Journal of Oleo Science
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doi : 10.5650/jos.ess22008
J. Oleo Sci. **71**, *(8) 1199-1206 (2022)*

Impact of Olive Oil Constituents on C-reactive Protein: *In silico* Evidence

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Abstract: Pain is a sensation a humans sense as a protective mechanism against physical injury. This sensation is closely related to inflammation. It ranges from mild to highly obnoxious. It is well-known that the levels of the inflammatory biomarker, C-reactive protein (CRP), increase manifold in acute inflammation and pain. Olive oil, known to have many phytochemicals, has been traditionally used to alleviate pain. Amongst major phenolic compounds in olive oil are oleuropein (OLE), hydroxytyrosol (HT), tyrosol, and oleocanthal. Whether the analgesic and anti-inflammatory properties in olive oil are due to any specific interections is not known. Therefore, this study aimed to elucidate the possible anti-inflammatory and anti-nociceptive properties in those major phenolic compounds by using molecular docking software MOE 2015, comparing the energy value and binding site of phenolic compounds to that of well-known synthetic non-steroidal anti-inflammatory drugs (NSAIDs) and phosphocholine. The docking experiment showed that all compounds could directly interact with CRP. Oleuropein had the most potent interaction with CRP (–7.7580), followed by indomethacin (–6.0775), oleocanthal (–5.5734), ibuprofen (–5.3857), phosphocholine (–4.3876), HT (–4.2782), and tyrosol (–4.2329). Interestingly, the present study found other phytochemicals in olive oil that can be exploited as potential, safe, and cost-effective lead compound(s) for analgesic and anti-inflammatory activity, as supported by its molecular docking data.

Key words: C-reactive protein, molecular docking, olive oil, oleuropein, hydroxytyrosol, tyrosol, oleocanthal

1 Introduction

C-reactive protein (CRP) is an inflammatory protein found in the liver[1]. CRP levels rise swiftly in reaction to trauma, inflammation, and infection and fall quickly when the condition is resolved[2]. As a result, CRP testing is routinely used to monitor various inflammatory conditions[3, 4]. In individuals with COVID-19, blood levels of IL-6 and CRP can be used to measure disease severity of inflammation[5]. CRP binds to polysaccharides on microorganisms, such as phosphocholine (PCh), and activates C1q, triggering the classical complement cascade of innate immunity[6].

Proteins (receptors) are the primary molecular targets in virtual screening (VS) to identify drug action quickly. Several substances (ligands), either synthetic drugs or phytochemicals, attach to protein targets to have beneficial or inhibitory effects, which aid in developing new and efficient therapeutics as lead molecules. VS exposes vast libraries of commercially available drug-like compounds that have been computationally tested against known structural targets and those projected to bind properly in experiments[7, 8].

At numerous stages of drug development, pharmaceutical companies have made significant advancements in computer-aided drug design, including identifying new hits, enhancing molecule binding affinity in hit-to-lead, and lead optimization[9]. In addition, *in silico* approaches are frequently used in current drug design to assist in the knowledge of drug-receptor interactions. By exposing the mechanism of drug-receptor interactions, computational methodologies have been devised in the literature to

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Accepted May 8, 2022 (received for review January 7, 2022)
Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://www.jstage.jst.go.jp/browse/jos/ http://mc.manusriptcentral.com/jjocs

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strongly support and facilitate the discovery of novel, more potent inhibitors[10, 11].

Furthermore, there has been an increase in the acceptance of herbal treatment[12]. The search for a safe anti-inflammatory drug with no or few side effects continues. *Olea europaea* L. is the most well-known plant in the *Olea* genus[13]. This species enjoys being consumed as food[14, 15]. The polyphenols generated by the olive tree (*Olea europaea*) are found mainly in the tree's leaves and drupes. They are essential as phytoalexins, molecules created by the plant to defend it from bacteria and fungi and prevent leaf-eating insects. Extra Virgin Olive Oil (EVOO) contains over 30 phenolic chemicals, including OLE (glycated and aglycone forms), oleocanthal, HT, and tyrosol. Although the health benefits of EVOO and olive leaf extracts have long been recognized, they have only lately been thoroughly investigated. More recent study has focused on the biological capabilities of these compounds, which were initially identified in 1950 and include antibacterial, hypoglycemic, vasodilator, antihypertensive, antioxidant, and anti-inflammatory effects[16]. Olives have extensive application in ethnomedicine. It is used to treat diabetes[17], hypertension[18], pain[19], inflammation[20], gastrointestinal diseases, rheumatism, and resolve constipation. Because of these qualities, the European Pharmacopoeia (Ph. Eur.) has included the alcoholic extract (80%) of olive leaves, which contains OLE, HT, tyrosol, apigenin, and verbascoside, among other minor components (Ph. Eur.)[21]. Because of deglycosylation by plant glycosidases during olive squeezing, the polyphenols produced by the olive tree are usually found in the glucose-free form (aglycones) in the lipid fraction and the water fraction (dispersed as minute droplets) of olive oil. The most common secoiridoid in olives is 3,4-dihydroxyphenylethanol-elenolic acid (3,4-DHPEA-EA), whose glucose-bound form is OLE is the main cause of the bitter taste of olive leaves and drupes. OLE and its derivatives HT, oleocanthal, and tyrosol are shown in Fig. 1 (a, b, c, and d, respectively).

In a recent study, olive extract demonstrated CRP-lowering activity[14, 22–25] and anti-inflammatory effects[26–29]. However, the active component for CRP-lowering activity is still unknown, which led us to design the present investigation to unravel the active constituent underlying the CRP-lowering effect in the anti-inflammatory action of olive.

We performed molecular docking experiments to investigate the manner of binding OLE, oleocanthal, HT, and tyrosol to CRP to obtain insight into the structural requirements for the inhibition. The findings of this study would help researchers better understand the inhibitory mode of these compounds and forecast their activities more quickly and precisely. These findings can also be used to deduce some valuable hints that would aid in the development of novel CRP–directed inhibitors with desirable therapeutic characteristics.

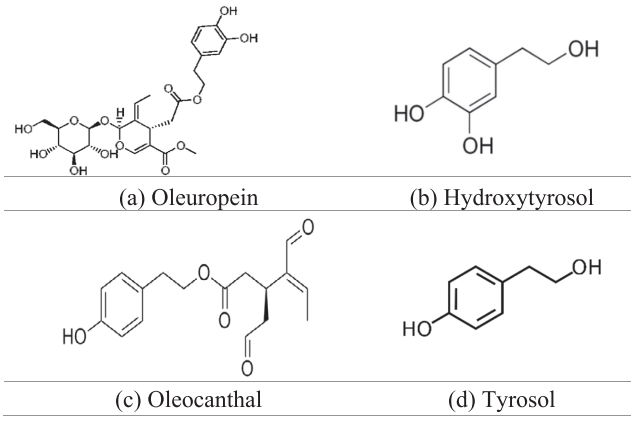


Fig. 1 Structures of OLE and its derivatives.

2 Material and Methods

2.1 *In silico* molecular docking analysis

MOE is a drug discovery software platform from Chemical Computing Group Inc., whose main application areas include structural-based design[30]. MOE was used to perform a docking operation following the stages outlined in the algorithm. The proposed study would employ the MOE 2015 program to investigate the binding behavior of an active ingredient in olive oil to CRP.

The canonical SMILES for OLE, HT, tyrosol and oleocanthal were downloaded from PubChem and produced using the MOE Builder interface before performing energy minimization. The water molecules in the complex have been removed. After they were inserted into the complex in their standard configuration, the energy of all hydrogen atoms was minimized using MOPAC (Molecular Orbital PACkage). The product was placed through a rigorous conformational scan for default values with a root mean squared (RMS) gradient of 0.001 kcal/mol. The false atoms were made from alpha spheres created while searching for an active pocket on the target. The energy used was limited to a bare minimum, while the residues and backbone remained constant. The MOE's docking performance was tested by redocking the cocrystallized structure of CRP (PDB ID 1B09) and PCh. The cocrystallized ligand, PCh, has been docked into the active protein site to ensure that this software regenerates the binding confirmation correctly. All compounds were docked using the procedure indicated above. Each molecule was given ten different conformations. The default settings for the remaining parameters have been used. The best confirmation of each target complex was chosen based on the lowest energy. The compound–target complex model was then used to deduce compound–enzyme interactions at the active site. The docking complex resembles the original crystal struc-

ture in appearance.

A total of 4 reported anti-inflammatory agents from olive oil were selected to perform the molecular docking studies to screen and identify the potent anti-inflammatory agents specifically for CRP[31, 32]. Molecular docking is essential for structure-based drug creation since it can reveal new chemical binding modes when used at the right binding location[33]. In this research, binding modes and interactions for active inhibitors of CRP (PDB ID 1B09) were explored via molecular docking experiments. These were chosen as target proteins in studies of anti-inflammatory and anti-nociceptive docking.

The step-by-step process of Target preparation in molecular docking using MOE 2015 is explained in Algorithm1, and its flowchart is shown in Fig. 2. However, the ligand preparation in molecular docking is demonstrated in Algorithm2, and its flowchart is shown in Fig. 3. For the Target-ligand complex, the docking process is illustrated in Algorithm3, and Fig. 4 shows the flowchart of a docking simulation.

3 Results

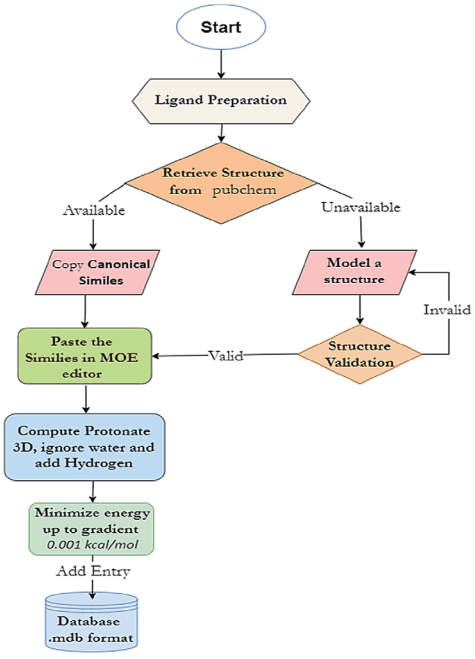
We studied the affinity of six ligands (OLE, indomethacin, oleocanthal, ibuprofen, HT, and tyrosol) as well as the standard ligand PCh to CRP. Docking experiments in Fig. 6 showed that all ligands could directly interact with CRP. Among these, OLE had the most potent interaction with CRP (energy = −7.7580) through two H bonding interactions to GluA14 (distance = 2.95, energy = −1.8) and GluA197 (distance = 3.07, energy = −1.2) residues of CRP, followed by indomethacin (energy = −6.0775); two H bonding interactions with LysB119 (distance = 2.93, energy = −6), SerB120 (distance = 2.97, energy = −3.2) residues and one π-cation interaction bond with GluA14, oleocanthal (energy = −5.5734); one H bonding interaction to GluA48 (distance = 3.59, energy = −0.6), ibuprofen (energy = −5.3857); one H bonding interaction to ArgA47 (distance = 2.98, energy = −2.8) residue and one ionic bond with ArgA47, PCh (energy = −4.3876); one H bonding interaction to LysB119 (distance = 3.33, energy = −3,9) residue and one ionic bond with LysB119, HT (energy = −4.2782); one H bonding interaction to ProA12 (distance = 3.02, energy = −2.3), and tyrosol (energy = −4.2329); one H bonding interaction to GluA14 (distance = 3.14, energy = −1.5) residue. According to the results mentioned above, OLE was found to have the strongest binding to CRP compared with the standard ligand PCh and drug indomethacin and ibuprofen.

Target Preparation

Algorithm1:

1. *Begin*
2. *Search Structure in RCSB*
3. *If Mol[1] is available*
4. *Then Download (Mol, PDB[2]) & goto Step 10*
5. *else*
6. *Model (Mol)*
7. *If Validate (Mol) is False*
8. *goto Step 6*
9. *else*
10. *Protonate (Mol)*
11. *Remove (Mol, Water)*
12. *Add (Mol, Hydrogen)*
13. *Minimize (E[3], 0.001)*
14. *Find (Mol, AS[4])*
15. *Save (Mol)*
16. *End if*
17. *End if*
18. *End*

FlowChart



[1] Molecule, [2] Protein Data Bank, [3] Energy, [4] Active Site

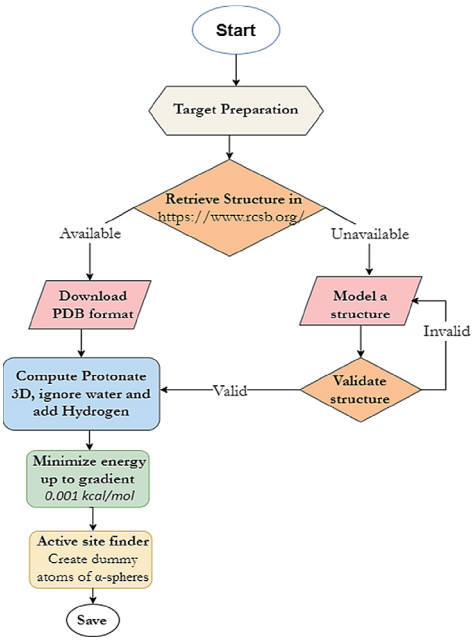
Fig. 2 Flowchart of target preparation.

Ligand Preparation

Algorithm2:

1. *Begin*
2. *Search Lig[5] in PubChem*
3. *If Lig is available*
4. *Then Copy (Lig, CS[6]) & goto Step 10*
5. *else*
6. *Model (Lig)*
7. *If Validate (Lig) is False*
8. *goto Step 6*
9. *else*
10. *Protonate (Lig)*
11. *Remove (Lig, Water)*
12. *Add (Lig, Hydrogen)*
13. *Minimize (E, 0.001)*
14. *Create (DB[7], mbd)*
15. *Add (DB, Entry)*
16. *End if*
17. *End if*
18. *End*

Flow chart



[5] Ligand, [6] Canonical Smilies, [7] Database

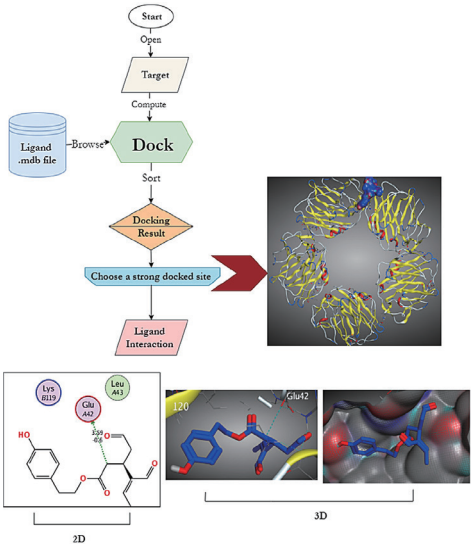
Fig. 3 Flowchart of ligand preparation.

Target-Ligand Complex

Algorithm3:

1. *Begin*
2. *If Mol is available*
3. *Open (Mol, moe) goto Step5*
4. *else execute Algorithm1*
5. *Compute Dock (Lig)*
6. *Sort (output, Dock)*
7. *Set He = Select Max[8] (E)*
8. *If Lig is available*
9. *Browse (P[9], He[10]) goto Step 11*
10. *else execute Algorithm2*
11. *Compute (Lig-interaction)*
12. *Save Figure (2d)*
13. *Save Figure (3d)*
14. *Else*
15. *End if*
16. *End if*
17. *End*

Flow chart



[8] Maximum, [9] Pose, [10] Highest Energy

Fig. 4 Flowchart of docking.

Seven ligands were being docked with active sites of CRP, where various positions of the ligand-CRP complex were evaluated to predict preference for the binding site and pose of ligand-target interaction. The best confirmation is chosen based on the lowest binding energy, and its comparative analysis is shown in Fig. 5. This study represented a significant contribution to understanding the effect of olive oil and its active constituent consumption on inflammation and nociception, with rigorous control of CRP. The results provide valuable clues about the beneficial effects of olive oil as revealed by plasmatic levels of selected markers of inflammation.

Analysis of OLE, indomethacin, oleocanthal, ibuprofen, PCh, HT, and tyrosol interactions at the active sites of CRP revealed a higher prevalence of hydrogen, ionic, and π-cation bonds. The results of the docking studies are summarized in Table 1.

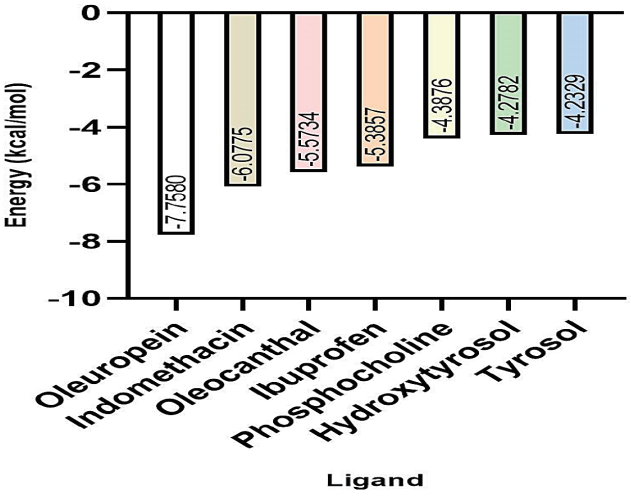


Fig. 5 Comparative analysis of binding energy.

4 Discussion

Following interventions with olive oil ingredients, results from the current study suggest that CRP was positively altered. We've long suspected that CRP has pro-inflammatory features. Binding to ligands exposed on cells or other autologous structures due to infection, inflammation, ischemia, and other diseases might worsen tissue damage and lead to more severe disease[34]. The excellent correlation of circulating CRP concentrations with the severity, extent, and progression of many different diseases and the prognostic relevance of these correlations support the idea that CRP is a marker of disease and plays a role in pathogenesis. The use of olive oil and its constituents precisely prevent CRP binding and its pro-inflammatory effects[35, 36]. Such medications could be a valuable tool for identifying whether elevated CRP production is due to inflammation alone and has anti-inflammatory and anti-nociceptive properties[37].

OLE has the strongest binding affinity compared to indomethacin, ibuprofen, and other olive constituents, which was used to determine which element of olive oil decreases CRP compared to other olive oil constituents and NSAIDs.

Without molecular docking, VS is insufficient; especially when a 3D structure of the drug target is available, docking is the most widely used VS technique. Docking is made up of two fundamental processes. Docking algorithms perform conformational sampling of tiny molecules in the first stage to position them in the 3D-binding site of receptors/proteins. Scoring functions calculate the binding affinity of each docked ligand confirmation within the binding site in the second phase. Scores or binding free energy are used to estimate binding affinities. During docking, scoring functions must distinguish correct binding states of ligands from non-native docked conformations and identify known potential inhibitors (active molecules) within a library of inert compounds with reasonable accuracy and precision.

Table 1 Interaction sites of CRP with the seven ligands.

Ligand	H-Bond			Ionic	π-Cation interaction
	Energy (kcal/mol)	Distance (Å)	Amino Acid		
OLE	− 1.8	2.95	GluA14	……	……
	− 1.2	3.07	GluA197		
Indomethacin	− 6.0	2.93	LysB119	……	GluA14
	− 3.2	2.97	SerB120		
Oleocanthal	− 0.6	3.59	GluA48	……	……
Ibuprofen	− 2.8	2.98	ArgA47	ArgA47	……
Phosphocholine	− 3.9	3.33	LysB119	LysB119	……
Hydroxytyrosol	− 2.3	3.02	ProA12	……	……
Tyrosol	− 1.5	3.14	GluA14	……	……

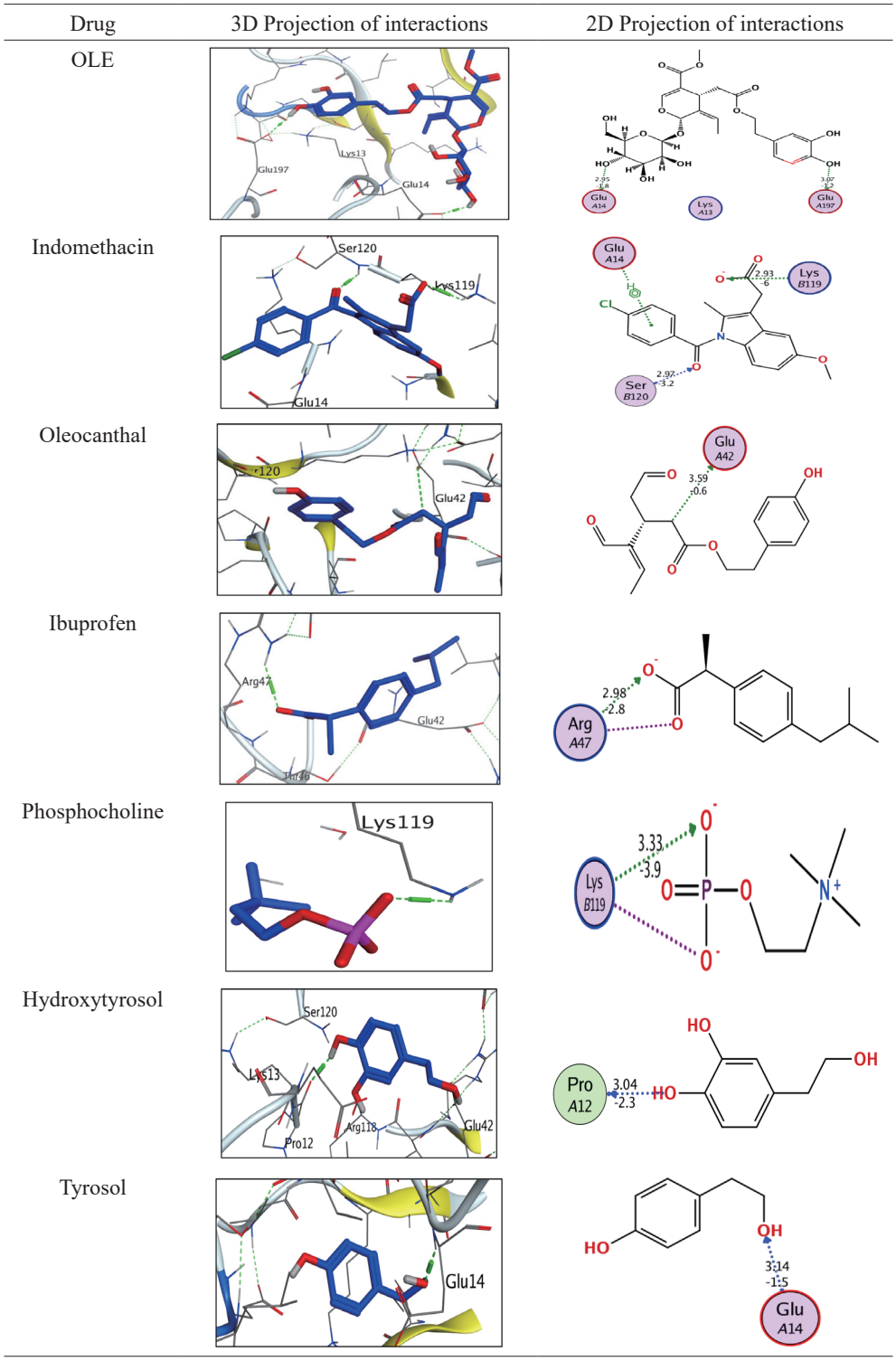


Fig. 6 Binding of ligands with the active sites of CRP and its interaction.

Research evaluating docking/scoring approaches has been undertaken, primarily focusing on ligand pose prediction and VS. Pose prediction accuracy has been attained for pharmacological targets. However, the scoring functions need to be improved to accurately forecast a ligand's genuine binding affinity for its receptor.

As a result, validating a docking approach before performing VS against a specific target of interest is highly suggested. This work aimed to develop an effective and acceptable VS procedure for data mining of CRP inhibitors.

5 Conclusions

In conclusion, an approach to VS under molecular docking and receptor ligand-binding affinity can be an easy screening method before identifying the efficacy of the same lead compound with potent therapeutic efficacies without any side effects. Herein, it was observed that available phytochemicals from *Olea europaea* can be used in future drug designing and development as anti-inflammatory and anti-nociceptive phytomedicine at a low cost. The present work also helps to identify exact compounds for future functional assays. It is suggested that the current data should be validated with *in vivo* and *in vitro* tests.

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