GMR
The Original

IN SILICO ELUCIDATION OF THE ANTI-INFLAMMATORY SYNERGISTIC ACTIVITY OF TERMINALIA CHEBULA AND VIRGIN COCONUT OIL BIOACTIVE IN MODULATING ACUTE LUNG INJURY PATHWAYS

Dr. Kamalakannan Solaiyappan[1], Dr. Sowjanya Bandlamudi[2*], Dr. Vaijayanthimala Ponnusamy[3], Dr. Deepalakkshmi Balakrishnan[4], Dr. Arathi M S[5], Dr. Archana Rajasundaram[6]

[1]Tutor, Department of Anatomy, Shri Sathya Sai Medical College and Research Institute, SBV Chennai Campus, Sri Balaji Vidyapeeth (Deemed to be university), Pondicherry - 607 402, India
[2]Professor & Head, Department of Anatomy, Shri Sathya Sai Medical College and Research Institute, SBV Chennai Campus, Sri Balaji Vidyapeeth (Deemed to be university), Pondicherry - 607 402, India
[3]Associate Professor, Department of Anatomy, Sri Lalithambigai Medical College and Hospital, Dr. M.G.R Educational and Research Institute (Deemed to be university), Chennai 600095 Tamil Nadu, India.
[4]Tutor, Department of Anatomy, Faculty of Allied Health Sciences, Sri Lalithambigai Medical College and Hospital, Dr. M.G.R Educational and Research Institute (Deemed to be university), Chennai 600095 Tamil Nadu, India.
[5]Professor, Department of Anatomy, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam, Chennai 603103, India
[6]Professor, Department of Anatomy, Sree Balaji Medical College and Hospital, Bharath University, Chromepet, Chennai 600 044, India.
*Corresponding Email ID: sowjanya.vneni@gmail.com

ABSTRACT

Background: There are few treatment options for acute lung injury (ALI), a potentially fatal inflammatory pulmonary condition. The current research work used an integrated In-silico method to target important inflammatory mediators implicated in ALI in order to examine the synergistic anti-inflammatory potential of Terminalia chebula and Virgin Coconut Oil (VCO) bioactives.

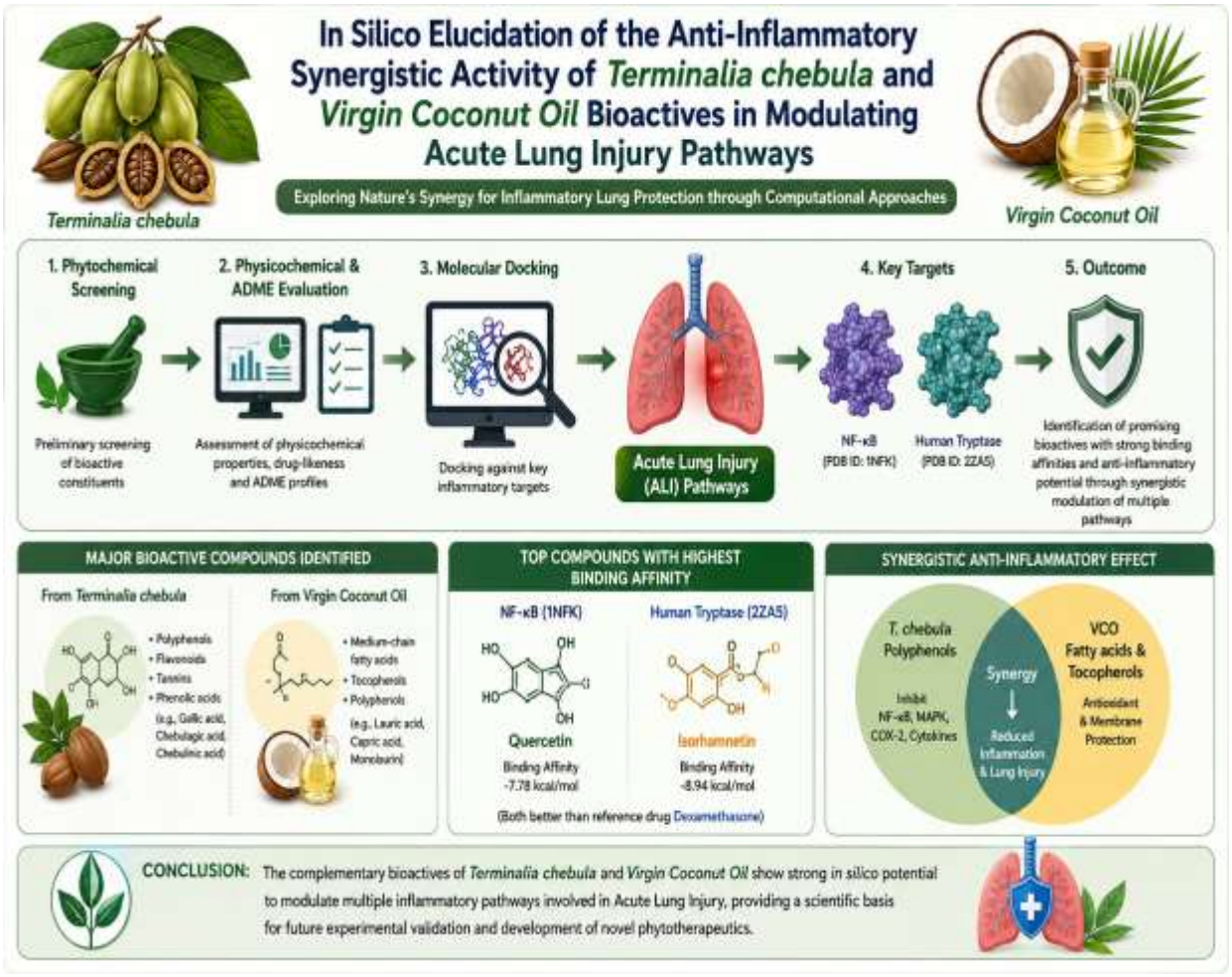
Method: To determine the primary bioactive components, preliminary phytochemical screening, physiochemical characterisation, and ADME profiles of a few chosen phytochemicals were assessed. Dexamethasone was utilised as the reference medication in molecular docking investigations against human tryptase (PDB ID: 2ZA5) and nuclear Factor Kappa-B (NF-κB; PDB ID: 1 NFK).

Key Findings: The presence of polyphenols, flavonoids, tannins, medium-chain fatty acids, and tocopherols with established anti-inflammatory properties was verified by GCMS results and phytochemical analysis. ADME analysis identified quercetin, kaempferol, isorhamnetin, gallic acid, ferulic acid, caffeic acid, linoleic acid, oleic acid, capric acid, monolaurin and tocopherols as promising pharmacologically active components. Molecular docking demonstrated strong binding affinities of quercetin towards NF-κB (-7.78 kcal/mol) and isorhamnetin towards human tryptases (-8.94 kcal/mol), surpassing the reference drug dexamethasone.

Conclusion: These results imply that VCO and T. chebula have complimentary bioactive components that can work in concert to modulate certain inflammatory pathways linked to ALI. In order to create new phytopharmaceutical treatments for inflammatory lung conditions, our work offers a scientific foundation for more molecular dynamics, experimental and clinical research.

KEYWORDS: Virgin coconut oil, Terminalia chebula, In-silico, Acute lung injury, anti-inflammatory

GRAPHICAL ABSTRACT



1. INTRODUCTION

Acute lung injury (ALI) is a type of inflammatory lung injury which causes a severe clinical syndrome involving the injury and inflammation of the alveolar epithelium, pulmonary edema, and infiltration of inflammatory cells with an "impairment of gas exchange.[1,2] ALI and its extreme form, ARDS, are responsible for a significant number of cases and deaths caused by pulmonary disease. Though there has been advancement in the management of patients in the intensive care unit, there are few options for effective drug treatment due to the complex nature and multiple components of this syndrome.[3,4] The cytokine storm theory describes the primary behavioural components of ALI as the excessive damage and inflammation of lung epithelium which are the advanced forms of oxidative stress and inflammation, and the dysfunction of the vascular endothelium stemming from the activation of multiple inflammatory pathways such as TNF-α, IL-1β, IL-6, NF-κB, COX-2, MAPK, and NLRP3 inflammation among other pathways.[5,6,7] The unrelenting activation of these mediators results in injury to the tissues, damage to the epithelial cells of the alveoli, and the lung progressively becomes unable to function.[8]

Natural products are excellent stimuli when aiming to uncover innovative treatments for inflammatory diseases due to their multiple active components and favourable safety profiles. T. chebula Retz. (Combretaceae) (Haritaki) is globally a highly valued medicinal plant within the ancient medical practices of Ayurveda, Siddha and Unani among others.[9,10] The fruit of T. chebula has been reported to elicit multiple actions such as antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, cardioprotective and immunomodulatory.[11,12,13] The diverse actions of this fruit have been largely attributed to its unique phytochemistry which includes hydrolysable tannins, phenolic acids, flavonoids, triterpenes and glycosides. Chebulagic acid, chebulinic acid, gallic acid, ellagic acid, corilagin, punicalagin, terchebulin, terflavin A and terflavin B are some of the bioactive constituents that have been shown to inhibit the synthesis of inflammatory mediators and reduce the oxidative burden by regulation of the NF-κB and MAPK pathways.[14]

VCO is obtained from the raw flesh of coconuts without the aid of solvents and has been used as a functional food, and more recently, as a therapeutic agent.[15] Compared to conventional coconut oil, VCO has a higher concentration

of active constituents that include medium chain fatty acids, tocopherols, polyphenols, and several other antioxidants.[16,17] Lauric, capric, and caprylic acids as well as myristic and oleic acids along with linoleic acid and monolaurin make up the primary constituents of VCO. VCO has been shown to possess many diverse actions including anti-inflammatory, antioxidant, antimicrobial and immunomodulatory.[18] Medium chain fatty acids alter the production of inflammatory cytokines, and improves membrane stability and reduces oxidative damage while tocopherols aid in scavenging free radicals and protection of cells.[19,20]

Recent studies on synergistic phytotherapy combinations of natural bioactive compounds that exert synergistic therapeutic effects through the modulation of multiple molecular targets have been made. Given the phytochemical synergies of T. chebula and VCO, their synergistic use may give a more effective approach to managing the diverse inflammatory pathways of ALI.[21] The polyphenols of T. chebula may inhibit some inflammatory cascade pathways. In contrast, the fatty acids and tocopherols in VCO may assist in permeating cell membranes, increasing bioavailability and providing a better antioxidant defense.[22] This combination may be more effective and may also have a better safety profile. Additionally, the use of computational technologies has revolutionized the early phases of drug discovery. The application of In Silico technologies has made it possible to evaluate and rank a large number of promising bioactive compounds in a short time span and relatively low cost, based on their physicochemical parameters, pharmacokinetics, and toxicodynamics. Additionally, the potential molecular interactions and the target-pathway relationships of the compounds can be analysed. The physicochemical evaluation has a large impact on the absorption and distribution of the bioactive compounds. It also predicts the metabolism and excretion in the case of toxicological evaluation.[23] The use of molecular docking helps explain the binding and interactions of the bioactive compounds with the targets of the disease related to the pathways and helps hypothesize the potential mechanisms of the compounds.

The current study aimed at detailed phytochemical screening and analysis of the physical and chemical properties of T. chebula and VCO, and later an In Silico study of the major bioactive compounds. An assessment was made on the physical and chemical properties and the behaviour and the drug-like characteristics of the key phytochemicals and the assessment was made to explore and identify lead compounds that possess the ability to regulate inflammatory pathways of ALI. The outcome of this study may help in the development of new models of Phytopharmaceuticals based on T. chebula and VCO that help in the treatment of Inflammatory pulmonary diseases.

2. MATERIALS AND METHODS

2.1 Preliminary Phytochemical Screening

The qualitative phytochemical screening of ethanolic extracts of T. chebula and VCO was carried out using standard procedures. For the detection of alkaloids, the test employed was Mayer's test; for steroids, the test employed was the Liebermann-Burchard test; for flavonoids, the test was the hydrochloric acid test; for saponins, it was the foam test; for phenols and tannins, the test was the ferric chloride test; for carbohydrates, the Molisch's test; for cardiac glycosides and glycosides, the Keller-Killiani test; for terpenoids, the Salkowski test; for proteins, the Xanthoprotein test; the Copper Acetate test was employed for fatty acids; and for tocopherols, the Emmerie-Engel reaction was employed.[24,25,26]

2.2 Physicochemical Evaluation

The determination of the physicochemical parameters of T. chebula powder sample was done as per standard procedure. The parameters determined include loss on drying, total ash, acid insoluble ash, water- and alcohol-soluble extractive values, pH, foreign matter content, and tannin content. The characterization of VCO was done based on the organoleptic properties and the physicochemical parameters of colour, odour, moisture content, refractive index, and acid value.[27,28]

2.3 GC-MS Analysis

The biochemicals of T. chebula and VCO were analysed using the Gas Chromatography-Mass Spectrometry (GC-MS) system that was equipped with a capillary column and a mass selective detector. The carrier gas was He, and the injector and detector temperature were set based on the manufacturer's suggested values. The mass spectra were acquired in the electron ionization mode for a selected mass range. The identification of the compounds was done by comparing the acquired mass spectra to those in the NIST 20 mass spectral library. Characterization of the identified compounds was carried out using retention times, molecular formulae, molecular weights, and similarity indices.[29,30]

2.4 In Silico Physicochemical and ADME Analysis

For the major phytoconstituents of T. chebula and VCO, the major phytoconstituents were run through some in silico studies. The in silico studies included calculating Log P, TPSA, and the number of hydrogen bond donors and acceptors, the number of rotatable bonds, violations of Lipinski's rule, penetration of the blood-brain barrier, and other parameters. The study also included ADME properties such as intestinal absorption, skin permeability, and

bioavailability, and the potential for the components to interact with and be substrates of the P-glycoprotein and CYP3A4 and CYP2D6 and the potential to induce hepatotoxicity.[31]

2.5 Molecular Docking Analysis:

The binding affinities of the active constituents were computed for the Nuclear Factor Kappa-B (Nf-Kb) P50 Homodimer and the Human Tryptase with a potent non-peptide inhibitor using AutoDock Tools 1.5.7. The active sites of the target proteins were prepared for docking using Molegro Molecular Viewer. Water molecules and non-covalently" bound ligands were removed from the Nf-Kb P50 Homodimer and the Human Tryptase 3D structures that were obtained from the Protein Data Bank (PDB) with the access numbers 1NFK and 2ZA5, respectively. Co-factors and bound ligands were removed. The structures were saved in .pdbqt format after adding polar and non-polar hydrogens. The structures were charged. We used Chemdraw Professional to create 2D mol files of the non-toxic active components of T. chebula and VCO, which were then, minimized using the MMFF94 force field in Avogadro. Preliminary studies on docking using Autodock Tools were conducted to test the methodology. Native ligands were redocked to their native binding sites, and the validation process confirmed that the root-mean-square deviation (RMSD) between the experimental and predicted poses was ≤ 2 Å. The grid box was adjusted to bring the active binding site into better focus. The binding energies (ΔG) were calculated in kcal/mol, and the protein-ligand interactions were studied using Biovia Discovery Studio 21.0.[31,32]

Molecular docking was executed with AutoDock Tools 1.5.7 to evaluate the binding affinity of the active constituents to the active pocket of Lipoxygenase. The preparation of target proteins for docking was performed with the help of the Molegro Molecular Viewer. Water molecules, metal atoms, co-crystallized ligands, and other non-covalently bound molecules were removed from lipoxygenase. It was downloaded from the Protein Data Bank (PDB) with an accession number of 1N8Q. The charges were increased, and the structure was saved in pdbqt format after the addition of polar and the combination of non-polar hydrogens. The non-toxic active constituents of Triphala churna were drawn to make 2D mol files of ligands in Chemdraw Professional. These 2D mol files were subjected to energy minimization in Avogadro. The docking studies in Autodock Tools were also performed as these studies were verified by the re-docking of native ligands in their original binding sites. Validation of the docking studies was performed by calculating the RMSD of experimental and predicted ligand positions with the acceptance value of 2 Å. The grid box was resized to increase the active binding site coverage. The binding energies (ΔG) of the ligands were calculated in kilocalories per mole (kcal/mol), and the docking results were analysed in Biovia Discovery Studio 21.0.

3.1 Preliminary Phytochemical Screening

Phytochemical analysis of T. chebula (table 1) confirmed the presence of alkaloids, steroids, flavonoids, phenols, tannins, coumarins, terpenoids and proteins, and the absence of saponins, carbohydrates and cardiac glycosides. Since acute "lung injury is associated with the augmentation of oxidative stress and the resultant inflammatory response, the presence of a wide variety of phenolic compounds and tannins may be responsible for the antioxidant potential and may help to control the inflammatory response.

Phytochemical screening of VCO (table 2) showed the presence of flavonoids, phenolic compounds, tannins, terpenoids, steroids, glycosides, fatty acids, and tocopherols. The presence of tocopherols and phenolic compounds indicates the presence of potent oxidant scavengers. Furthermore, the presence of lauric acid, capric acid and caprylic acid may further be responsible for the anti-inflammatory and modulating response.

The presence of phenolic compounds and tannins in T. chebula and fatty acids and tocopherols in VCO may exert combined therapeutic effects for lung inflammatory conditions.

3.2 Physicochemical Evaluation of T. chebula

The physicochemistry of T. chebula demonstrates the quality characteristics of the product as indicated on Table 3. The loss on drying was 6.12% that demonstrates low moisture content with low probability of microbial contamination during storage. The total ash value of 2.85% indicates low inorganic impurities and improved quality of the raw material. The acid insoluble ash value of 1.30% indicates low siliceous contamination. The water-soluble extractive value was 62.32% which was higher as compared to the alcohol-soluble extractive value (40.91%). This shows the presence of water-soluble constituents such as tannins, gallic acid derivatives and hydrolysable poly phenols. The pH of the aqueous solution was recorded to be 3.8 indicating the acid nature of the plant material that was due to the presence of phenolic acids. It was also recorded that the tannin content was 54mg/100mg powder. This shows that T. chebula is a good source of tannin.

3.3 Physicochemical Evaluation of VCO

VCO appeared to be a colourless to pale-yellow liquid with a coconut odour (Table 4). The moisture content of VCO was between 0.10–0.18%, and indicated low susceptibility to oxidation and excellent stability during storage. The observed refractive index values at 1.448 - 1.450 confirmed that the VCO used in this study was of good quality as per the established standards. The acid value of 0.35 mg KOH/g showed low extent of hydrolytic degradation,

indicating good quality of the oil. It can therefore be concluded that the VCO used in this study was of good quality and preserved its natural bioactive constituents.

3.4 GC-MS Analysis

The GC-MS analysis revealed the presence of several bioactive compounds from T. chebula and VCO. The major compounds identified were fatty acid esters, long chain alcohols, and saturated fatty acid derivatives. Chromatographic analysis identified isopropyl myristate (tetradecanoic acid isopropyl ester) at 12.93 min, 270 Da with 91% similarity. A peak at 13.22 min was accounted for by n-pentadecanol and other long chain fatty alcohols (hexadecanol and tetradecanol) with 96% similarity. A peak at 13.29 min, with 94% similarity, was identified as hexadecanol (cetyl alcohol). Hexadecanoic acid (methyl palmitate) was present and was shown to elute at a retention time of 13.41 min with 270 Da and 89% similarity.

The compounds identified have been shown to have antioxidant, anti-inflammatory, and membrane-stabilizing activities and may have additional modulatory effects. The fatty acid esters and long chain alcohols are shown to enhance membrane permeability and may contribute to the biological activity of the formulation.

The phytochemical, physicochemical, and GC-MS analyses showed that the active ingredients present have the potential to work synergistically. It is therefore proposed that the combination of hydrolysable tannins from T. chebula, medium-chain fatty acids, and lipid-soluble antioxidants in VCO can be used to modulate inflammatory pathways in ALI.

3.5 In Silico Physicochemical Properties

The study analyzed thirty phytoconstituents of T. chebula and VCO. The study noted the following differences in the physicochemical properties of the compounds: molecular weight and lipophilicity, polarity, and blood-brain barrier (BBB) permeability (Table 5).

It was noted that hydrolysable tannins such as chebulagic acid, Chebulinic acid, punicalagin, terchebulin, terflavin A, terflavin B, and tannic acid had high molecular weights (MM) of 634 to 1701 Daltons and TPSA values of greater than 300 A^2. These hydrolysable tannins showed a number of Lipinski violations and low BBB permeability, indicating limited bioavailability. In contrast, gallic acid, ethyl gallate, kaempferol, and quercetin, isorhamnetin, ferulic acid, and caffeic acid, showed molecular weights less than 350 Daltons and had no Lipinski violations, indicating good drug-like qualities. Kaempferol, quercetin, and isorhamnetin, had log P values in the range of 1.9 to 2.3 and BBB permeability of about -1, indicating good membrane permeability.

From the compounds derived from VCO, lauric acid, capric acid, caprylic acid, oleic acid, linoleic acid, and monolaurin had low TPSA values and good lipophilic characteristics indicating good membrane permeability. It was noted that tocopherols had the highest bioavailability by virtue of their BBB permeability (0.739).

3.6 In-Silico ADME Analysis

As illustrated in Table 6, ADME analysis indicates that hydrolysable tannins have low values of predicted gastrointestinal absorption and are classified as P-glycoprotein substrates which may restrict their systemic availability. The bioavailability of hydrolysable tannins is in the range of 0.11 to 0.17, exhibiting low oral bioavailability. On the other hand, gallic acid, ethyl gallate, kaempferol, quercetin, isorhamnetin, and ferulic and caffeic acid showed gastrointestinal absorption and Caco-2 permeability values and bioavailability" scores ranging from 0.55 to 0.85, indicating good absorption and bioavailability.

ADME analysis of fatty acids showed that VCO and its fatty acid derivatives, such as lauric acid, capric acid, palmitic acid, stearic acid, oleic, and linoleic acid, exhibited high gastrointestinal absorption and bioavailability of 0.85. With respect to the pharmacokinetics of the other compounds that were analyzed in this study, it was predicted that most of the compounds are not hepatotoxic, with the exception of arjungenin, which was predicted to be hepatotoxic. None of the selected compounds were predicted to inhibit CYP2D6, therefore reducing the risks for in vivo drug-drug interactions.

Based on the results of the ADME analysis, the most promising compounds are kaempferol, quercetin, isorhamnetin, ferulic acid, caffeic acid, gallic acid, ethyl gallate, lauric acid, capric acid, linoleic acid, monolaurin, and tocopherols, due to their high "gastrointestinal absorption and bioavailability and predicted lack of toxicity. All of these compounds were therefore selected for further molecular docking studies to target and interact with the inflammatory pathways of ALI.

In-silico ADME prediction revealed significant difference in the pharmacokinetics of phytoconstituents (Table 6). Majority of hydrolysable tannins (chebulagic acid, chebulinic acid, corilagin, punicalagin, chebulanin, terchebulin, terflavin A, terflavin B, and tannic acid) were poorly absorbed in the GI tract, major P-glycoprotein substrates, with poor bioavailability (0.11–0.17), thus implying that although biologically active, they are poorly absorbed in vivo. On the other hand, flavonoids and phenolic acids including quercetin, kaempferol, isorhamnetin, gallic acid, ferulic acid, caffeic acid, and ethyl gallate showed high gastrointestinal absorption, good permeation through Caco-2 cells, good bioavailability (0.55–0.85), and non-hepatotoxicity. Also, majority of medium chain fatty acids in VCO such as lauric

acid, capric acid, caprylic acid, oleic acid, linoleic acid, and monolaurin had favourable ADME attributes with high gastrointestinal absorption and bioavailability. Majority of compounds did not inhibit CYP3A4 and CYP2D6 enzymes, thus posing less risk of drug–drug interaction, except arjungenin which was predicted to be hepatotoxic. Overall, the ADME profiling suggested that quercetin, kaempferol, isorhamnetin, gallic acid, ferulic acid, caffeic acid, lauric acid, capric acid, linoleic acid, monolaurin

3.7 Molecular Docking Analysis:

This molecular docking study aimed to understand how the non-toxic phytoconstituents of T. chebula and VCO bind, interact, and potentially inhibit two key inflammatory biomolecules, namely, Nuclear Factor Kappa-B (NF-κB; PDB ID: 1NFK) and Human Tryptase (PDB ID: 1NFK and 2ZA5). Parameters such as binding affinity (kcal/mol), inhibition constant (Ki), and hydrogen bond interactions were evaluated to determine the relative anti-inflammatory potential of the tested phytochemicals. The findings are presented in Tables 7 and 8. The 2D and 3D representations of the docking studies of the non-toxic phytoconstituents of T. chebula and VCO against NF-κB and Human Tryptase are illustrated in Figures 1 to 30.

3.7.1 Docking against Nuclear Factor Kappa-B (NF-κB, PDB ID: 1NFK)

From the docking studies of NF-κB (PDB ID: 1NFK), it was found that there was no uniformity in the binding affinities of the phytoconstituents (Table 7). Quercetin was found to be the most strongly interacting phytoconstituent with NF-κB, with a binding affinity of -7.78 kcal/mol and an inhibition constant of 2.0 nM. Quercetin was also found to form six hydrogen bonds with NF-κB at the following residues: SER40, SER34, GLY14, SER25, ARG18, and GLY28.

Linoleic acid showed excellent binding with a docking score of -7.34 kcal/mol, an inhibition constant of 4.17 nM, and formed interactions with HIS26 and ARG18. The binding affinity of isorhamnetin was -7.32 kcal/mol, and a Ki of 4.32 nM, with formation of hydrogen bonds with CYS78, LYS76, ALA97, and VAL93. Oleic acid and Kaempferol showed binding affinities of -7.10 kcal/mol, and inhibition constants of 6.28 nM, and 6.22 nM, respectively. Kaempferol interacted with GLY95, CYS78, LYS76, and ASN98, while oleic acid interacted with HIS26 and ARG18. Among the phenolic acids, caffeic acid showed a better binding score of -6.20 kcal/mol than ferulic acid (-6.04 kcal/mol), and gallic acid (-5.81 kcal/mol). Caffeic acid interacted with HIS26, ARG18, and GLY28, while ferulic acid interacted with LYS234, LYS203, ASN209, and LYS209. Gallic acid interacted with LYS234, LYS203, SER202, ASN209, and SER208, and although its docking score is the lowest, it suggests the most stabilization of all the phenolic acids. The fatty acids of VCO showed moderate binding. Capric acid, tocopherols, caprylic acid, and lauric acid showed binding affinities of -6.15, -6.13, -5.88, and -5.85 kcal/mol, respectively. Monolaurin, and ethyl gallate, showed the weakest binding affinities of -4.98 and -4.47 kcal/mol, respectively.

The reference drug dexamethasone had a derived binding strength of −5.61 kcal/mol with an inhibition constant of 76.6 nM and formed hydrogen bonds with ASN62 and ARG13. Interestingly, a number of phytoconstituents including quercetin, linoleic acid, isorhamnetin, kaempferol, oleic acid, caffeic acid, capric acid, ferulic acid, and tocopherols, had better docking scores than dexamethasone suggesting that these may be better NF-κB inhibitors.[33]

3.7.2 Docking against Human Tryptase (PDB ID: 2ZA5)

From the phytoconstituents examined, numerous compounds showed a strong binding affinity toward human tryptase, as evidenced by the docking study (Table 8). Isorhamnetin had the best binding affinity of all of the compounds examined, with a value of -8.94 kcal/mol and interactions with ASP49, LYS51, VAL46, PRO48, HIS80, and ILE77. This was the second best binding affinity and was accomplished by quercetin (-8.86 kcal/mol) and kaempferol (-8.75 kcal/mol). With respect to the active site, quercetin formed hydrogen bonds with PRO48, ILE77, VAL46, PHE83, ASP49, and LYS51, while kaempferol and PRO242, With a binding affinity of −8.23 kcal/mol, tocopherols formed hydrogen bonds with VAL59, ILE77, LYS51, and PRO48. Monolaurin scored a favorable docking score of −7.63 kcal/mol, with an exceptionally low inhibition constant of 2.55 nM, formed interactions with GLN191, ARG138, ASP132, and CYS190, indicating excellent inhibitory potential. Among the fatty acids, capric and linoleic acids, with a binding affinity of −6.38 kcal/mol, were followed by gallic and oleic acids with −6.57 and −6.53 kcal/mol, respectively. These compounds predominantly formed interactions with LYS201, TRP206, LYS11, and other adjacent residues. Caffeic and ferulic acids and caprylic acid bound in the range of −5.71 to −6.07 kcal/mol, while lauric acid and ethyl gallate showed poor interactions with docking scores of −4.84 and −4.45 kcal/mol, respectively.

Dexamethasone, as a reference ligand, showed a binding affinity of −6.90 kcal/mol and an inhibition constant of 8.8 nM with the interaction of TYR232. In comparison, isorhamnetin, quercetin, kaempferol, tocopherols and monolaurin exhibited better binding affinities, indicating their potential of stronger binding to human tryptase and potential as anti-inflammatory compounds.[34]

4. DISCUSSION

ALI leads to lung impairment and alveolar epithelial damage from prolonged activation of signalling pathways caused by inflammatory mediators and oxidative stress. The complexity of ALI has led to the development of therapies targeting multiple pathways employing different natural products. This study aimed to evaluate the potential of T.”

chebula and VCO to synergistically inhibit inflammatory pathways by integrating phytochemical, physicochemical, GC–MS, ADME, and molecular docking studies.[35] The results indicate that the phytoconstituents of the two natural products had excellent drug-like properties and the potential to target key inflammatory mediators of ALI. Preliminary phytochemical screening demonstrated that T. chebula is abundant in phenols, flavonoids and steroids, and has tannins, terpenoids, and coumarins, while VCO is rich in flavonoids, phenolics, fatty acids and terpenoids along with tocopherols, steroids and glycosides. Presence of these classes of constituents is of "particular interest because polyphenols and flavonoids are well-known as inhibitors of inflammatory signaling pathways, and medium-chain fatty acids and tocopherols have important roles in membrane stabilization and defense against oxidative stress. The available literature suggests that the polyphenolic constituents inhibit some of the NF-κB and MAPK pathway activations as well as the NLRP3 inflammasome, and consequently, the production of the inflammatory cytokines TNF-α, IL-1β and IL-6. Additionally, the tocopherol and fatty acid constituents exert antioxidant effects by scavenging free radicals and by maintaining cellular membrane integrity. The phytochemical constituents evaluated in this study therefore, provide an excellent scientific rationale for the proposed additive anti-inflammatory effects of this combined therapy.

The physicochemical assessment also verified the quality and authenticity of the chosen materials. The presence of medicinal polyphenols in T. chebula, which are of voluminous medicinal importance, can be attributed to the moisture content, ash values, high extractive values, and abundant tannin contents. VCO contains very low moisture content and acid values indicating hydrolytic degradation of VCO is minimal and the VCO possesses very good oxidative stability. Also, these parameters satisfying the pharmacopoeial standards preserve bioactive constituents during the storage of the formulation, thus enhancing the reproducibility of the formulation and the reliability of the therapeutic effect.

GC–MS analysis showed the presence of long-chain alcohols, fatty acid esters, and saturated fatty acid derivatives among the active constituents. This analysis showed that methyl palmitate, hexadecanol, pentadecanol, isopropyl myristate, and hexadecanol are linked to antimicrobial, anti-inflammatory, membrane-protective, and antioxidant activities. The presence of these lipid-derived compounds and tannins and phenolic compounds suggests that there is a synergistic effect in which polyphenols inhibit inflammatory mediators and the lipid constituents improve membrane permeability and enhance cellular absorption of the compounds. The synergistic effect is recognized to be important in the development of phytopharmaceuticals to enhance the therapeutic effect of the formulation.[37]

ADME and Drug-likeness assessments are a necessary part of identifying and optimizing lead compounds in early stage drug discovery. Although a number of hydrolysable tannins have shown biological activity, the hydrolysable tannins displayed high molecular weights, high TPSA, and multiple Lipinski's rule violations, showing poor intestinal absorption and, as a result, poor systemic bioavailability. These results are in line with previous studies that have shown the large polyphenolic compounds have poor membrane permeability, despite their biological activity. In contrast, the polyphenols quercetin and kaempferol, along with isorhamnetin, methylecaffeate, ferulic acid, gallic acid, and ethyl gallate, fulfilled Lipinski's rule of five, exhibited good intestinal absorption and permeability through Caco-2 cells, and had good bioavailability and no hepatotoxicity.[38] In addition, the medium-chain fatty acids from VCO showed high predicted intestinal absorption and bioavailability, and so are good candidates for use in therapeutic applications.

While high-molecular-weight tannins may have strong biological activities, these observations suggest that smaller phenolic molecules and fatty acid derivatives may be more promising tobe drug leads.

Molecular docking with NF-κB (PDB ID: 1NFK) revealed that many phytoconstituents have the potential to interact with the transcription factor that regulates the expression of inflammation-related genes. Out of all the tested compounds, quercetin had the highest binding affinity at -7.78 kcal/mol. This was followed by linoleic acid at -7.34 kcal/mol, isorhamnetin at -7.32 kcal/mol, kaempferol at -7.10 kcal/mol, and oleic acid at -7.10 kcal/mol. All of these compounds formed several hydrogen bonds with key amino acid residues of NF-κB (ARG18, HIS26, SER25, SER34, SER40, GLY14, CYS78, and LYS76), which indicates the formation of stable ligand-protein complexes. It is worth noting that several of these phytoconstituents had stronger binding affinities to NF-κB than the anti-inflammatory drug dexamethasone, which may indicate that they have a stronger potential to inhibit the activation of NF-κB compared to dexamethasone. Given that NF-κB is the primary regulator of all pro-inflammatory cytokines during ALI, the inhibition of NF-κB may help prevent the recruitment of pro-inflammatory cells to the lungs, decrease the release of inflammatory cytokines, and reduce the damaging effects of inflammation to the lungs. Human tryptase is another important target of interest due to its implication in mast cell activation, increased vascular permeability, bronchoconstriction and inflammation of the lungs. Docking studies of human tryptase revealed stronger interactions than those of NF-κB. The highest binding affinity was that of isorhamnetin at -8.94 kcal/mol, followed by quercetin at -8.86 kcal/mol, kaempferol at -8.75 kcal/mol, tocopherols at -8.23 kcal/mol, and monolaurin at -7.63 kcal/mol. All of these compounds interacted

Inhibition of human tryptase activity may minimize mast cell-related inflammatory response and lessen pulmonary oedema caused by ALI.[39,40] The strong interactions of the flavonoids with lipid-derived components support the idea of the combined formulation having a synergistic effect of multi-target inhibition on inflammation.

One of the most important results in this study is that the strongest inhibitors of both NF- κB and human tryptase were identified as flavonoids. Fatty acids were shown to be of importance in the development of favourable pharmacokinetics and also related anti-inflammatory actions. This suggests a complementary activity of the two sources. It was shown that T. chebula has potent polyphenolic inhibitors of inflammatory pathway signalling, and that VCO has highly bioavailable lipid component(s) that may enhance membrane permeability, increase the stability of the active compounds, provide an additional protective effect against oxidative damage, and exert a synergistic multi-target effect. It was shown that this type of synergistic effect is advantageous in the treatment of many complex inflammatory diseases, such as ALI, in which the inhibition of several interdependent pathways of inflammation is more therapeutically relevant than that of a single pathway. Even though there is a strong basis for the anti-inflammatory activity of the two substances used in combination, there are some limitations to this study. In-silico ADME predictions must be validated experimentally. In order to extend this work to the clinic, a rational approach would be to perform the studies that are mentioned above. However, the work presented here is a good basis for the use of VCO and T. chebula.

5. CONCLUSION

This study demonstrated the ability of T. chebula and VCO as complementary agents to manage ALI by targeting multiple anti-inflammatory pathways. Phytochemical screenings and GC-MS analyses showed high concentrations of polyphenols, flavonoids, tannins, medium chain fatty acids, tocopherols, and other active agents having inflammation and oxidation related bioactivities. In addition, the computational analyses of the physicochemical and ADME properties showed that some of the identified constituents, in particular quercetin, kaempferol, isorhamnetin, caffeic acid, ferulic acid, gallic acid, linoleic and oleic and capric and lauric acid, and tocopherols, had drug like properties, good biopharmaceutical potential, and low toxicity. From the results of molecular docking studies, quercetin was the strongest NF-κB inhibitor, whereas isorhamnetin, was the strongest binder to human tryptase. Several phytoconstituents showed stronger binding affinities than dexamethasone and therefore have the potential to inhibit the primary inflammatory pathways related to ALI. The combination of polyphenolic constituents of T. chebula and the lipids of VCO, highly complements one another and provides a synergistic effect to therapy by that targets inflammatory pathways, while simultaneously reducing oxidative stress and stabilizing cellular membranes.

The current study provides solid groundwork for the first phytopharmaceutical formulation combining T. chebula and VCO as a potential solution for inflammatory pulmonary conditions. Further studies involving molecular dynamics simulations, in vitro studies, mechanisms of action, and pharmacological research in proposed models of ALI and subsequent clinical studies are necessary to assess the potential of this synergistic formulation" as a natural product for anti-inflammatory purposes.

6. Contribution of Authors

Conceptualization: Kamalakannan S; Methodology: Kamalakannan S, Sowjanya Bandlamudi; Software: Kamalakannan S; Validation: Sowjanya Bandlamudi, Vaijayanthimala P; Formal analysis: Vaijayanthimala P, Deepalakkshmi B; Investigation: Kamalakannan S, Sowjanya Bandlamudi; Resources: Vaijayanthimala P, Deepalakkshmi B; Data curation: Kamalakannan S; Writing – Original Draft Preparation: Kamalakannan S; Writing – Review & Editing: Vaijayanthimala P, Deepalakkshmi B; Visualization: Kamalakannan S; Supervision: Sowjanya Bandlamudi. All authors have read and agreed to the published version of the manuscript.

7. Funding

This research was not funded by any agency.

8. Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

9. Conflict of Interest

The authors declare that they have no competing interests.

10. Acknowledgements

We are extremely grateful to the department of anatomy, shri sathya sai medical college and research institute, for providing us laboratory facilities, computational facilities, and technical help for conducting this research work. We are also thankful to all the concerned faculty members and staffs of the department for their valuable help and suggestions.

REFERENCES

1. Liu H, Dong J, Xu C, Ni Y, Ye Z, Sun Z, Fan H, Chen Y. Acute lung injury: pathogenesis and treatment. Journal of translational medicine. 2025 Aug 18;23(1):926.
2. Long ME, Mallampalli RK, Horowitz JC. Pathogenesis of pneumonia and acute lung injury. Clinical Science. 2022 May;136(10):747-69.
3. Verma N, Hochhegger B, Mukhopadhyay S, Mohammed TL. Acute lung injury. Journal of Thoracic Imaging. 2025 May 1;40(3):e0820.
4. Lv K, Liang Q. Macrophages in sepsis-induced acute lung injury: exosomal modulation and therapeutic potential. Frontiers in Immunology. 2025 Jan 7;15:1518008.
5. Hou F, Shi N, Yuan H, Li B, Xiao J, Xiao K, Xie L. Targeting alveolar macrophages: a promising intervention for pulmonary infection and acute lung injury. Cellular & Molecular Biology Letters. 2025 Jun 14;30(1):69.
6. Liu D, Weng S, Fu C, Guo R, Chen M, Shi B, Weng J. Autophagy in acute lung injury. Cell Biochemistry and Biophysics. 2025 Jun;83(2):1415-25.
7. Liu Z, Huang X, Guo HY, Zhang LW, Quan YS, Chen FE, Shen QK, Quan ZS. Design, synthesis fusidic acid derivatives alleviate acute lung injury via inhibiting MAPK/NF-κB/NLRP3 pathway. European Journal of Medicinal Chemistry. 2023 Nov 5;259:115697.
8. Chen C, Li L, Liu X, Zhang D, Liu Y, Li Y. 23-O-acetylshengmanol-3-O-α-L-arabinoside alleviates lipopolysaccharide-induced acute lung injury through inhibiting IκB/NF-κB and MAPK/AP-1 signaling pathways. Journal of ethnopharmacology. 2023 Jan 10;300:115725.
9. Wang C, Zhang H, Wang X, Wang X, Li X, Li C, Wang Y, Zhang M. Comprehensive review on fruit of Terminalia chebula: traditional uses, phytochemistry, pharmacology, toxicity, and pharmacokinetics. Molecules. 2024 Nov 24;29(23):5547.
10. Bulbul MR, Chowdhury MN, Naima TA, Sami SA, Imtiaj MS, Huda N, Uddin MG. A comprehensive review on the diverse pharmacological perspectives of Terminalia chebula Retz. Heliyon. 2022 Aug 1;8(8).
11. Bairwa VK, Kashyap AK, Meena P, Jain BP. Triphala's characteristics and potential therapeutic uses in modern health. International journal of physiology, pathophysiology and pharmacology. 2025 Apr 25;17(2):19.
12. Perera WP, Liyanage JA, Dissanayake KG, Gunathilaka H, Weerakoon WM, Wanigasekara DN, Fernando WS, Rajapaksha RM, Liyanage RP, Perera BT. Antiviral potential of selected medicinal herbs and their isolated natural products. BioMed research international. 2021;2021(1):7872406.
13. Gao H, Lu H, Fang N, Su J, Li R, Wang W, Zhang Y. The potential of Terminalia chebula in alleviating mild cognitive impairment: A review. Frontiers in Pharmacology. 2024 Oct 18;15:1484040.
14. Ekambaram SP, Aruldhas J, Srinivasan A, Erusappan T. Modulation of NF-κB and MAPK signalling pathways by hydrolysable tannin fraction from Terminalia chebula fruits contributes to its anti-inflammatory action in RAW 264.7 cells. Journal of Pharmacy and Pharmacology. 2022 May 1;74(5):718-29.
15. Rao YL, Pai MM, Krishnaprasad PR, Pai MV, Murlimanju BV, Mohan A, Prabhu LV, Vadgaonkar R. Virgin coconut oil–its methods of extraction, properties and clinical usage: a review. La Clinica Terapeutica. 2024 Apr 2;175(2).
16. Voon PT, Ng CM, Ng YT, Wong YJ, Yap SY, Leong SL, Yong XS, Lee SW. Health effects of various edible vegetable oils: An umbrella review. Advances in Nutrition. 2024 Sep 1;15(9):100276.
17. Zeng YQ, He JT, Hu BY, Li W, Deng J, Lin QL, Fang Y. Virgin coconut oil: A comprehensive review of antioxidant activity and mechanisms contributed by phenolic compounds. Critical Reviews in Food Science and Nutrition. 2024 Feb 5;64(4):1052-75.
18. Zhang Y, Wang Y, Li L, Hong X, Xia H, Sohouli MH, Xu Z. The effect of virgin coconut oil (VCO) on cardiovascular disease risk factors: a systematic review and meta-analysis. Diabetology & Metabolic Syndrome. 2025 Dec 24;17(1):456.
19. Ajibare AJ, Akintoye OO, Folawiyo MA, Babalola KT, Omotuyi OI, Oladun BT, Aransi-Ola KT, Odetayo AF, Olayaki LA. Therapeutic potential of virgin coconut oil in mitigating sodium benzoate-model of male infertility: Role of Nrf2/Hmox-1/NF-kB signaling pathway. Iranian Journal of Basic Medical Sciences. 2024;27(5):543.
20. Deepika NP, Sharma R, Banerjee S, Verma T, Prajapati PK, Muhasina KM, Parihar VS, Kondengadan SM, Duraiswamy B. Neuroprotective effect of Urukku Velichenna (Traditionally processed virgin coconut oil) in Parkinson's disease: Bridging ethnopharmacology and experimental validation using in vitro, in vivo, and in silico models. Journal of Ethnopharmacology. 2026 Oct 28;369:121933.
21. Wang Y, Yuan Y, Wang W, He Y, Zhong H, Zhou X, Chen Y, Cai XJ, Liu LQ. Mechanisms underlying the therapeutic effects of Qingfeiyin in treating acute lung injury based on GEO datasets, network pharmacology and molecular docking. Computers in biology and medicine. 2022 Jun 1;145:105454.
22. Hu Q, Zhang S, Yang Y, Yao JQ, Tang WF, Lyon CJ, Hu TY, Wan MH. Extracellular vesicles in the pathogenesis and treatment of acute lung injury. Military Medical Research. 2022 Nov 1;9(1):61.

23. Li Z, Bu Y, Wang C, Yu Y, Han L, Liu C, Chen G, Li C, Zhang Y, Cao H, Ma Z. Extracellular vesicle-packaged GBP2 from macrophages aggravates sepsis-induced acute lung injury by promoting ferroptosis in pulmonary vascular endothelial cells. Redox Biology. 2025 May 1;82:103614.
24. Suchalatha S, Devi CS. Antioxidant activity of ethanolic extract of Terminalia chebula fruit against isoproterenol-induced oxidative stress in rats. Indian Journal of Biochemistry and Biophysics. 2005 Aug 1;42(4):246.
25. Eltimamy M, Elshamarka M, Aboelsaad M, Sayed M, Moawad H. Effects of alcoholic extract of Terminalia Chebula dried fruit on blood biochemical profile in diabetic rats. Journal of Diabetes & Metabolic Disorders. 2022 Jun;21(1):159-70.
26. Manalo RV, Silvestre MA, Barbosa AL, Medina PM. Coconut (Cocos nucifera) ethanolic leaf extract reduces amyloid-β (1-42) aggregation and paralysis prevalence in transgenic Caenorhabditis elegans independently of free radical scavenging and acetylcholinesterase inhibition. Biomedicines. 2017 Apr 21;5(2):17.
27. Dhivya LS, Haritha M, Anjana GV, Priya D. In silico screening of Triphala churna against bacterial agents.
28. Ashokkumar D. Pharmacognostical investigations on triphala churnam. Ancient Science of Life. 2007 Jan 1;26(3):40-4.
29. Li Y. Analytical methods for the analysis of volatile natural products. Natural Product Reports. 2023;40(4):922-56.
30. Akter R, Khan SS, Kabir MT, Halder S. GC-MS-employed phytochemical characterization, synergistic antioxidant, and cytotoxic potential of Triphala methanol extract at non-equivalent ratios of its constituents. Saudi Journal of Biological Sciences. 2022 Jun 1;29(6):103287.
31. Trismayanti NM, Dian H. In silico identification of natural compounds from virgin coconut oil as potential ligand peroxisome proliferator-activated receptor-gamma as preventive food leads against colitis: Is it really work?. Journal of Advanced Pharmaceutical Technology & Research. 2023 Jan 1;14(1):39-45.
32. Li H, Bao S, Bai L, Li Z, Wang Q, Narenhu N, Xiao X, Bao L, Bai T. Terminalia chebula Extract Alleviates Lipopolysaccharide-Induced Acute Lung Injury by Modulating the Interleukin-17-Dependent Mitogen-Activated Protein Kinase/Nuclear Factor-Kappa B Signaling Pathways. Phytotherapy Research. 2026 May;40(5):2965-85.
33. Sharma M, Gari M, Sharma A, Aneesha VA, Lingaraju MC, Singh V, Shanker K, Singh TU. Terminalia chebula fruit extract attenuates LPS-induced acute lung injury in mouse model. Tissue and Cell. 2025 Jun 1;94:102805.
34. Pradeep S, Patil SM, Dharmashekara C, Jain A, Ramu R, Shirahatti PS, Mandal SP, Reddy P, Srinivasa C, Patil SS, Ortega-Castro J. Molecular insights into the in silico discovery of corilagin from Terminalia chebula as a potential dual inhibitor of SARS-CoV-2 structural proteins. Journal of Biomolecular Structure and Dynamics. 2023 Dec 21;41(20):10869-84.
35. Abdollahi F, Hadizadeh F, Farhadian S, Assaran-Darban R, Shakour N. In-silico identification of COX-2 inhibitory phytochemicals from traditional medicinal plants: molecular docking, dynamics, and safety predictions. In Silico Pharmacology. 2025 Sep 16;13(3):133.
36. Prasad YM, Gowda SR, Shantamurthy N, Sehar AE, Razack SA, Srichairatanakool S, Ravikumar Y. Computational Identification of Triphala-Derived Sterol Compounds as Putative Agonists of the Human Takeda G Protein-Coupled Receptor (TGR5). International Journal of Molecular Sciences. 2026 Mar 30;27(7):3130.
37. Soni M, Babu YS, Kumar V, Singh B, Brahma M, Maruthi M, Kumar A, Kumar V. In-silico novel thioredoxin-interacting protein inhibitors from Syzygium aromaticum and Terminalia chebula and their validations to combat arsenic-Induced toxicity in Vero cells. Toxicology Reports. 2025 Jun 1;14:102027.
38. Ghosh G, Duyne GV, Ghosh S, Sigler PB. Structure of NF-κB p50 homodimer bound to a κB site. Nature. 1995 Jan 26;373(6512):303-10.
39. Costanzo MJ, Yabut SC, Zhang HC, White KB, de Garavilla L, Wang Y, Minor LK, Tounge BA, Barnakov AN, Lewandowski F, Milligan C. Potent, nonpeptide inhibitors of human mast cell tryptase. Synthesis and biological evaluation of novel spirocyclic piperidine amide derivatives. Bioorganic & medicinal chemistry letters. 2008 Mar 15;18(6):2114-21.
40. Pradeep S, Patil SM, Dharmashekara C, Jain A, Ramu R, Shirahatti PS, Mandal SP, Reddy P, Srinivasa C, Patil SS, Ortega-Castro J. Molecular insights into the in silico discovery of corilagin from Terminalia chebula as a potential dual inhibitor of SARS-CoV-2 structural proteins. Journal of Biomolecular Structure and Dynamics. 2023 Dec 21;41(20):10869-84.

Tables

Table 1. PreliminaryPhytochemical Screening of T. chebula Extract

S.NO.	Chemical Constituents	Identification Test	Results of Ethanolic Extract
1	Alkaloids	Mayer's Test	Present
2	Steroids	Liebermann Test	Present

3	Flavonoids	Hydrochloric acid	Present
4	Saponin	Foam test	Absent
5	Phenols	Ferric chloride test	Present
6	Tannins	Ferric chloride test	Present
7	Carbohydrates	Molisch's test	Absent
8	Coumarins	Ferric chloride test	Present
9	Cardiac glycosides	Kellers-killani Test	Absent
10	Terpenoids	Salkowiski Test	Present
11	Proteins	Xanthoprotein test	Present

Table 2. Preliminary Phytochemical Screening of VCO

S.NO.	Chemical Constituents	Identification Test	Results
1	Alkaloids	Mayer's Test	Absent
2	Flavonoids	Hydrochloric acid	Present
3	Phenolic Compounds	Ferric chloride test	Present
4	Tannins	Ferric chloride test	Present
5	Terpenoids	Salkowiski Test	Present
6	Steroids	Ferric chloride test	Present
7	Glycosides	Kellers-killani Test	Present
8	Saponins	Foam test	Absent
9	Fatty Acids	Copper Acetate Test	Present
10	Tocopherols (Vitamin E)	Emmerie–Engel Reaction	Present
11	Proteins	Xanthoprotein test	Absent

Table 3. Physicochemical Parameters of T. chebula Powder

S. No.	Parameter	Result (% w/w)
1	Loss on Drying (LOD)	6.12
2	Total Ash	2.85
3	Acid Insoluble Ash	1.30
4	Water Soluble Extractive	62.32
5	Alcohol Soluble Extractive	40.91
6	pH (1% Solution)	3.8
7	Foreign Matter	1.2
8	Tannin Content	54mg/100 mg powder

Table 4. Physicochemical Parameters of VCO

S. No.	Parameter	Result (% w/w)
1	Colour	Colourless to Pale Yellow
2	Odor	Characteristic Coconut Aroma
3	Moisture Content (%)	0.10 – 0.18
4	Refractive Index (40°C)	1.448 – 1.450
5	Acid Value (mg KOH/g)	0.35

Table 5. In Silico-Physicochemical parameters of T. chebula and VCO.

S.No.	Compounds	TPSA	Log P	Molecular Weight	nOH	nOHNH	nviolations	nrotb	BBB (log BB)
01.	Chebulagic acid	447.09	-0.0017	954.664	13	26	0	5	-3.708
02.	Chebulinic acid	447.09	0.0179	956.68	13	26	3	9	-4.459
03.	Gallic acid	97.99	0.5016	170.12	4	5	0	1	-1.102
04.	Ellagic acid	141.34	1.3128	302.19	4	8	0	0	-1.272
05.	Chebulic acid	198.89	-0.3139	356.239	6	8	2	5	-1.786
06.	Corilagin	310.66	-0.2965	634.455	11	18	3	2	-2.828
07.	Punicalagin	526.60	1.8012	1084.72	17	30	3	2	-4.107
08.	Chebulanin	313.57	-1.9451	652.47	9	19	3	6	2.581
09.	Terchebulin	507.76	2.3783	1084.72	16	30	3	0	-4.345
10.	Terflavin A	518.76	1.9782	1086.73	17	30	3	8	-4.897
11.	Terflavin B	385.24	0.0348	784.54	13	22	3	8	-3.845
12.	Tannic acid	444.18	4.8381	1701.206	25	46	3	16	-8.62
13.	Ethyl gallate	86.99	0.9801	198.17	3	5	0	3	-1.098
14.	Kaempferol	111.13	2.284	286.24	4	6	0	1	-0.939
15.	Quercetin	131.36	1.988	302.24	5	7	0	1	-1.098
16.	Isorhamnetin	120.36	2.291	316.26	4	7	0	2	-1.135
17.	Arjunglucoside I	197.37	1.4061	666.84	8	11	3	5	-1.281
18.	Arjungenin	118.22	4.1476	501.70	5	6	1	2	-0.802
19.	Lauric acid	37.30	3.9919	200.32	1	2	0	10	0.057
20.	Myristic acid	37.30	4.7721	228.37	1	2	0	12	-0.027
21.	Caprylic acid	40.13	-1.8992	182.30	0	2	0	6	-0.108
22.	Capric acid	37.30	2.8309	195.25	1	2	0	8	0.12
23.	Palmitic acid	37.30	5.5523	256.42	1	2	1	14	-0.111
24.	Stearic acid	37.30	6.3325	284.48	1	2	1	16	-0.195
25.	Oleic acid	37.30	6.1085	282.46	1	2	1	15	-0.168
26.	Linoleic acid	37.30	5.8845	280.45	1	2	1	14	-0.142
27.	Monolaurin	66.76	2.8037	274.40	2	4	0	14	-0.658

28.	Tocopherols	29.46	8.5318	416.68	1	2	1	12	0.739
29.	Ferulic acid	66.76	1.4986	194.18	2	4	0	3	-0.239
30.	Caffeic acid	77.76	1.1956	180.16	3	4	0	2	-0.647

Table 6. In SilicoADME properties of T. chebula and VCO.

S.No.	Compounds	GIA (% Absorbed)	P-gp S	CaCo2 (log Papp in 10-6 cm/s)	CYP 3A4	CYP 2D6	Log Kp (-cm/s)	BA	Hepatotoxicity
01.	Chebulagic acid	LOW	YES	-2.229	NO	NO	**-2.735**	0.11	NO
02.	Chebulinic acid	low	yes	-2.242	no	no	-11.67	0.11	no
03.	Gallic acid	high	no	-0.081	no	no	-2.735	0.56	no
04.	Ellagic acid	no	no	0.335	no	no	-5.76	0.55	no
05.	Chebulic acid	low	no	-1.258	no	no	-9.05	0.11	no
06.	Corilagin	low	yes	-1.337	no	no	-10.12	0.17	no
07.	Punicalagin	low	yes	-2.224	no	no	-10.29	0.17	no
08.	Chebulanin	low	yes	-1.182	no	no	-11.47	0.11	no
09.	Terchebulin	low	yes	-2.096	no	no	-9.90	0.17	no
10.	Terflavin A	low	yes	-2.429	no	no	-9.88	0.17	no
11.	Terflavin B	low	yes	-1.939	no	no	-9.49	0.17	no
12.	Tannic acid	Low	yes	-3.581	no	no	-9.49	0.17	no
13.	Ethyl gallate	high	no	-0.004	no	no	-6.59	0.55	no
14.	Kaempferol	high	no	0.032	no	no	-6.67	0.55	no
15.	Quercetin	high	no	-0.229	no	no	-7.01	0.55	no
16.	Isorhamnetin	high	no	-0.003	no	no	-6.87	0.55	no
17.	Arjunglucoside I	low	yes	-0.611	yes	no	-8.46	0.17	no
18.	Arjungenin	high	yes	0.401	yes	no	-6.18	0.56	yes
19.	Lauric acid	high	no	1.562	no	no	-4.54	0.85	no
20.	Myristic acid	high	yes	1.56	no	no	-3.35	0.85	no
21.	Caprylic acid	high	no	1.453	No	no	-4.41	0.55	no
22.	Capric acid	high	no	1.571	no	no	-4.59	0.85	no
23.	Palmitic acid	high	no	1.558	yes	no	-2.77	0.85	no

24.	Stearic acid	high	no	1.556	Yes	no	-2.19	0.85	no
25.	Oleic acid	high	no	1.563	yes	no	-2.60	0.85	no
26.	Linoleic acid	high	no	1.57	yes	no	-3.05	0.85	no
27.	Monolaurin	high	no	0.69	no	no	-5.02	0.55	no
28.	Tocopherols	low	yes	1.458	yes	no	-1.51	0.55	no
29.	Ferulic acid	high	no	0.176	no	no	-6.41	0.85	no
30.	Caffeic acid	high	no	0.634	no	no	-6.58	0.56	no

⁎GIA - Gastrointestinal Absorption ; P - gp S - P glycoprotein Substrate ; Log Kp - Skin Permeability; BA – Bioavailability

Table 7. Binding Affinity and Interaction of Non-Toxic T. chebula and VCO targeting Nuclear Factor Kappa-B (Nf-Kb)

COMPOUND NAME	BINDING AFFINITY PDB ID: 1NFK (-Kcal/mol)	INHIBITION CONSTANT (nm)	HYDROGEN BOND INTERACTIONS
CAFFEIC ACID	6.2	28.47	HIS 26, ARG 18, GLY28
CAPRIC ACID	6.15	31.06	LYS 76, HIS 77
CAPRYLIC ACID	5.88	48.61	LYS 234,LYS 203
ETHYL GALLATE	4.47	526	ASP 168, ASN 62, ASN 65, LYS 64,GLU 166
FERULIC ACID	6.04	37.44	LYS 234, LYS 203, ASN 209
GALLIC ACID	5.81	54.87	LYS 234, LYS 203, SER 202, ASN 209, SER 208
ISORHAMNETIN	7.32	4.32	CYS 78, LYS 76, ALA 97, VAL 93
KAEMPFEROL	7.1	6.28	GLY 95, CYS 78, LYS 76, ASN 98
LAURIC ACID	5.85	51.46	LYS 203
LINOLEIC ACID	7.34	4.17	HIS 26, ARG 18
MONOLAURIN	4.98	224.51	LEU 29, ASN 98
OLEIC ACID	7.1	6.22	HIS 26, ARG 18
QUERCETIN	7.78	2.0	SER 40, SER 34, GLY 14, SER 25, ARG 18, GLY 28
TOCOPHEROLS	6.13	31.91	VAL 235
DEXAMETHASONE	5.61	76.6	ASN 62, ARG 13

Table 8. Binding Affinity and Interaction of Non-Toxic T. chebula and VCO targeting human tryptase

COMPOUND NAME	BINDING AFFINITY PDB ID: 2ZA5 (-Kcal/mol)	INHIBITION CONSTANT (nm)	HYDROGEN BOND INTERACTIONS
Caffeic Acid	5.71	65.39	LYS 201, TRP 206, LYS 11, SER 10
Capric Acid	6.38	20.92	LYS 201, TRP 206, LYS 11
Caprylic Acid	6.07	35.63	TRP 206, LYS 201, LYS 11
Ethyl Gallate	4.45	549.46	PRO 73
Ferulic Acid	5.72	63.68	LYS 11, LYS 201
Gallic Acid	6.57	15.18	LYS 201, TRP 206, LYS 11, SER 10, TRP 12
Isorhamnetin	8.94	280.27	ASP 49, LYS 51, VAL 46, PRO 48, HIS 80, ILE 77
Kaempferol	8.75	387.1	PRO 242, HIS 35, THR 109, LEU 112
Lauric Acid	4.84	282.65	ASP 136, ASN 135, CYS 190, CYS 218
Linoleic Acid	6.38	20.91	PRO 224, GLU 216, ASP 159, CYS 158
Monolaurin	7.63	2.55	GLN 191, ARG 138, ASP 132, CYS 190
Oleic Acid	6.53	16.34	TRP 206, LYS 11, LYS 201
Quercetin	8.86	320.97	PRO 48, ILE 77, VAL 46, PHE 83, ASP 49, LYS 51.
Tocopherols	8.23	928.79	VAL 59, ILE 77, LYS 51, PRO 48
Dexamethasone	6.9	8.8	TYR 232

Figures

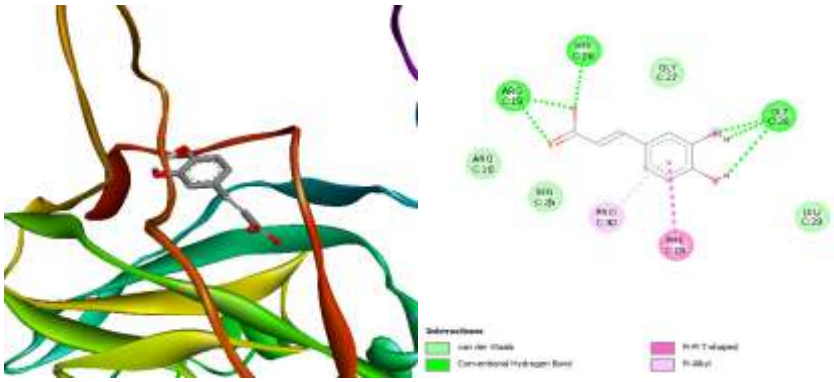


Fig 1. 2D and 3D Visualization of Caffeic Acid Targeting Nuclear Factor Kappa-B (Nf-Kb)

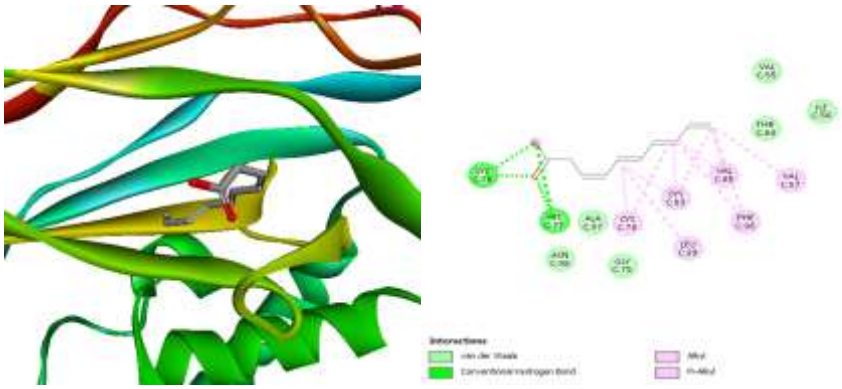


Fig 2. 2D and 3D Visualization of Capric Acid Targeting Nuclear Factor Kappa-B (Nf-Kb)

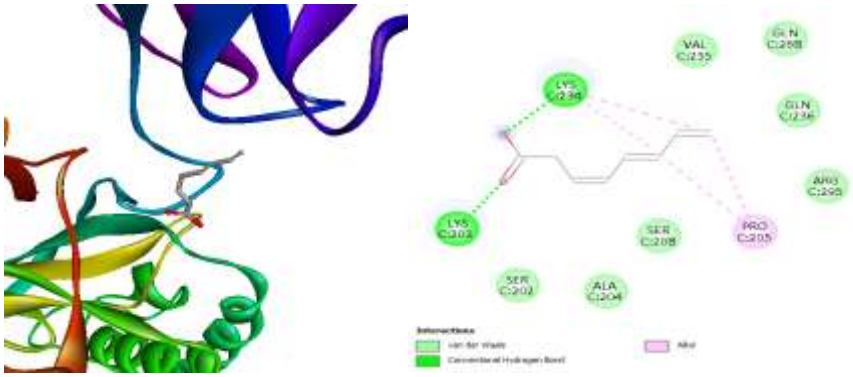


Fig 3. 2D and 3D Visualization of Caprylic Acid Targeting Nuclear Factor Kappa-B (Nf-Kb)

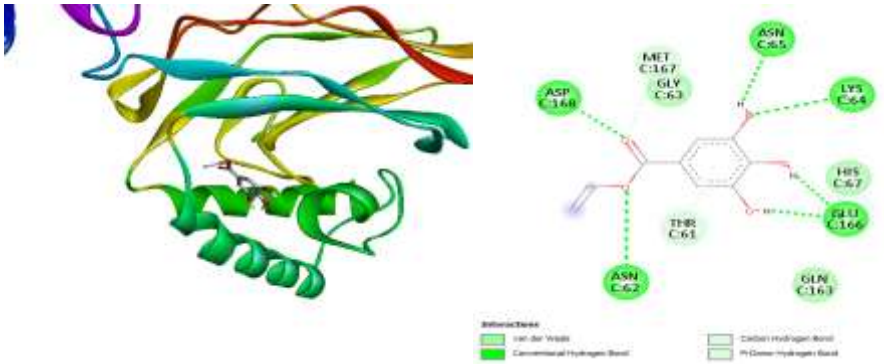


Fig 4. 2D and 3D Visualization of Ethyl Gallate Targeting Nuclear Factor Kappa-B (Nf-Kb)

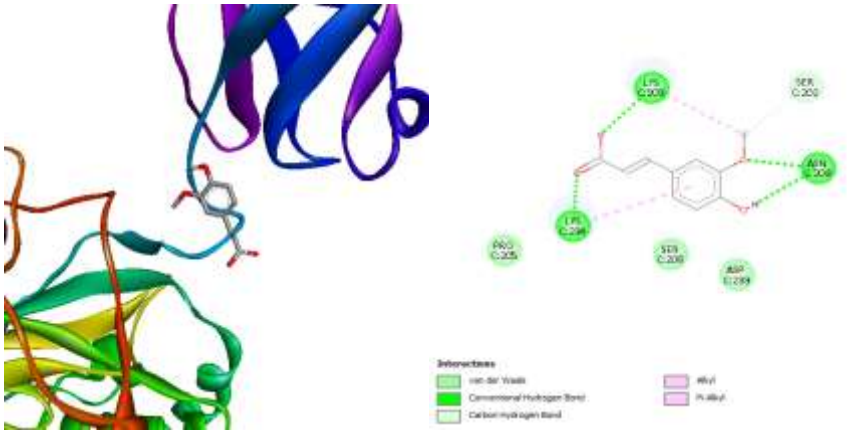


Fig 5. 2D and 3D Visualization of Ferulic Acid Targeting Nuclear Factor Kappa-B (Nf-Kb)

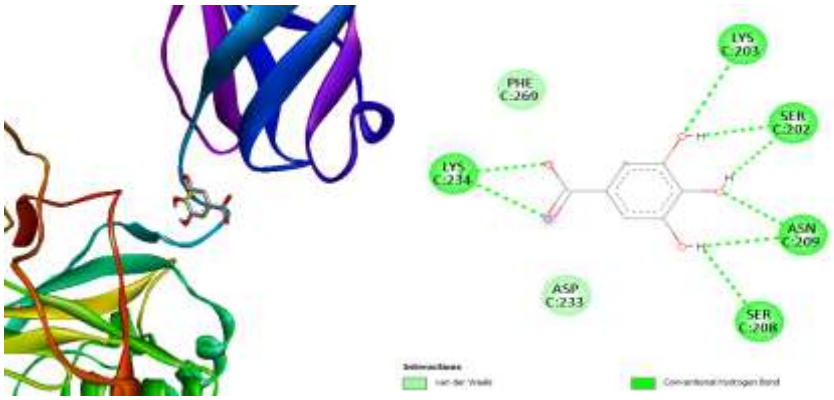


Fig 6. 2D and 3D Visualization of Gallic Acid Targeting Nuclear Factor Kappa-B (Nf-Kb)

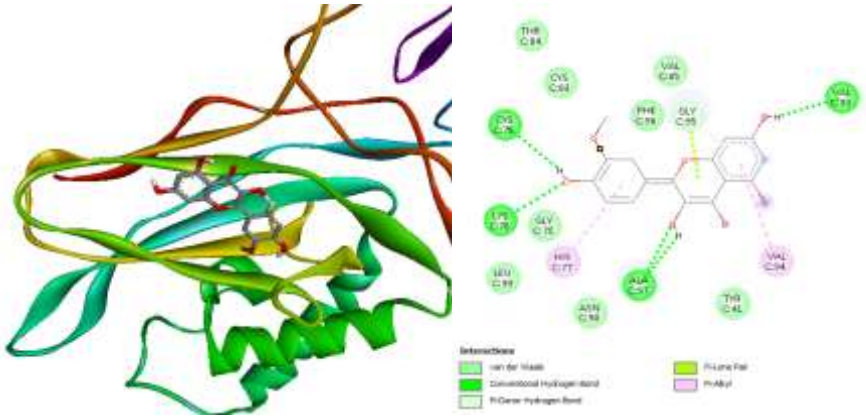


Fig 7. 2D and 3D Visualization of Isorhamnetin Targeting Nuclear Factor Kappa-B (Nf-Kb)

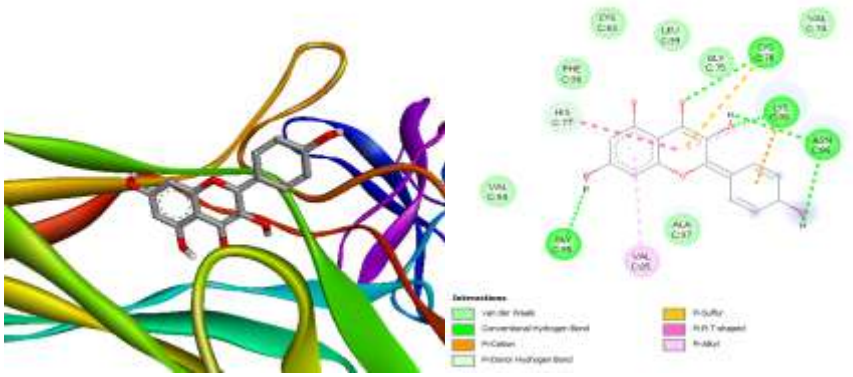


Fig 8. 2D and 3D Visualization of Kaempferol Targeting Nuclear Factor Kappa-B (Nf-Kb)

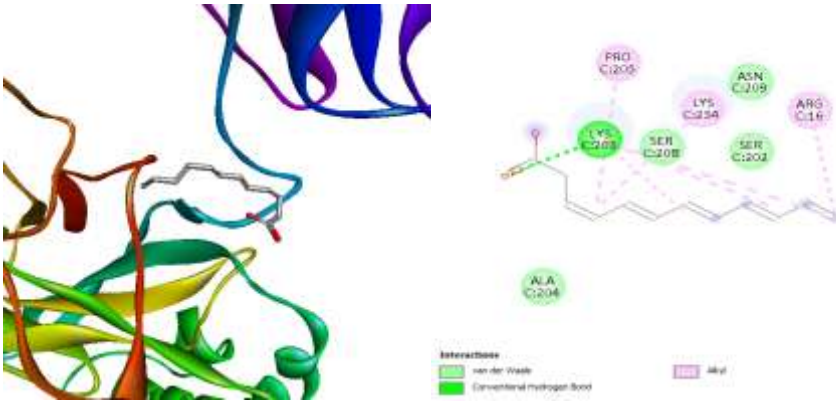


Fig 9. 2D and 3D Visualization of Lauric Acid Targeting Nuclear Factor Kappa-B (Nf-Kb)

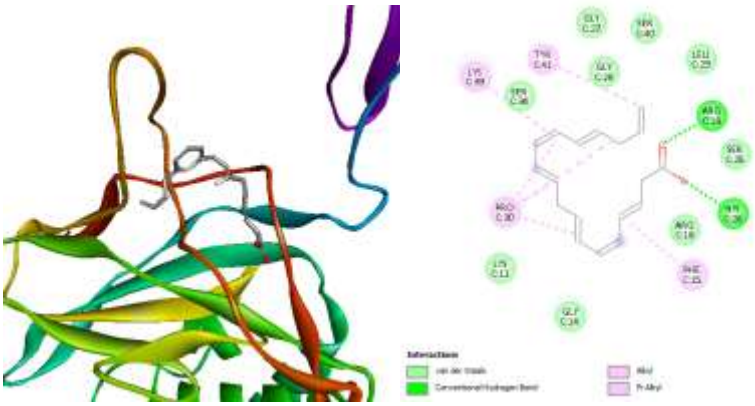


Fig 10. 2D and 3D Visualization of Linoleic Acid Targeting Nuclear Factor Kappa-B (Nf-Kb)

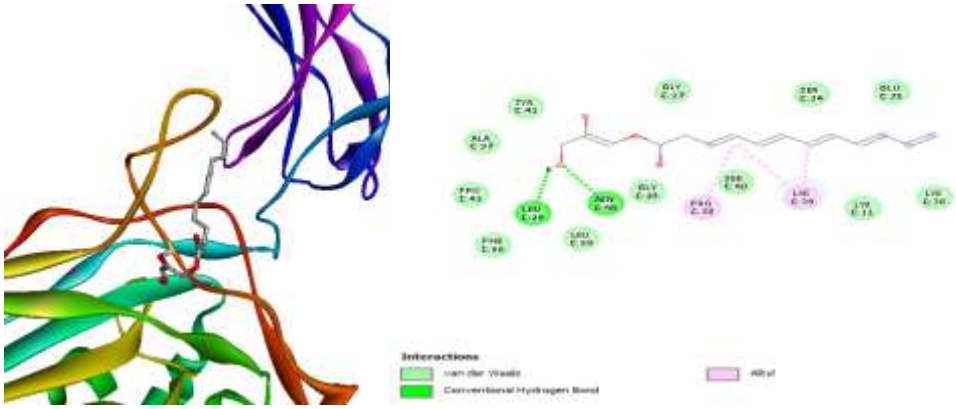


Fig 11. 2D and 3D Visualization of Monolaurin Targeting Nuclear Factor Kappa-B (Nf-Kb)

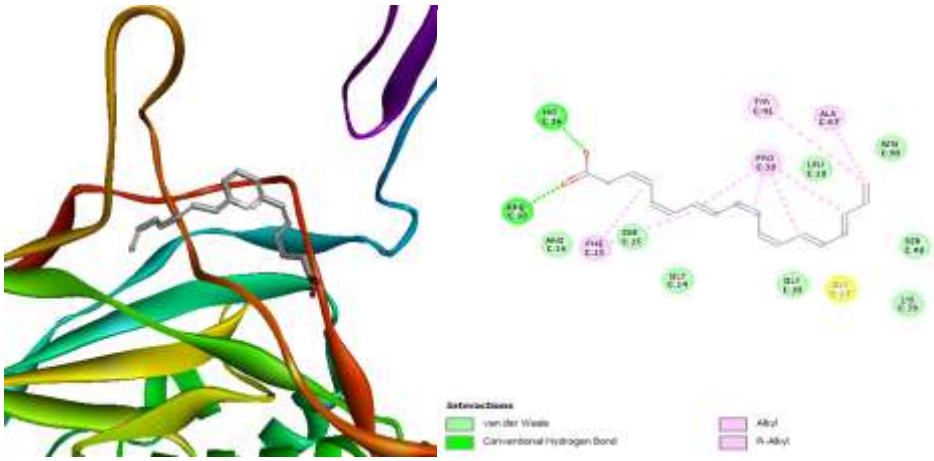


Fig 12. 2D and 3D Visualization of Oleic Acid Targeting Nuclear Factor Kappa-B (Nf-Kb)

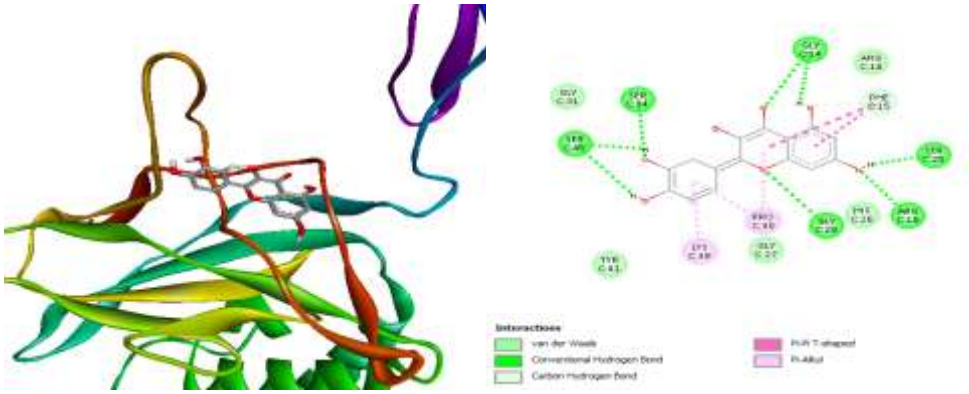


Fig 13. 2D and 3D Visualization of Quercetin Targeting Nuclear Factor Kappa-B (Nf-Kb)

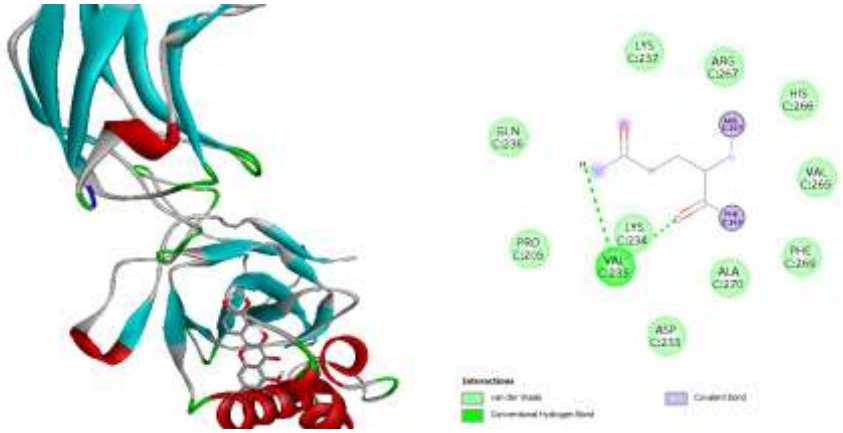


Fig 14. 2D and 3D Visualization of Tocopherol Targeting Nuclear Factor Kappa-B (Nf-Kb)

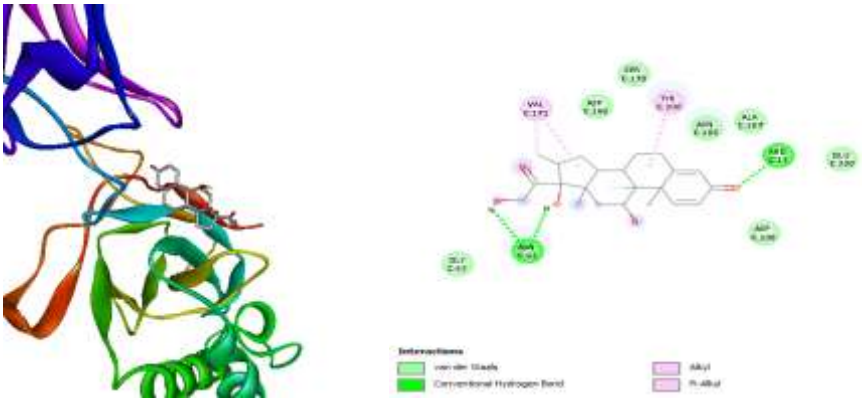


Fig 15. 2D and 3D Visualization of Dexamethasone Targeting Nuclear Factor Kappa-B (Nf-Kb)

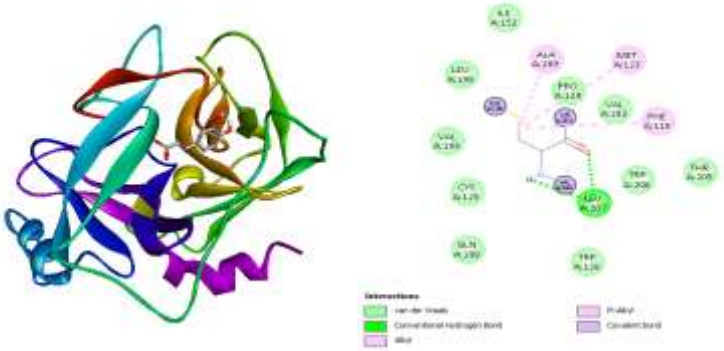


Fig 16. 2D and 3D Visualization of Caffeic Acid Targeting Human Tryptase

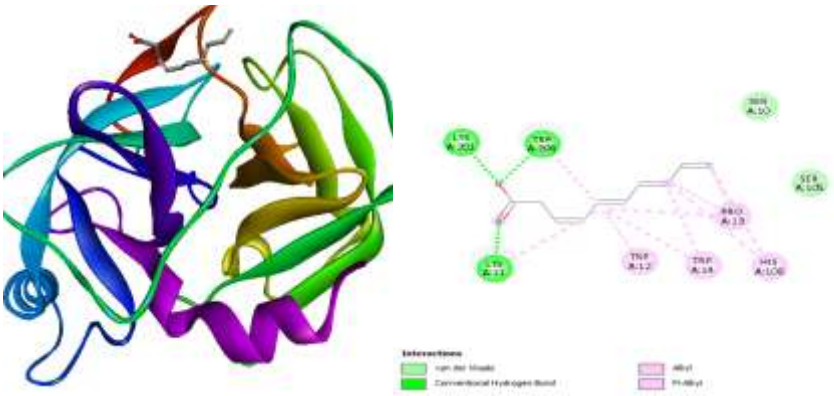


Fig 17. 2D and 3D Visualization of Capric Acid Targeting Human Tryptase

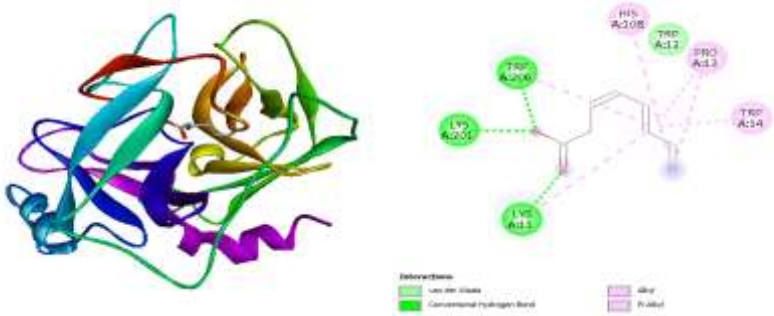


Fig 18. 2D and 3D Visualization of Caprylic Acid Targeting Human Tryptase

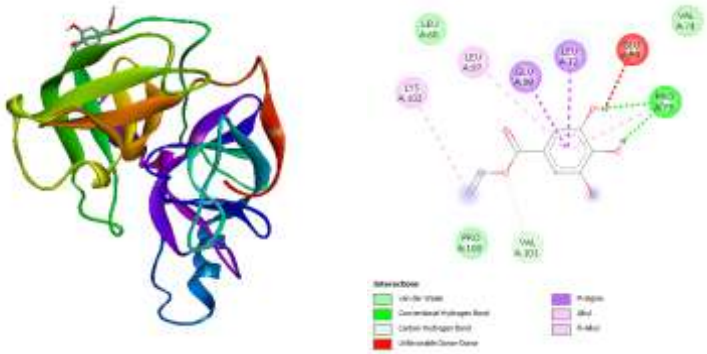


Fig 19. 2D and 3D Visualization of Ethyl Gallate Targeting Human Tryptase

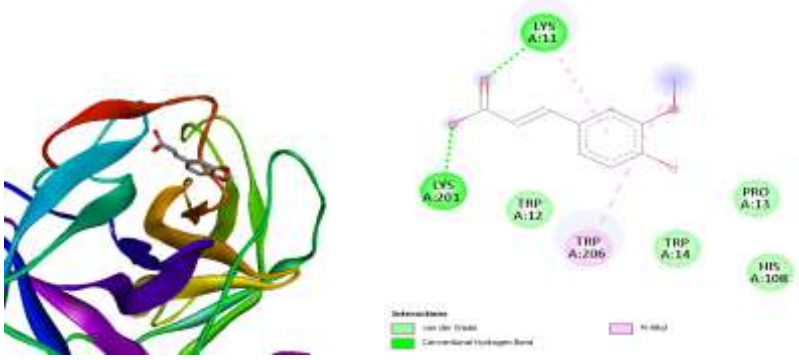


Fig 20. 2D and 3D Visualization of Ferulic Acid Targeting Human Tryptase

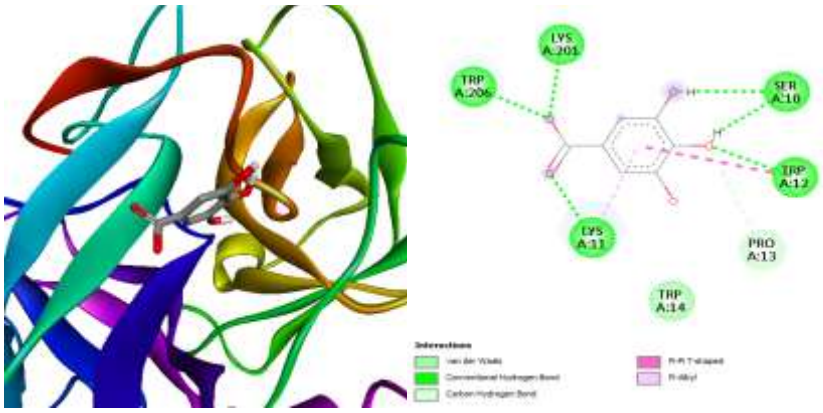


Fig 21. 2D and 3D Visualization of Gallic Acid Targeting Human Tryptase

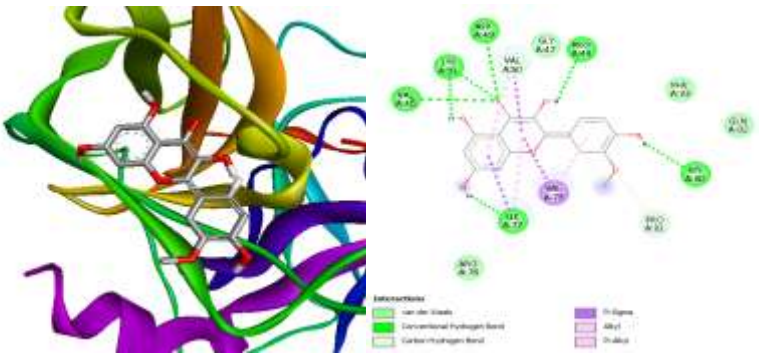


Fig 22. 2D and 3D Visualization of Isorhamnetin Targeting Human Tryptase

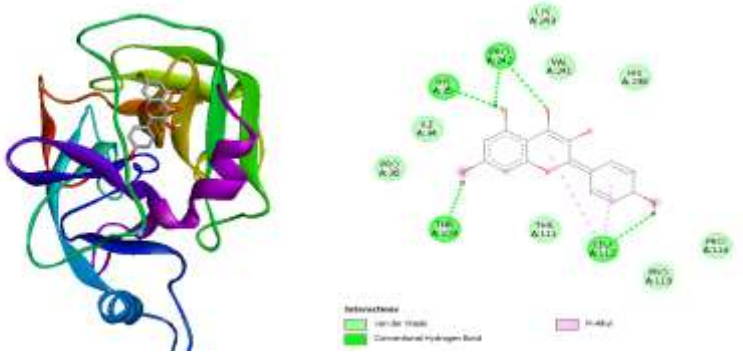


Fig 23. 2D and 3D Visualization of Kaempferol Targeting Human Tryptase

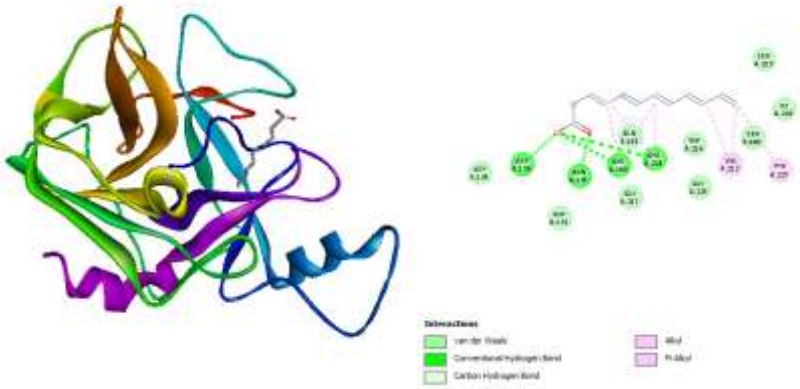


Fig 24. 2D and 3D Visualization of Lauric Acid Targeting Human Tryptase

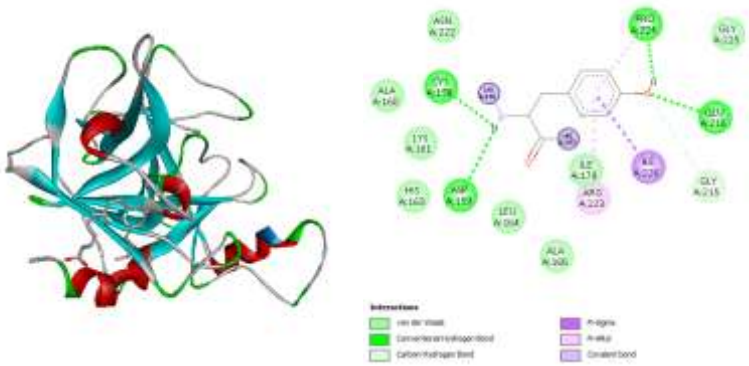


Fig 25. 2D and 3D Visualization of Linoleic Acid Targeting Human Tryptase

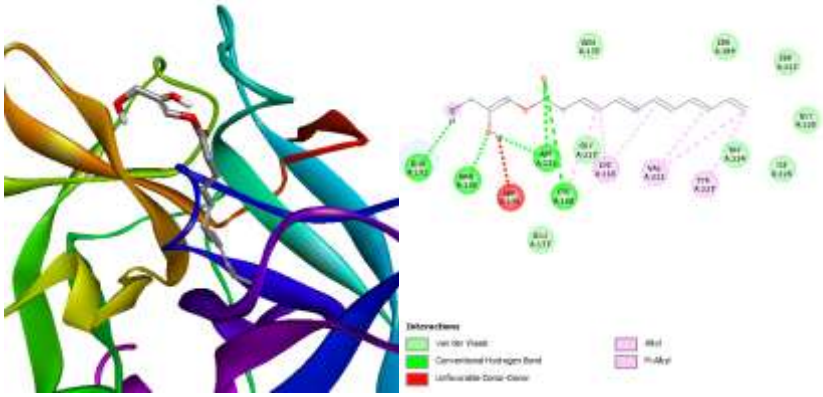


Fig 26. 2D and 3D Visualization of Monolaurin Targeting Human Tryptase

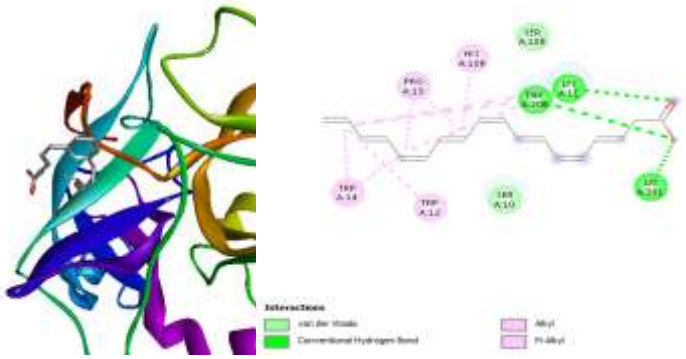


Fig 27. 2D and 3D Visualization of Oleic Acid Targeting Human Tryptase

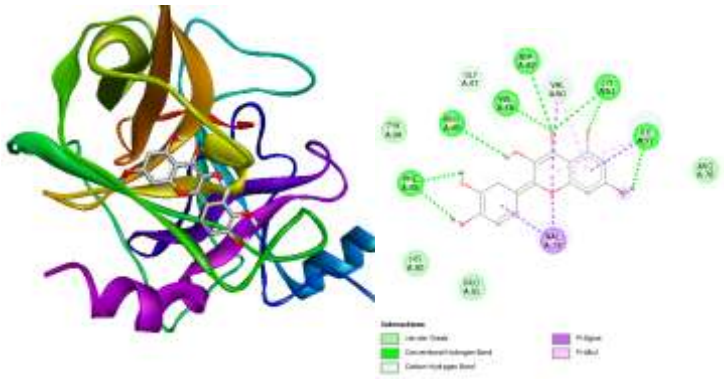


Fig 28. 2D and 3D Visualization of Quercetin Targeting Human Tryptase

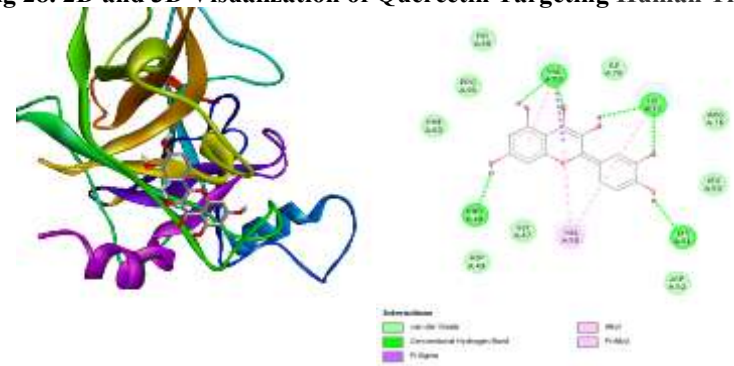


Fig 29. 2D and 3D Visualization of Tocopherol Targeting Human Tryptase

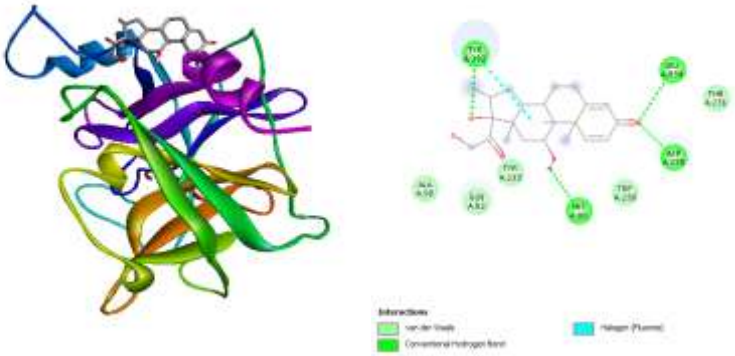


Fig 30. 2D and 3D Visualization of Dexamethasone Targeting Human Tryptase