinnamaldehyde with SARS-CoV-2

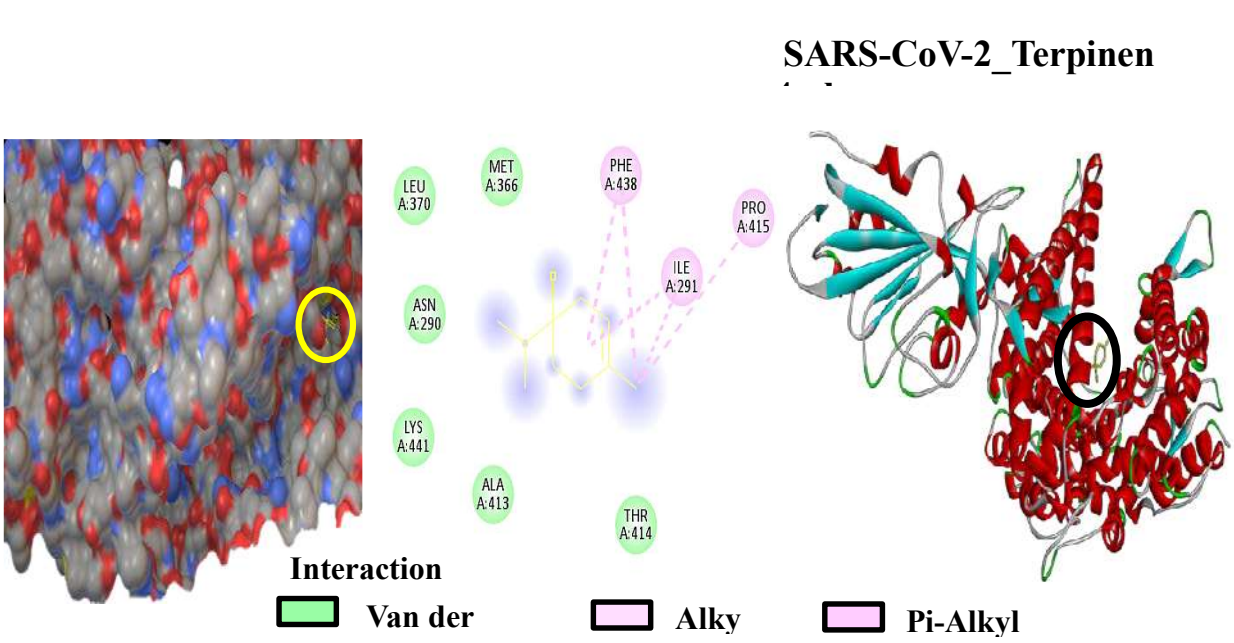


Fig. 10 Molecular interactions of ligand terpinen-4-ol with SARS-CoV-2

Tables:

Table 1: Molecular properties and structure of active components

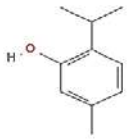
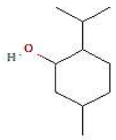
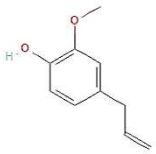
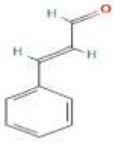
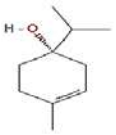
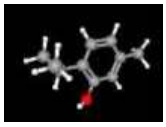
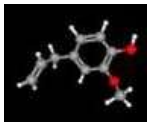
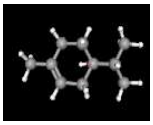
Properties	Thymol	Menthol	Eugenol	Cinnamaldehyd	Terpinen 4-ol
PubChem ID	6989	1254	3314	637511	2724161
Molecular Formula	C10H14O	C10H20O	C10H12O2	C9H8O	C10H18O
Molecular Weight	150.22	156.26	164.2	132.16	154.25
Log P	3.3	3	2	1.9	2.2
2 D structure					
3 D structure					

Table 2: Molecular interactions of active component with Multi virulent *E. coli* and SARS-CoV-2

	Organism Name: Multivirulent *E. coli* (PDB ID 1BXW)				
			Type of Interaction		
Compound Name	**Affinity (kcal/mol)**	**Hydrogen Bond**	**Pi alkyl**	**Van dar Waals**	**Pi Sigma**
Thymol	-3.9	Tyr 63, Thr 30	Lys 64	-	-
Menthol	-3.4	-	Liu 79, Phe51	-	Tyr 43
Eugenol	-4.5	ASN 33, Thr 30, His 31	Tyr 63	-	-
Cinnamaldehyde	-3.8	His 31, Thr 30	-	-	Tyr 63
Terpinen 4-ol	-3.9	-	-	Met 61, Asn 33, Pro 62	Tyr63
Organism Name: SARS-CoV-2 (PDB ID: 6M0J)					
			Residual Interaction		
Compound Name	**Affinity (kcal/mol)**	**Hydrogen Bond**	**Pi alkyl**	**Van dar Waals**	**Pi Sigma**
Thymol	-7.2	-	Pro 415	Ala413, Met 366, Asn 290, Asn 437, Thr 434, His 540, Glu 435, Phe 428	Ile 291
Menthol	-5.2	Ser 420	-	Asp 543, Ser 545, Asn 546	-
Eugenol	-6.5	-	His A540, Lys A 541	-	ILE 291
Cinnamaldehyde	-6.5	Asn 290	Pro 415	Thr 414, His 540, Glu 435, Thr 434, Phe 428, Pro 289, Asn 437, Ile291	-
Terpinen 4-ol	-5.8	-	Phe 438, Pro 415	Met 366, Leu 370, Asn 290, Lys 441, Ala 413, Thr 414	-

Table 3: ADMET and toxicity analysis of active compounds

Properties	Thymol	Menthol	Eugenol	Cinnamaldehyde	Terpinen 4-ol
Blood Brain Barrier (BBB)	5.53	6.25	2.25	1.26	5.53
CaCO2	50.8	39.49	46.88	23.78	50.8
CYP_3A4 inhibition	Non	Inhibitor	Non	Non	Non
Human Intenstinal Absorption (HIA (%))	100	100	96.77	100	100
Plasma protein binding (PPB)	100	100	100	52.89	100
P glycoprotein (Pgp) inhibition	Non	Non	Non	Non	Non
	Toxicity				
AMES Test	Mutagen	Mutagen	Mutagen	Mutagen	Mutagen
Carcino_Mouse	Negative	Negative	Positive	Negative	Positive
Carcino_Rate	Negative	Positive	Positive	Negative	Negative

Table 4: Application/health benefits of active components of essential oils

Active Ingredients	Plausible application/health benefits	Exemplary Studies (PMID)	Clinical trials.gov.in
Thymol	antioxidant	29799283	Thymol on Netrin-1 on Obese Patients NCT05427721 (Phase 2)
	anti-inflammatory	26553130, 24785965	A Study to Evaluate the Effect and Tolerance of Musclin™ (Thymol) in Healthy Subjects NCT03767504
	Antimicrobial, antiviral	26546890, 36093954	-
	Antidiabetic, antiobesity, Neuropharmacological, antihyperglycemic and hypolipidemic	26680107, 29635674, 24486068, 29069225, 26007642	-
Menthol	Antipruritics, Pain and analgesia	29524352, 36277488	1. Addiction and Behavior Related to Menthol Cigarette Substitutes NCT04844762 2.Menthol for PDT Pain Relief NCT02984072 (Phase 4)
	Anticancer	35467269, 35950244	Topical Menthol Application in Chemotherapy-Related Peripheral Neuropathy in Patients with Breast CancerNCT05429814
Eugenol	Preventing chronic diseases such as cancer, inflammatory reactions, and other conditions	27771920	Using a Mouthwash Containing Propolis, Clove Oil and Chlorhexidine to Improve the Caries Risk of high-risk Patients NCT03553628
	Anti-inflammatory, anti-oxidant	30425782	Endodontic Medications for Irreversible Pulpitis: Articaine or Eugenol NCT03472456
	Antiviral	36636828, 36450916, 36151910,35684363, 35836934	-
Cinnamaldehyde	Antioxidant	31735467	Safety and tolerability of cinnamaldehyde in orabase for oral candidiasis treatment: phase I clinical trial (PMID: 35305150)
	Antiviral, antibacterial. antifungal, anticancer, anti-diabetic	35508082, 34722758, 27771918, 17140783	The Effect of Capsaicin and Cinnamaldehyde on Intestinal Permeability. NCT01667523
Terpinen 4-ol	antibacterial, antifungal, antiviral	32630600, 31111285, 19843207, 35744913	Demodex Blepharitis Treatment Study NCT01647217 (Phase 1)
	Anticancer, anti-arthritic	35322742, 28781645, 27275783, 35320496	-

References:

1. V.V. Ranade, V.M. Bhandari, S. Nagarajan, V.P. Sarvothaman, A.T. Simpson, Hydrodynamic cavitation: Devices, Design, and Applications. Disinfection of water, Wiley 145, 2022.
2. M. Cerecedo, C. Dopazo, Rafael Gomez-Lus., Ultrason. Sonochem., 48, 71, 2018.
3. WHO, Quantitative microbial risk assessment: application for water safety management, WHO Press., 187, 2016 (http://www.WHO.).
4. WHO, WHO guidelines for drinking water quality: first addendum to the fourth edition, 2017.
5. M. Elfaky, M. Abdel-Hamid, E. Khalifa, W.A. Alshareef, R.A. Mosbah, S.T., Elazab, M.M. Ghoneim, M.M. Al-Sanea, M.M. Bendary, Appl. Microbiolo. Biotech. 106, 691, 2022.
6. T.P.K. Murthy, T. Joshi, S. Gunnan, N. Kulkarni, V.P. Kumar, S.B. Gowrishankar, Curr. Res. Green Sustain. Chem. 4, 10059, 2021.
7. H.N. Tran, G.T. Le, D.T. Nguyen, R.S. Juang, R.J. Bhatnagar, E.C. Lima, H.M.N. Iqbal, A.K. Sarmah, H.P. Environ Res. 193, 110265, 2021.
8. M.B. Mane, V.M. Bhandari, V.V. Ranade, J. Water Process Eng. 43, 102280, 2021.
9. M.B. Mane, V.M. Bhandari, K. Balapure, V.V. Ranade, Ultrason. Sonochem. 61, 104820, 2020.
10. M.B Mane, V.M Bhandari, K. Balapure, V.V. Ranade. Ultrasonics Sonochemistry, 69, 105272, 2020
11. Dixit, D., Bhandari, V.M., Mane, M.B., Balapure, K. Biochem. Eng. J. 187, 108631, 2022.
12. P. Jain, V.M. Bhandari, K. Balapure, J. Jena, V.V. Ranade, D.J. Killedar, J. Environ. Manage. 242, 210, 2019.
13. V.V. Ranade, V.M. Bhandari, Industrial Wastewater Treatment, Recycling, and Reuse, in: Ind. Wastewater Treat. Recycl. Reuse, Elsevier, 1–80, 2014.
14. F. Nazzaro, F. Fratianni, R. Coppola, V. Feo, pharmaceuticals (Basel).10, 86, 2017.
15. X.Y.Meng, H.-X. Zhang, M. Mezei, M. Cui, Curr Comput Aided Drug Des. 7, 146, 2011.
16. M.A. Mbock, R.G. Kamkumo, R. Shukla, W.F. Fouatio, P.V.T. Fokou, F.N. Tsofack, C.D. Noussi, R. Fifen, A.E. Nkengfack, T.R. Singh, B.L. Ndjakou, N. Sewald, F.F. Boyom, J.J.E. Ngang, O. Boyomo, Dimo, J. Ethnopharmacol. 307, 116209, 2023.
17. L. Ralte, L. Khiangte, N.M. Thangjam, A. Kumar, Y.T. Singh, Sci. Rep. 12, 3395,

2022.

18. V. Puthanveedu, K. Muraleedharan, Struct. Chem. 33, 1423, 2022.
19. S.A. Kulkarni, S.K. Nagarajan, V. Ramesh, V. Palaniyandi, S.P. Selvam, T. Madhavan, J. Mol. Struct. 1221, 128823, 2020.
20. M. Aliye, A. Dekebo, H. Tesso, T. Abdo, R. Eswaramoorthy, Y. Melaku, Ocimum cufodontii. Sci. Rep. 11, 10101, 2021.
21. G.M. Morris, H. Ruth, W. Lindstrom, R.K. Belew, D.S. Goodsell, A.J. Olson, J. Comput. Chem. 30, 2785, 2009.
22. T. Hou, S. Sana, H. Li, Y. Xing, A. Nanda, Vasudeva Reddy Netala, Zhijun, Sankar Sana, Z. S., Li, H., Y. Xing, A. Nanda, V. Reddy Netala, Z. Zhang, Food Bioscience 47, 101716, 2022.
23. M.Y.Wu, D.Q. Dai, H. Yan, H. Proteins Struct. Funct. Bioinforma. 80, 2137, 2012.
24. V. Jani, S. Koulgi, V.N. Uppuladinne, U, Sonavane, R, Joshi, ChemRxiv. 2020.
25. F. Li, W. Li, S.C. Farzan, M. Harrison, Science 309, 1864, 2005.
26. M. Sahlan, L.K. Dewi, D.K. Pratami, K. Lischer, H. Hermansyah, Int. J. Technol. 14, 387,2023.
27. F. Benencia, M.C. Courreges, Phytother Res. 14, 1099, 2000.
28. A. Terstappen, Trends Pharmacol. Sci. 22, 23, 2001.
29. F. Nazzaro, F. Fratianni, L. De Martino, R. Coppola, V. De Feo, Pharmaceuticals. 6, 1451, 2013.
30. G. Sahar Elbager, Abdelrahman H. Abdelmoneim, et al., ChemRxiv, 12235181, 2020.
31. Finch and Pillans, Aust Prescr 37, 137–139, 2014.
32. A. Kowalczyk, M. Przychodna, S. Sopata, A. Bodalska, I. Fecka, Molecules. 25, 4125, 2020.
33 M. Sun, R. Yao, Y. You, S. Deng, W. Gao, Front. Environ. Sci. Eng. 1, 95, 2007.
34. L. Deng, M. Taxipalati, P. Sun, F. Que, H. Zhang, Colloids Surfaces B: Biointerfaces, 136, 859, 2015.
35. K. Rajamanickam, T. Rathinavel, V. Periyannan, S. Ammashi, S. Marimuthu, M. Nasir Iqbal, J. Biomol. Struct. Dyn. 7386, 2022.
36. E. Zhou, Y. Fu, Z. Wei, Y. Yu, X. Zhang, Z. Yang, Fitoterapia 96, 131, 2014.
37. M. Rafiul Haque, S.H. Ansari, A.K. Najmi, M.A. Ahmad. Toxicol Mech Methods. 24,116, 2014.
38. I. Padhy, P. Paul, T. Sharma, S. Banerjee, A. Mondal, Life 12, 1795, 2022.
39. A. Hussain, K. Brahmbhatt, A. Priyani, M. Ahmed, T.A. Rizvi, C. Sharma, Cancer

Biother. Radiopharm. 26, 519, 2011.

40. J.N. Barboza, C. da Silva Maia Bezerra Filho, R.O. Silva, J.V.R. Medeiros, D.P. de Sousa, Oxid. Med. Cell. Longev. 3957262, 2018.
41. F. Naz, I. Khan, S. Baammi, A. Islam, J. Biomol. Struct. Dyn. 41, 10161, 2023.
42. J. H. Hoofnagle, J. Serrano, J.E. Knoben, V.J. Navarro, Hepatology 57,873, 2013.
43. A. Nag, R. Banerjee, S. Paul, R. Kundu, Computers in Bio Medicine 146, 105552, 2022.
44. A. Garozzo, R. Timpanaro, B. Bisignano, P.M. Furneri, G. Bisignano, A. Castro, Lett. Appl. Microbiol. 49, 806, 2009.
45. D.D.N. Alves, R.X. Martins E.D.S Ferreira, A.F. Alves, J.C. Andrade, T.M. De, Batista, J.G. Lazarini, L.S. Amorim, P.L. Rosalen, D.F. Farias, R.D. De Castro, Biomed Res. Int. 2305695, 2021.
46. P. Subash Babu, S. Prabuseenivasan, S. Ignacimuthu, Phytomedicine 14, 15, 2007.
47. W. Cao, R. Tian, R. Pan, B. Sun, C. Xiao, Y. Chen, Z. Zeng, S. Lei, Bioengineered 13, 8643, 2022.
48. K. Nakayama, S. Murata, H. Ito, K. Iwasaki, M.O. Villareal, Y.W. Zheng, H. Matsui, H. Isoda, N. Ohkohchi. Oncol. Lett. 14, 2015, 2017.
49. I.A. Freires, C. Denny, B. Benso, S.M. De Alencar, P.L. Rosalen, Molecules, 20, 7329, 2015.