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Original Research Article

Prediction of Antiinflammatory Effects of *Rosmarinus officinalis* L. in Osteoarthritis Through Inhibition in PGE2-R, COX-2, and IL-1β: an *In Silico* Study

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ABSTRACT

Osteoarthritis can cause inflammation, stiffness and pain in the joints. Phytochemical compounds of *Rosmarinus officinalis* L. (RO) such as Carnosol (CAR), Carnosic Acid (CA), Rosmarinic Acid (RA), and Micromeric Acid (MA) have been proven to be anti-inflammatory alternative drugs. This study was conducted to predict anti-inflammatory effects through the inhibition of several inflammatory mediators in osteoarthritis such as Prostaglandin E2 Receptor (PGE2-R), Cyclooxygenase-2 (COX-2) and Interleukin 1 beta (IL-1β) by identifying the binding affinity, hydrogen bond distance, RMSDAll, and RMSDLigMove of ligand complexes with proteins. Phytochemical compounds of RO were subjected to molecular docking using PyRx 0.8 software with the AutoDock Vina method then analyzed using Biovia Discovery Studio Visualizer 2021 software. The results show that the binding affinity value of molecular docking ligands with PGE2-R showed CAR (-8.70 kcal/mol), CA(-7.30 kcal/mol), RA(-6.80 kcal/mol), and MA (-8.20 kcal/mol). The binding affinity values of molecular docking of ligands with COX-2 are in the following order: CAR (-7.90 kcal/mol), CA (-7.60 kcal/mol), RA (-7.20 kcal/mol), MA (-8.80 kcal/mol). The binding affinity values of molecular docking of ligands with IL-1β are in the following order: CAR (-8.20 kcal/mol), CA (-8.10 kcal/mol), RA (-6.60 kcal/mol), MA (-7.40 kcal/mol). Finally, a molecular dynamics simulation experiment using YASARA software showed that RMSDAll and RMSDLigMove values of the ligands were better than potassium diclofenac. The study concluded that the phytochemical compounds of *Rosmarinus officinalis* L could inhibit PGE2-R, COX-2, and IL-1β with more negative binding affinity than potassium diclofenac.

Keywords: Molecular docking, Molecular dynamic, Rosmarinus officinalis L, Prostaglandin E2-Receptor, Cyclooxygenase-2, Interleukin-1 beta

Introduction

Osteoarthritis (OA) is a common musculoskeletal condition with synovitis, subchronic bone sclerosis and cartilage loss. Its disease progression depends on several risk factors. Disruption of the cytokine balance that supports proinflammatory cytokines is one of the most important factors in the pathogenesis of OA.[1] There are several inflammatory mediators in the pathogenesis of OA such as Interleukin-1 beta (IL-1β), Tumor Necrosis Factor-alpha (TNF-α) and Interleukin-6 (IL-6),[2] while arachidonic acid metabolites mediate inflammation and pain.[3] Cyclooxygenase (COX) metabolises arachidonic acid to form Prostaglandin H2 (PGH2), and then Prostaglandin E (PGE) synthase will be metabolised PGH2 into Prostaglandin E2 (PGE2).
COX isoforms consist of two kinds, the first one encoded by Prostaglandin-Endoperoxide Synthase (PTGS1) is COX-1, which can be found in most tissues. The second one encoded by PTGS2 is COX-2, it is induced by various cytokines and growth factors.[4,5]

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OA management usually involves the use of non-steroidal anti-inflammatory drugs (NSAIDs) as the primary pharmacological treatments of choice. NSAIDs have often been associated with unwanted side effects when used for a long term[6], such as peptic ulcers (38%), gastrointestinal (GI) bleeding 6%, GI tract erosion 5% and intestinal obstruction (4%).[7] Other adverse effects of NSAIDs affect the gastric mucosa, hematologic system, hepatic system, renal system and cardiovascular system. Gastrointestinal damage is caused by NSAIDs by inhibiting COX-1 which induces a decrease in gastric mucosa production. Nephrotoxicity can also occur and cause a decrease in prostaglandin levels which is essential for the vasodilation of the renal arterioles.[8] Among the various NSAIDs, diclofenac has a higher rate of hepatotoxic effects.[9]
Meanwhile, the anti-inflammatory activity of natural products has attracted wide attention and developed year by year, one of which is Rosmarinic acid (RA). The anti-inflammatory effects of RA have been revealed through *in vitro* and *in vivo* studies of various inflammatory diseases like arthritis, colitis, and atopic dermatitis.[10] Hu *et al* reported an effect of RA on OA in rat chondrocytes that were incubated with RA and isolated from rat cartilage.[11] It was found that RA can inhibit IL-1β and IL-6 secretion. Moreover, it also reported that RA can inhibit the Aggrecan (ACAN) and Collagen 2 (COL2), the main parts of cartilage Extra Cellular Matrix (ECM) gene expression that is induced by IL-1β. The study reported that using supercritical fluid extraction, the main phytochemical yield of RA consisted of Carnosic Acid (CA) (8.30% dry weight), Micromeric Acid (MA) (4.70%) and Carnosol (CAR) (1.00%).[12]
This study used molecular docking and molecular dynamics approaches to identify the binding affinity, hydrogen bonding distance, and Root

Mean Square Deviation (RMSD) of protein-ligand complexes. Molecular docking aims to predict protein-ligand complexes using computer modelling.[13] Molecular docking occurs through two main stages, the process of sample ligand retrieval and protein-ligand complex assessment,[14] the more negative binding affinity value, the stronger the ligand-protein bond. Meanwhile, molecular dynamics (MD) is the simulation of protein-ligand complexes in body systems (*in vivo*) that are influenced by biological factors.[15] It is used to determine the stability of the protein-ligand complex bond.

However, efforts to carry out comprehensive analysis at the molecular level, including target enzymes, are still lacking, and need to be more examined. The use of phytochemicals of *Rosmarinus officinalis* L. (Carnosol, Carnosic acid, Rosmarinic acid, and Micromeric acid) has not been studied specifically regarding the anti-inflammatory effects in inhibiting PGE2, COX-2 and IL-1β as the important inflammatory mediators in OA. In search of novelty, natural, safe, with proven scientific effectiveness and new dimensions of biocompatibility, an *in silico* study was performed to uncover the phytochemical profile of RO that plays a role in inflammatory pain, especially in important inflammation-related pathways via the COX-2, PGE2-R and its effect on IL-1β excretion using molecular docking and molecular dynamic analysis.

Materials and Methods

Protein Modelling and Ligand Preparation

FASTA proteins were downloaded from https://www.ncbi.nlm.nih.gov/ and then modelled using https://swissmodel.expasy.org/interactive. Protein modelling results were selected that had a sequence identity close to 100%. PGE2-R, COX-2, and IL-1β proteins were downloaded in pdb format. The ligands used in this study were downloaded from https://pubchem.ncbi.nlm.nih.gov/ with CID 5315615, 323275, 46886716, 73242194, and 3033. The 3D structures of the ligands were downloaded in pdb format.

Molecular Docking

Molecular docking was processed by the PyRx 0.8 software with the AutoDock Vina method released in 2021. The ligands were inputted in PyRx and were minimised to obtain their pdb format. The target protein(s) was loaded into the PyRx software and maximised. Then the docking was done with the Vina software embedded in PyRx. The data obtained from this process were binding affinity and RMSD data. Drug candidates that have been processed by molecular docking were saved in pdb format.

Combining Ligand Protein Complex

PyMOL Molecular Graphics System, Version 2.0 Schrodinger, LLC released in 2021 software was used to combine the ligand protein complex. It is used to visualize protein molecules in 3D. The criteria for ligand selection is based on the binding affinity value, the more negative the value of the binding affinity the higher the bonding of the ligand and protein. Drug candidates from molecular docking results were inputted into PyMol software and combined with proteins. The ligand-protein complex was then saved in pdb format.

Visualization of Ligand-Protein Complex

The ligand-protein complex was visualized by Biovia Discovery Studio Visualizer Software, Version 21.1.0.20298. It is a software for simulations and mutations of protein systems released in 2021. It is used to visualise the ligand interaction and 2D diagram of the ligand-protein complex. On ligand interaction, hydrogen bond distance analysis was performed.

Molecular Dynamics

The ligand-protein complexes resulting from molecular docking were subjected to molecular dynamics simulation using YASARA (Yet Another Scientific Artificial Reality Application) software Watching Nature@Work ™ 2021 with AMBER14 force field and the system was conditioned in a way similar to the physiological conditions of cells (pH = 7.4, 37°C, 1 atm, and 0.9% salt content) for 20 ns.[16]

Results and Discussion

The molecular docking data of drug candidates with PGE2-R is shown in Table 1. From the table, the drug candidate Carnosol has resulted in a binding affinity of -8.7 kcal/mol which was more than diclofenac potassium (-6.00), Carnosic acid has a binding affinity of -7.30 kcal/mol, also more than diclofenac potassium (-6.00 kcal/mol). Rosmarinic acid (-6.80 kcal/mol) and Micromeric acid (-8.20 kcal/mol) also have binding affinity scores more than diclofenac potassium (-6.00), with each having RMSD values of 0.000 Å. Diclofenac potassium's hydrogen bond distance was 2.30012 Å (N: UNK1:H - N: UNK1:O) and 2.15002 Å (N: UNK1:H - A: ASP90:OD1). The hydrogen bond distance of drug candidate Rosmarinic acid was 2.2053 Å (N: UNK1:H - A: SER171:O). Whereas, in the drug candidate Micromeric acid, the hydrogen bond distance was more than 2.7000 Å and in Carnosol and Carnosic acid no hydrogen bonds were found. In each drug candidate and comparator, hydrophobic bonds were found. Figure 1 shows the visualisation of docking results between the ligands and PGE2-R protein. The drug compound formed hydrogen bonds with amino acid residue A: ASP90 and pi-donor hydrogen bonds to amino acid residue A: SER313. Hydrophilic bonds in the form of stacked amide-pi bonds were formed with amino acid residue A: GLY131 and pi-alkyl bonds with amino acid residues A: CYS312, A: LEU316, A: VAL309, and A: LEU132.

Similarly, the molecular docking data of drug candidates with COX-2 is shown in Table 2. From the data, it was found that Carnosol has a binding affinity of -7.90 kcal/mol, Carnosic acid (-7.60 kcal/mol), Rosmarinic acid (-7.20 kcal/mol) and Micromeric acid (-8.80 kcal/mol) compared to diclofenac potassium (-6.80 kcal/mol), the standard anti-inflammatory agent, with all having RMSD values of 0.000 Å. The hydrogen bond distance of Diclofenac potassium was 2.30075 Å (N: UNK1:H - A: SER105:OG). The screened candidate molecules present with similar hydrogen bond distances. Rosmarinic acid has hydrogen bonds distance of 2.17120 Å (N: UNK1: H - A: VAL577: O) and 2.27156 Å (N: UNK1: H - A: ARG414: O). Carnosol and Micromeric acid have a hydrogen bond distance of more than 2,7000 Å, whereas Carnosic acid has no hydrogen bonds.

Figure 2 shows the visualisation of docking results between ligands and COX-2 protein. In the diclofenac potassium drug compound, a hydrophobic bond was formed in the form of a Pi-Sigma bond with amino acid residue A: VAL74. Pi-Pi T-shaped bond with amino acid residue A: TYR101. Alkyl and pi-alkyl bonds were also formed with amino acid residue A: ILE98. Carnosol formed Pi-sigma bonds with amino acid residue A: ILE174, alkyl and pi-alkyl bonds with amino acid residues A: LYS172 and A: ARG171. Carnosic acid formed Alkyl and Pi-Alkyl bonds with amino acid residues A: PRO148 and A: LEU157. Rosmarinic acid formed unfavourable donor-donor bonds with amino acid residues A: ASP379, A: ILE416, A: ALA581, A: GLN415, A: VAL577, A: PHE173 and pi-alkyl bond with amino acid residue A: PRO378. While Micromeric acid formed alkyl bonds with amino acid residues A: ARG171, A: LYS172, A: ALA581, and A: ILE174. Table 3 shows the molecular docking data of drug candidates with IL-1□. From the data, Carnosol has a very potent binding affinity value of -8.20 kcal/mol, Carnosic acid = -8.10 kcal/mol, Rosmarinic acid = -6.60 kcal/mol and Micromeric acid = -7.40 compared to diclofenac potassium (-6.50 kcal/mo), with RMSD values of 0.000 Å. The hydrogen bond distance of Diclofenac potassium was 2.63026 Å compared to that of Carnosic acid = 2.01787 Å

(N:UNK1:H-A: ILE52:O) and Micromeric acid = 1.73175 Å (N:UNK1:H-A: ASP89:OD2). While Carnosol has a hydrogen bond distance >2,7000 Å, there were no hydrogen bonds with Rosmarinic acid. However, all the molecules and standard agent formed hydrophobic bonds. Figure 3 shows the visualisation of docking results between ligand and IL-1 protein. Diclofenac potassium formed a hydrophobic bond in the form of a Pi-cation bond with amino acid residue A: LYS76 and pi-sulfur with amino acid residues A: LYS73 and A: CYS81. Carnosol formed a pi-anion bond with amino acid residue A: ASP89 and alkyl bonds with amino acid residues A: LYS73 and A: LEU44. Carnosic acid formed alkyl and pi-alkyl bonds with amino acid residues A: PHE93, A: LEU50, and A: ARG51. Rosmarinic acid formed a pi-alkyl bond with amino acid residue A: LEU44. Meanwhile, Micromeric acid formed an unfavourable donor-donor bond with the amino acid residue A: LYS73 and Alkyl and pi-alkyl bonds with amino acid residues A: LYS76 and A: PHE85.

Table 1: Molecular docking data of drug candidates against PGE2-R

Drug Candidates	Binding Affinity	RMSD score	Hydrogen Bond Distance (Å)	Hydrogen Bond	Hydrophobic Bond
Diclofenac potassium [b]	-6.00	0.00	2.30012 2.15002 3.96334	N: UNK1: H – N: UNK1: O N: UNK1: H – A: ASP90: OD1 A: SER313: OG – N: UNK1	N: UNK1 – A: LEU132 N: UNK1 – A: VAL309 N: UNK1 – A: CYS312 N: UNK1 – A: LEU316 A: THR164: CG2 – N: UNK1 A: ALA137 – N: UNK1 A: LEU160 – N: UNK1
Carnosol [a]	-8.70	0.00	-	-	N: UNK1 – A: ILE40 N: UNK1: C – A: ILE140 N: UNK1: C – A: LEU144 N: UNK1: C – A: ILE168 N: UNK1– A: ALA137 A: VAL97 – N: UNK1 N: UNK1 – A: VAL97
Carnosic acid [a]	-7.30	0.00	-	-	A: PHE219 – N: UNK1 A: PHE216 – N: UNK1
Rosmarinic acid [a]	-6.80	0.00	2.20253	N: UNK1: H – A: SER171: O	A: VAL97 – N: UNK1 N: UNK1 – A: LEU124 A: TRP194 – N: UNK1: C
Micromeric acid [a]	-8.20	0.00	3.27979 3.18393	A: THR94: OG1 – N: UNK1: O A: SER98: OG – N: UNK1: O	

[a] Phytopharmaceutical ingredients of *Rosmarinus oficinalis* L as drug candidates
[b] NSAID Drugs as standard drugs

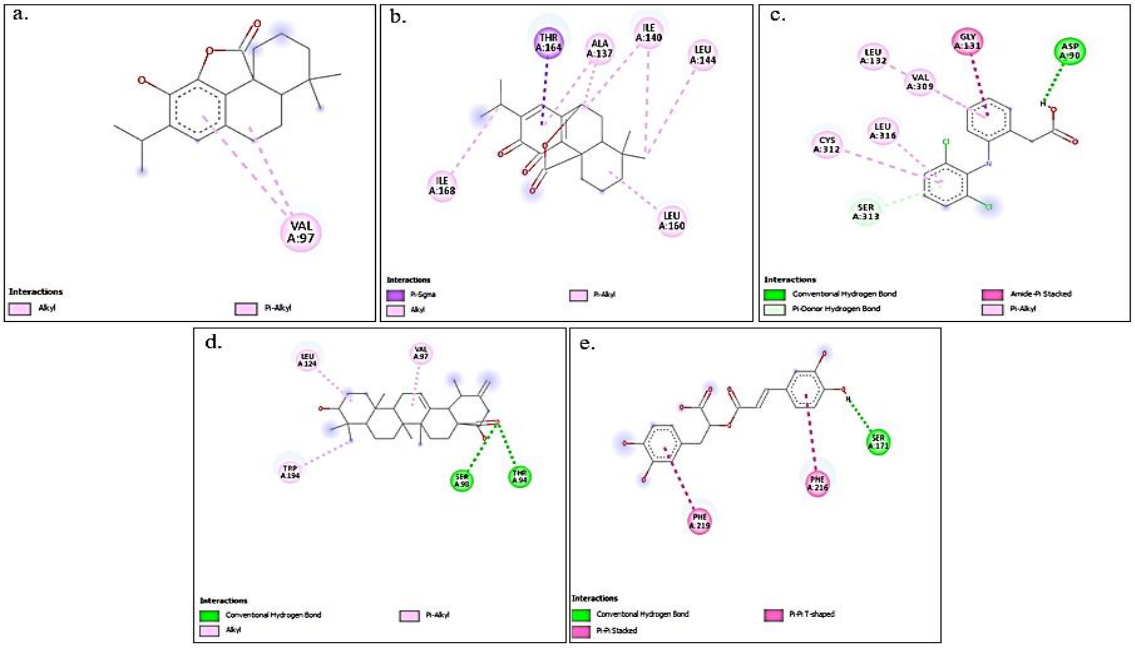

Figure 1: 2D structure diagram of ligand complex and PGE2-R protein docking results. (a) Diclofenac potassium, (b) Carnosol, (c) Carnosic acid, (d) Rosmarinic acid, and (e) Micromeric acid.

The analysis of the RMSD All value (complex-ligand stability) and RMSDLigMove (ligand movement stability when binding to the receptor) molecular dynamic results is presented in Table 4. For the RMSD All value, all protein-compound complexes

show RMSD values more than 3 Å. Figure 4 (a) shows the results of the molecular dynamic RMSDAll value of the ligand_PGE2R complex. The data shows that the RMSDAll values of the ligands Carnosol, Carnosic acid, Rosmarinic acid, and Micromeric acid on average show values of more than 3 Å. The same thing is also shown in the potassium diclofenac_PGE2R drug complex of more than 3 Å. Figure 4 (b) shows the molecular dynamic results in the form of RMSDLigMove. The data shows that the average value of RMSDLigMove in the Carnosol, Carnosic acid, Rosmarinic acid, and Micromeric acid ligand complexes was more than 3 Å. A similar result was observed with Potassium diclofenac with an average RMSDLigMove value of 3 Å. This value indicates that the protein-compound complex binding was not stable.

Table 2: Molecular docking data of drug candidates against COX-2

Drug Candidates	Binding Affinity	RMSD score	Hydrogen Bond Distance (Å)	Hydrogen Bond	Hydrophobic Bond
Diclofenac potassium [b]	-6.80	0.00	3.08510	A: ARG106: NE – N: UNK1: O	A: VAL74: CG1 – N: UNK1
			2.84234	A: ARG106: NH2 – N: UNK1: O	A: VAL74: CG2 – N: UNK1 A: TYR101 – N: UNK1
			2.30075	N: UNK1: H – A: SER105: OG	N: UNK1: Cl – A: ILE98 A: TYR101 – N: UNK1: Cl N: UNK1 – A: ILE98
Carnosol [a]	-7.90	0.00	3.30800	A: ARG419: NH1 – N: UNK1: O	A: ILE174: CD1 – N: UNK1 A: LYS172 – N: UNK1 A: ALA581 – N: UNK1: C
			3.42571	N: UNK1: C – A: ALA581: O	N: UNK1 – A: ILE174 N: UNK1: C – A: ARG171 N: UNK1: C – A: ILE174 N: UNK1 – A: ALA581 N: UNK1: C – A: LEU157 N: UNK1: C – A: VAL141 N: UNK1: C – A: LYS445 A: PRO148 – N: UNK1 N: UNK1 – A: PRO148
Carnosic acid [a]	-7.60	0.00	-	-	
Rosmarinic acid [a]	-7.20	0.00	3.08090	A: THR380: N – N: UNK1: O	
			2.17120	N: UNK1: H – A: VAL577: O	A: VAL577: CG1 – N: UNK1
			2.27156	N: UNK1: H – A: ARG414: O	A: PHE173 – N: UNK1
			3.45699	A: GLN415: CA – N: UNK1: O	N: UNK1 – A: PRO378
			3.56899	A: GLN415: NE2 – N: UNK1	
Micromeric acid [a]	-8.80	0.00	3.26384	A: ARG585: NE – N: UNK1: O	A: ARG171 – N: UNK1
			3.37317	A: ARG585: NH2 – N: UNK1: O	A: LYS172 – N: UNK1 A: ILE174 – N: UNK1 A: ALA581 – N: UNK1: C
			2.93919	A: ARG585: NH2 – N: UNK1: O	N: UNK1: C – A: ARG171 N: UNK1: C – A: LYS172 N: UNK1: C – A: ILE174

a Phytopharmaceutical ingredients of Rosmarinus oficinalis L as drug candidates
b NSAID Drugs as standard drugs

Table 3: Molecular docking data of drug candidates against IL-1β

Drug Candidates	Binding Affinity	RMSD score	Hydrogen Bond Distance (Å)	Hydrogen Bond	Hydrophobic Bond
Diclofenac potassium [b]	-6.50	0.00	2.63026	N: UNK1: H – A: GLE83: O	N: UNK1 – A: LYS76 N: UNK1 – A: LYS73
Carnosol [a]	-8.20	0.00	3.67127	A: PHE85: CA – N: UNK1: O	A: LYS73 – N: UNK1 N: UNK1 – A: LEU44
Carnosic acid [a]	-8.10	0.00	2.01787	N: UNK1: H – A: ILE52: O	N: UNK1: C – A: ARG51 A: LEU50 – N: UNK1
			3.84868	A: ILE52: N – N: UNK1	A: ARG51 – N: UNK1 N: UNK1 – A:ARG51 A: PHE93 – N: UNK1 A: PHE93 – N: UNK1: C
Rosmarinic acid [a]	-6.60	0.00	-	-	N: UNK1 – N: UNK1 N: UNK1 – A: LEU44 A: LYS73 – N: UNK1
Micromeric acid [a]	-7.40	0.00	1.73175	N: UNK1: H – A: ASP89: OD2	A: LYS76 – N: UNK1 N: UNK1: C – A: LYS76 A: PHE85 – N: UNK1C

[a] Phytopharmaceutical ingredients of *Rosmarinus oficinalis* L as drug candidates
[b] NSAID Drugs as standard drugs

Figure 5a shows the molecular dynamic results of the ligand and COX-2 protein complex. From these data, it was found that the average RMSDAll value of Carnosol, Carnosic acid, Rosmarinic acid, and Micromeric acid compounds was more than 3 Å. The same result was observed with Potassium diclofenac with an RMSDAll value of more than 3 Å. Figure 5b shows the molecular dynamic results of the ligand and COX-2 protein complex. From these data, it was found that the average RMSDLigMove value for Carnosol, Carnosic acid, Rosmarinic acid, and Micromeric acid was more than 3 Å. The same result was observed with Potassium diclofenac with an RMSDLigMove value of more than 3 Å. This value indicates that the protein-compound complex binding was not stable.

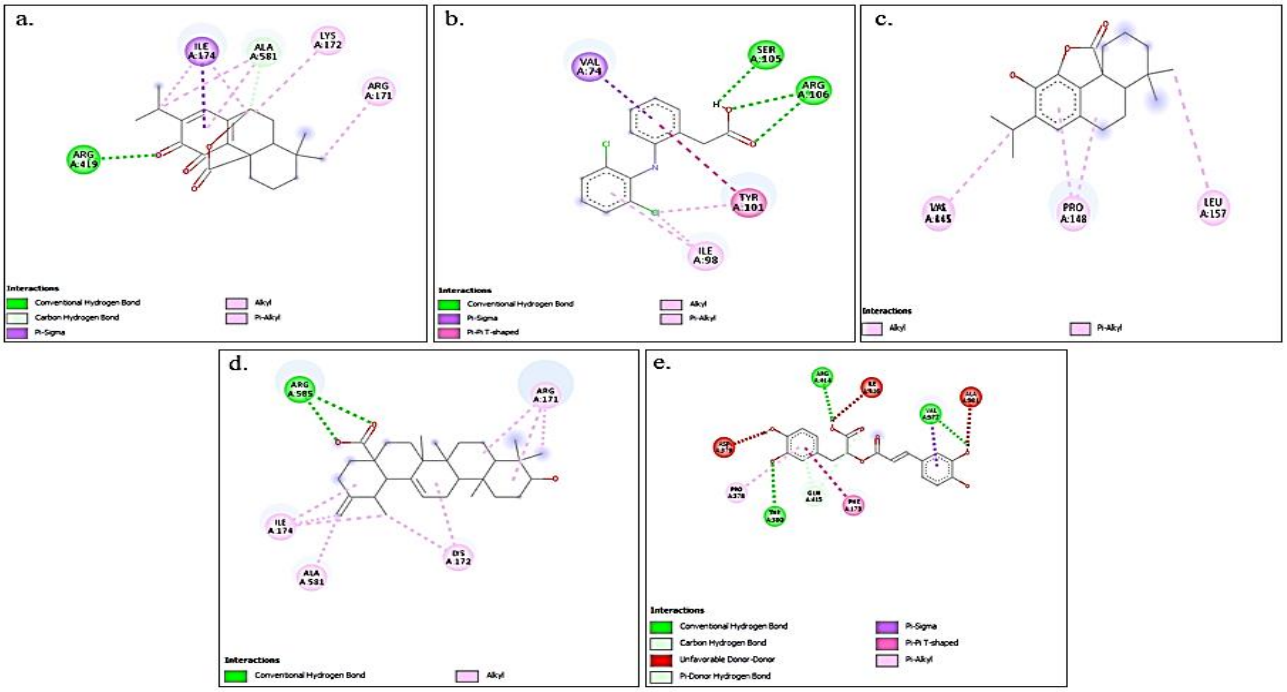

Figure 2: 2D structure diagram of ligand complex and COX-2 protein docking results. (a) Diclofenac potassium, (b) Carnosol, (c) Carnosic acid, (d) Rosmarinic acid, and (e) Micromeric acid.

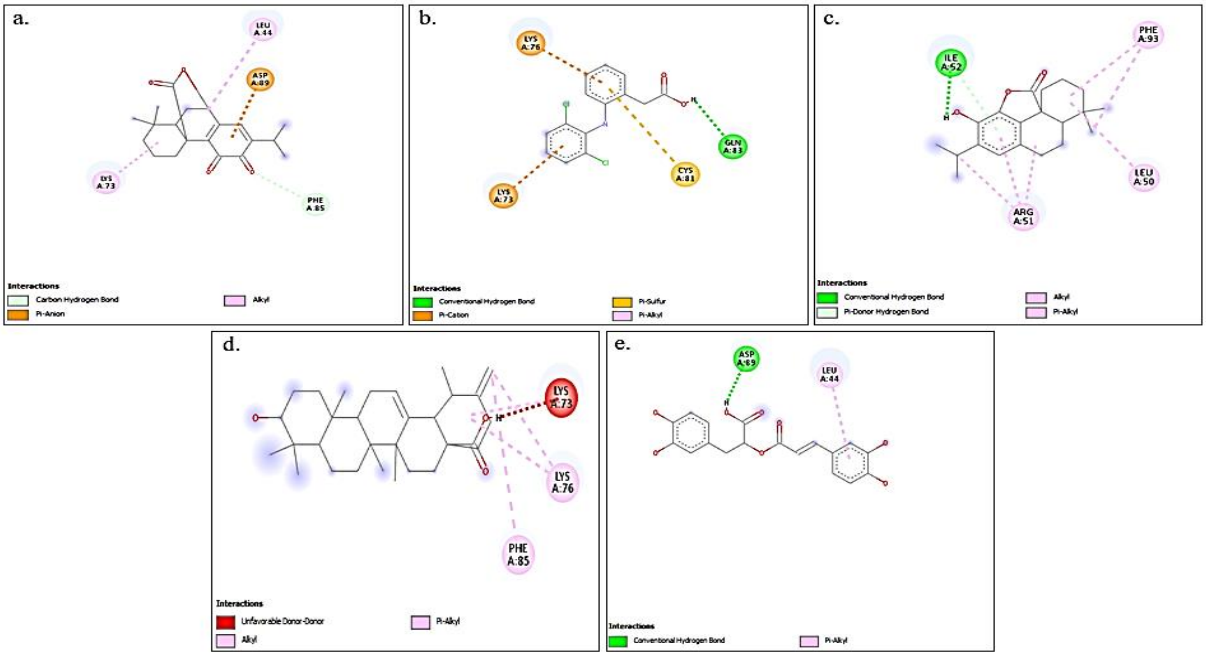

Figure 3: 2D structure diagram of ligand complex and IL-1□ protein docking results. (a) Diclofenac potassium, (b) Carnosol, (c) Carnosic acid, (d) Rosmarinic acid, and (e) Micromeric acid

Figure 6a shows the results of the molecular dynamic RMSDAll value of ligand_IL-1□ complex. The data shows that the RMSDAll values of Carnosic acid, Rosmarinic acid, and Micromeric acid ligands on average show values of more than 3 Å, the same as diclofenac potassium. In the Carnosol ligand, the RMSDAll value was more than 3 Å, compared to diclofenac

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potassium. The RMSDAll value of Carnosol was lower. Figure 6b shows the result of the molecular dynamic RMSDLigMove value of ligand_IL-1□ complex. The data shows the RMSDLigMove value of Carnosic acid and Rosmarinic acid ligands on average of more than 3 Å with the same as diclofenac potassium. In Carnosol and Micromeric acid ligands, the RMSDLigMove value was more than 3 Å when compared to diclofenac potassium, the RMSDLigMove value of Carnosol was lower. Molecular docking was used to predict the model of interaction between molecules of drug candidate and protein target at the atomic level. This method allows researchers to characterise the behaviour of the drug candidate molecule at the binding site of the protein target. In addition, it can be used to elucidate the basic biochemical process between the drug candidate molecule and the target protein.[17] The molecular docking process involves two basic processes: prediction of the ligand's conformation, position and orientation at the site (pose), also assessment of binding affinity.[18] Meanwhile, molecular dynamic simulation is effectively used to assess the structure-function relationship of drug candidate molecule complexes with proteins.19 Molecular docking simulations between drug candidate molecules and proteins are shown in the following order PGE2-R (Table 1), COX-2 (Table 2), and IL-1□ (Table 3). I t showed binding affinity and RMSD values of drug candidate compounds (Carnosol, Carnosic acid, Rosmarinic acid, and Micromeric acid) are higher than the control (Diclofenac potassium) in complexes with PGE-2, COX-2 and also IL-1□. It shows that the drug candidate compounds can compete for PGE2 binding to PGE2-R so that an inflammatory cascade is not formed.20 In addition, Carnosol, Carnosic acid, Rosmarinic acid, and Micromeric acid compounds can inhibit COX-2 and IL-1□ which results in the inhibition of the inflammatory cascade.21,22 The RMSD value of the molecular docking simulation of PGE2-R, COX-2, and IL-1□ showed a value of 0.00 Å (Table 1-3) which is less than 2.00 Å. This RMSD value indicates the validity of the molecular docking simulation process of drug candidate molecules with PGE2-R protein is good. RMSD value is the value of the difference between the crystal coordinates of the ligand with the predicted coordinates of molecular docking simulation results to validate the molecular docking simulation process. RMSD value is considered good if < 2.00 Å.23 Hydrogen bonds were formed in the molecular and protein complexes of PGE2-R (Table 1), COX-2 (Table 2), and IL-1□ (Table 3). There was one hydrogen bond with a bond distance < 2.7 Å in the Rosmarinic acid complex with PGE2- R (Table 1). While in the complex of Rosmarinic acid with COX-2, two hydrogen bonds were formed with a bond distance < 2.7 Å (Table 2). Table 3 shows that there are two complexes, namely Carnosic acid with IL-1□ and Micromeric acid with IL-1□, which have one hydrogen bond with a bond distance < 2.7 Å. Hydrogen bonds are considered stable and have a strong strength and must have a bond distance < 2.7 Å. The hydrogen bond formed will have weak stability and break easily if the hydrogen bond distance is > 2.7 Å.24 Weak and strong hydrogen bonds can become even more stable due to the presence of pi bonds, covalent bonds, and hydrophobic bonds. These bonds serve to stabilise weak or strong hydrogen bonds.25 Hydrogen bonds affect the binding energy value. The greater number of hydrogen bonds formed in the protein molecular complex causes a decrease in the binding energy value. This will cause a decrease in the inhibition constant which results in only a small concentration of ligand needed to inhibit the protein.26 Hydrogen bonds are bonds between hydrogen atoms in one molecule with one element (N, O, F) in another molecule and are the strongest dipole-dipole forces.27 Hydrogen bonds play an important role in determining the structure, biological properties, cell organisation and function of a molecular complex.[28]

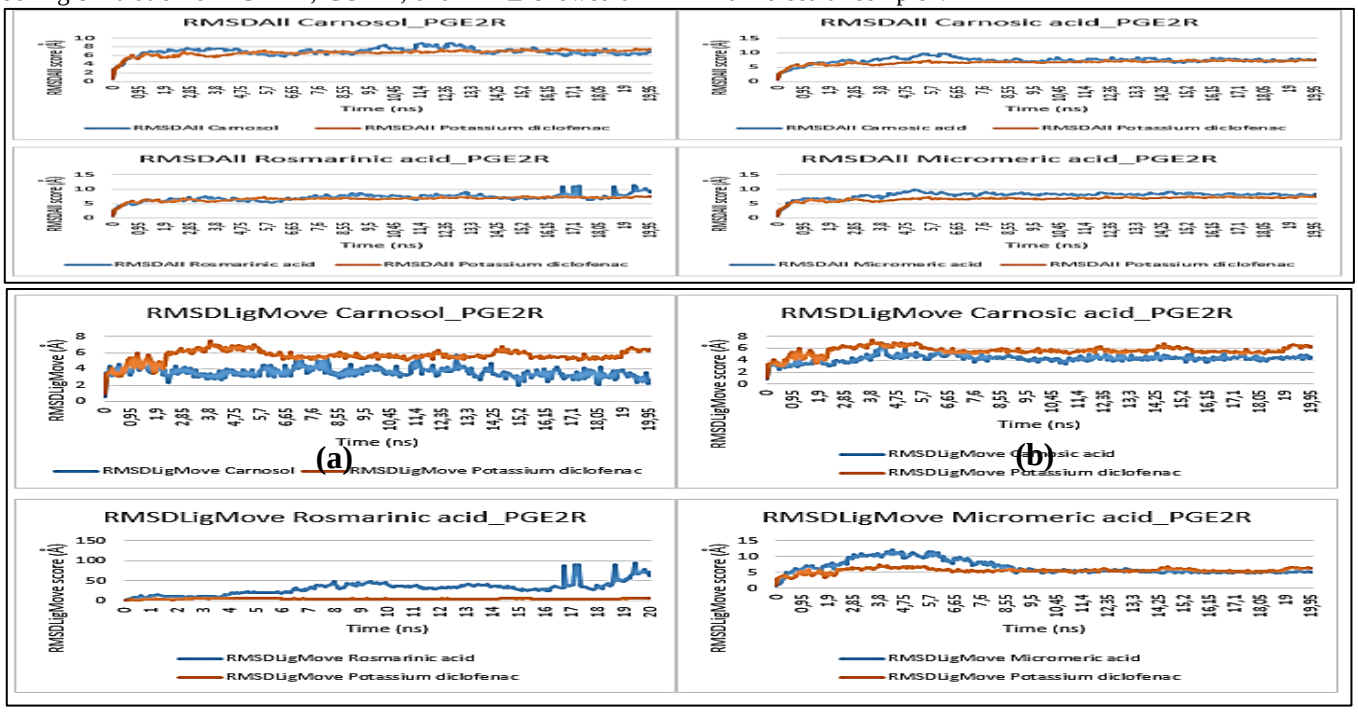

Figure 4: Molecular dynamics of ligand complex with PGE2-R. (a) RMSDAll (complex-ligand stability) value of complex ligand with PGE2-R, (b) RMSDLigMove (ligand movement stability when binding to the receptor) value of complex ligand with PGE2-R.

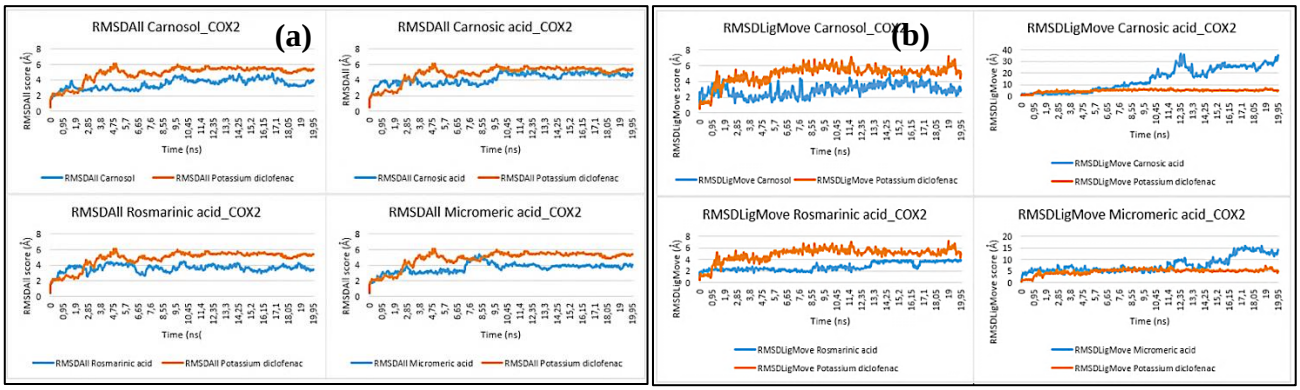

Figure 5: Molecular dynamics of ligand complex with COX-2. (a) RMSDAll (complex-ligand stability) value of complex ligand with COX-2, (b) RMSDLigMove (ligand movement stability when binding to the receptor) value of complex ligand with COX-2.

Molecular dynamics simulation shows the RMSDAll value that can assess the stability of the complex and ligand. The RMSDAll value of the PGE2-R_ligand complex shows an average value of more than 3 Å. A compound can be accepted in the body system if it has an RMSDAll value of less than 3 Å.19 This indicates that the ligand as a drug candidate has low binding stability with the PGE2-R protein complex. The RMSDAll values of the ligands were similar to the anti-pain drug potassium diclofenac. The COX-2 complex also showed an RMSDAll value of more than 3 Å. When compared to the anti-inflammatory drug potassium diclofenac, the RMSDAll value of the ligands was still below the RMSDAll value of potassium diclofenac. This indicates that the ligand's binding stability with the COX-2 complex was better than the binding stability of potassium diclofenac with the COX-2 complex.

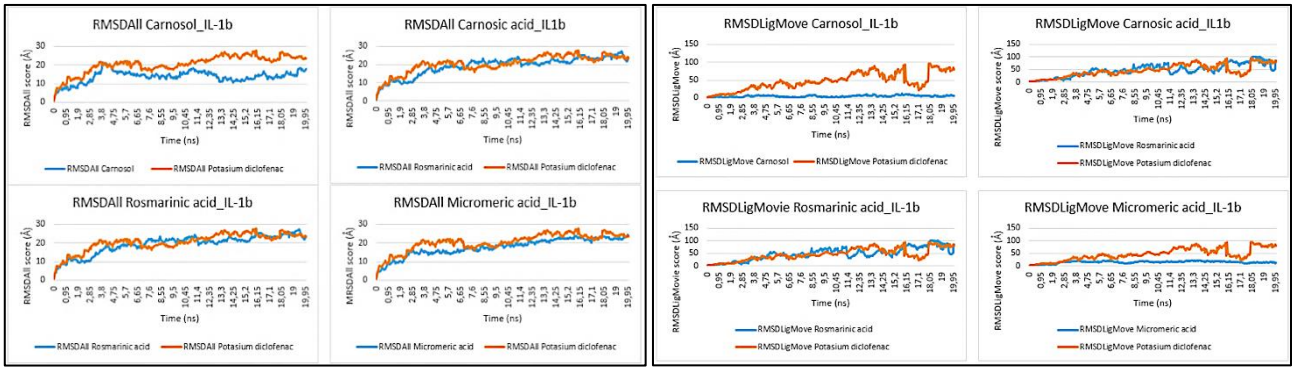

Figure 6: Molecular dynamics of ligand complex with IL-1β. (a) RMSDAll (complex-ligand stability) score of the complex ligand with IL-1*β*, (b) RMSDLigMove (ligand movement stability when binding to the receptor) score of the complex ligand with IL-1*β*.

Conclusion

The phytopharmaceutical ingredients of *Rosmarinus officinalis* L (Carnosol, Carnosic acid, Rosmarinic acid, and Micromeric acid) could inhibit PGE2-R, COX-2, and IL-1*β* with more negative binding affinity than potassium diclofenac in this *in silico* study. Molecular dynamic modelling showed that the bonds of Carnosol, Carnosic acid, Rosmarinic acid, and Micromeric acid with proteins PGE2-R, COX-2, and IL-1*β*, respectively were more stable than the bonds of potassium diclofenac with these proteins. This study concluded that *Rosmarinus officinalis L.* can be used as a drug candidate for osteoarthritis through inhibition of PGE2, COX-2, and IL-1*β* receptors.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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