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An Insight of Co-Encapsulation *Nigella sativa* and *Cosmos caudatus* Kunth Extracts as Anti-Inflammatory Agent Through *In Silico* Study

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Abstract

This study analyzes anti-inflammatory activity from extracts of *Nigella sativa* and *Cosmos caudatus* Kunth co-encapsulated through *in silico* molecular docking. The LC-MS results revealed that extracts of *N. sativa* mostly contained thymoquinone and alpha-hederin, whereas quercetin and kaempferol were the major compounds in *C. caudatus* K. Nevertheless, the bioactive compounds are usually susceptible to degradation by exposure to light, heat, oxygen, which may limit its biological activity. Therefore, encapsulation is one of the promising techniques to protect bioactive compounds. Ligands were encapsulated with chitosan and sodium tripolyphosphate as wall materials. Cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) as the target enzymes were docked with a combination of these active compounds (non-encapsulated and encapsulated), using the HEX 8.0 program, and visualized using the Discovery studio visualizer software v16.1.0.15350. Interestingly, docking results of the combination of encapsulated ligands showed no interactions to COX-1 but interacted with COX-2. Therefore, co-encapsulation of extracts combinations has been suggested to act as anti-inflammatory agents targeted specifically to the COX-2 enzyme. The total energy of the encapsulated of combination of extract compounds to COX-2 were -1425.88 (mol/cal) for thymoquinone + quercetin; -1435.87 (mol/cal) for thymoquinone + kaempferol; 1175.97 (mol/cal) for quercetin + alpha hederin; -957.74 (mol/cal) for kaempferol + alpha hederin; and -283.3 (mol/cal) for diclofenac sodium, as a control NSAID drug. These suggest that encapsulated active compounds in *N. sativa* and *C. caudatus* K. have potency as a drug candidate for the selective NSAIDs category, which can be subjected to further *in vitro* and *in vivo* studies.

1. Introduction

Black cumin (*N. sativa*) is an herbal plant found in the Mediterranean and Southeast Asian countries, including Indonesia. The active compounds in black cumin have anticancer, antioxidant, and anti-inflammatory activity [1, 2, 3, 4]. Apart from black cumin, another medicinal plant with various benefits is *C. caudatus* K., locally known as *kenikir*. *C. caudatus* K. is a plant originating from the plains of America, and currently, its distribution is extensive, especially in tropical regions, *i.e.*, Indonesia [5, 6]. The bioactivity of the active compounds in *C. caudatus* K has great benefits, including antioxidant, antibacterial, antiedema, antifungal, anti-inflammatory, anti-tumor, and antiviral [7, 8].

The stability of natural bioactive compounds, *i.e.*, preservation of their functional properties, could be improved using encapsulation techniques, such as spray drying, spray cooling, coacervation, extrusion, and polymerization [9, 10]. Encapsulation is the process of coating the core material in solid, liquid, or gas particles using a coating material. This process aims to protect the core material in bioactive compounds from various environmental influences such as light, oxygen, water, and temperature [9, 10]. The encapsulation technique can

also increase the absorption of the drug when entering the body; hence, drugs can function optimally [10]. The coatings on encapsulation can be conducted using the ionic gelation technique, prepared using crosslinker polymer [11]. Encapsulation can be carried out by chitosan and sodium tripolyphosphate (Na-TPP), which act as crosslinker and coating agents that will protect the active compounds [11].

Drug activity in the human body plays an essential role in preventing and treating diseases. One of them is the anti-inflammatory activity of a drug which has a vital role in the early prevention of disease. Chronic inflammation is closely related to various diseases, such as arthritis, atherosclerosis, and cancer [12]. Inflammation is the sign of pathological or abnormality in tissue to alert as a trouble signal to the system. The inflammatory treatment strategy is to involve non-steroid drugs that act on the arachidonic acid pathway by inhibiting both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes [13, 14]. COX-1 is present in many tissues and produces prostaglandins that predominantly regulate normal cellular activities [15]. COX-2 activity is usually undetectable in most tissues but can be rapidly induced by proinflammatory cytokines or growth factors. Inhibition of COX-2 prevents high levels of local production of prostanoids, which results in reduced pain, edema, inflammation, and fever [16]. The selective NSAIDs inhibit COX-2 favorably, hence, reducing the unwanted effects of nonselective NSAIDs on the upper GI tract mucosa [17].

To the best of our knowledge, there are no studies of co-encapsulation for the combination of extracts from *N. sativa* and *C. caudatus* K. This approach allows management of the biologically active compounds of both *N. sativa* and *C. caudatus* K., in a single formulation, with many applications. One of those is as an anti-inflammatory candidate.

As an initial study, *in silico* analysis, drug candidates must be applied to determine their anti-inflammatory activity [18, 19]. *In silico* molecular docking demonstrates an interaction between two or more molecules in the most stable state and can be used as an initial examination in determining drug candidates based on the interaction of the proposed drug (ligands) with the drug target [20]. This study aims to determine the effect of the combination of active compounds on *N. sativa* and *C. caudatus* K. co-encapsulated with chitosan and Na-TPP in inhibiting COX-1 and COX-2 enzymes by *in silico* molecular docking to determine their anti-inflammatory activity. Molecular docking was carried out on the main bioactive compounds in the extracts of *N. sativa* and *C. caudatus* K. The presence of the compounds in *N. sativa* and *C. caudatus* K. is analyzed with liquid chromatography-mass spectroscopy (LC-MS).

2. Methodology

2.1. Extraction and LC-MS Analysis

The *N. sativa* and *C. caudatus* K. dried plants were obtained from Materia Medica, Batu, East Java, Indonesia. The species of the plants was confirmed by the letter of species determination, signed by a taxonomist from the herbarium unit of Materia Medica. The dried plants were crushed into powder. Ethyl alcohol pro analysis (p.a) was purchased from Sigma-Aldrich. The extracts of *N. sativa* and *C. caudatus* K. were prepared by the maceration method. A 100 gram of dried plant powder was immersed in 400 mL ethyl alcohol for 3 x 24 h. The resultant extracts were filtered using filter paper. The obtained filtrate was then concentrated with a rotary evaporator vacuum at 50°C. Extracts were stored at 4°C for further analysis.

The extracts from *N. sativa* and *C. caudatus* K were characterized using LC-MS. Liquid chromatographic separation was conducted in the analytical and instrumental chemistry laboratory of the Chemical Engineering Department, Politeknik Negeri Malang (POLINEMA). Columns used were a Hypersil Gold (50 mm x 2.1 mm x 1.9 µm), UHPLC brand ACCELLA type 1250 made by Thermo Scientific, consisting of a vacuum degasser and a quartener pump, a thermostatic autosampler controlled by a PC through the X-Calibur 2.1 program. Solvents used were solvent A = 0.1% formic acid in water and solvent B = 0.1% formic acid in acetonitrile. A mobile phase gradient was applied, with a flowrate of 300 µl/min, with the following settings: 0.0-0.6 min 15% B; 0.6-5.0 min 65% B; 3.0-3.5 min 95% B; 3.5-4.0 min 95% B; 4.0-4.5 min15% B and 4.5-6.0 min 15% B. The injection volume was 2 µl. The column was controlled at 30°C, and the autosampler compartment was set for 16°C. The MS/MS Triple Q (quadrupole) mass spectrometer was TSQ QUANTUM ACCESS MAX from Thermo Finnigan with ESI (electrospray ionization) ionization method. The source was controlled by TSQ Tune software, operated in positive ion mode. The ESI ionization conditions were as follows: 3 kV spray voltage; evaporation temperature 250°C; capillary temperature 300°C; nitrogen as sheath gas pressure at 40 psi, and aux gas pressure 10 psi with argon gas.

2.2. Ligands Encapsulation with Chitosan and Na-TPP

The preparation of co-encapsulation of *N. sativa* and *C. caudatus* K. extracts was carried out by weighing 500 mg of extracts mixture in a 1:1 ratio. The extracts were then dissolved in 17.5 mL of ethyl alcohol, then a 50 mL of 1% chitosan solution (v/v) in glacial acetic acid solution was added. An aliquot of Na-TPP solution (200 mL, 0.5%) was added gradually while stirring at a steady speed for one hour. The microencapsulated colloid of *N. sativa* and *C. caudatus* K. extracts was freeze-dried until the solution became microcapsules powder.

2.3. Ligands and Receptor Preparation

The structure of ligands was obtained from the PubChem NCBI database. The ligands used were compounds from *N. sativa* and *C. caudatus* K extracts: thymoquinone (CID: 10281), quercetin (CID: 5280343), kaempferol (CID: 5280863), alpha-hederin (73296), chitosan (CID: 71853), sodium tripolyphosphate (CID: 24455), and diclofenac sodium (CID: 5018304). The three-dimensional structures of ligands were prepared by minimizing the binding energy using PyRx software and change the SDF format to PDB format. Protein Data Bank (PDB) from https://www.rscb.org was used to acquire a

database to get the three-dimensional structure of COX-1 (PDB ID: 6Y3C) and COX-2 (PDB ID: 5F19). Water and other ligands bound to receptor target were removed using Discovery studio visualizer v16.1.0.15350 software [21].

2.4. Molecular Docking

COX-1 and COX-2 enzymes were docked to ligand combinations from *N. sativa* and *C. caudatus* K. extracts without encapsulation (a1-d1). For the encapsulated ligands, ligands combinations were docked to chitosan first. Thus, a ligand combination-chitosan was formed. After that, the ligan combination-chitosan complexes were docked with Na-TPP, resulting in ligand combinations-chitosan-Na-TPP complexes. This microcapsule complex was then docked to the target enzymes, COX-1 or COX-2. The encapsulated ligands with chitosan and Na-TPP compounds were designated as a2, c2, and d2; and diclofenac sodium as a reference ligand was also docked to COX-1 and COX-2 enzyme and designated as e. Molecular docking was established using Hex 8.0.0 software. Analyses of the molecular interactions of all ligands and receptors were conducted by Discovery studio visualizer v16.1.0.15350 software [21]. The amino acid residues, hydrogen bonds, van der Waals forces, and energy binding in the interactions between ligands and receptors were analyzed. The two-dimensional ligands and receptor interactions were visualized by the LigPlot+ program [22].

3. Results and Discussion

Extracts of *N. sativa* seed and *C. caudatus* K. leaves were characterized by the LC-MS analysis (Figures 1-2). The major compounds in *N. sativa* extract were thymoquinone and alpha-hederin. *C. caudatus* K. extracts were mostly contained quercetin and kaempferol as their active compounds (Figure 2). The retention time (RT) for the active compound shows peak of thymoquinone ($C_{10}H_{12}O_2$) at RT = 0.76 min, m/z 164.50-165.50, alpha-hederin ($C_{41}H_{66}O_{12}$) had RT = 1.37 min, m/z 751.50-752.50 (Figure 1). The peak of quercetin ($C_{15}H_{10}O_7$) appeared at RT = 2.70 min, m/z 300.50-301.50; kaempferol ($C_{15}H_{10}O_6$) peak at RT = 2.79 min, m/z 285.50-286.50 (Figure 2). These mass spectra agreed with the previously published literature reporting of mass spectrometry of thymoquinone, alpha-hederin [23], quercetin, and kaempferol [24]. The prominent peaks in the active compounds of each extract resulted from the LC-MS analysis became the standard for selecting compounds (ligands) in the subsequent *in silico* study.

The ligands-receptor interaction presented by the binding sites on amino acid residues and the types of formation of chemical bonds is shown in Figures 3 and 4. Five hydrogen bonds, two electrostatic bonds, and seven hydrophobic bonds, with the amino acid residues involving Gln44, Gly471, Lys468, His43, Arg83, Leu123, Tyr64, and Val119 were shown in the interactions of non-encapsulated thymoquinone + quercetin to COX-1, with the binding energy of -303.19 cal/mol (Figure 3a1). In the interactions of non-encapsulated thymoquinone + kaempferol to COX-1, there were two hydrogen bonds and eight hydrophobic bonds with amino acid residues Arg120, Val119, Pro84, Leu115, and Val116, and had -299.29 cal/mol binding energy (Figure 3b1). The amino acid residue Arg120 is located close to the active site of the COX-1 enzyme and is one of the amino acid residues that affect COX-1 inhibition [25]. In Figure 3c1, the binding energy of -406.53 cal/mol resulted in a complex of non-encapsulated quercetin + alpha hederin, with four hydrogen bonds and ten hydrophobic bonds formed from Leu115, Leu123, Arg83, Val119, Arg79, and Tyr64 (Figure 3c1). Four hydrogen bonds and one Pi-sigma bond in a non-encapsulated kaempferol + alpha-hederin interacted with Gly552, and the binding energy was -177.40 cal/mol (Figure 3d1). Interestingly, when encapsulated ligands and diclofenac sodium were docked to COX-1, as shown in Figures 3a2, 3b2, 3c2, 3d2, and 3e, there were no bonds formed between those ligands and receptor target, which were also confirmed by the two-dimensional conformation. The value of the resulted binding energy from the molecular dockings may occur from the presence of bonds within ligands and the solvent's influence during the docking process [26].

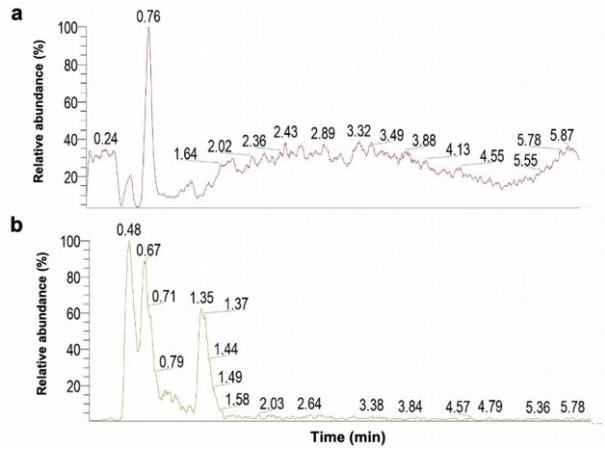

Figure 1. The LC-MS results from *N.sativa* seed extract: (a) thymoquinone; (b) alpha-hederin.

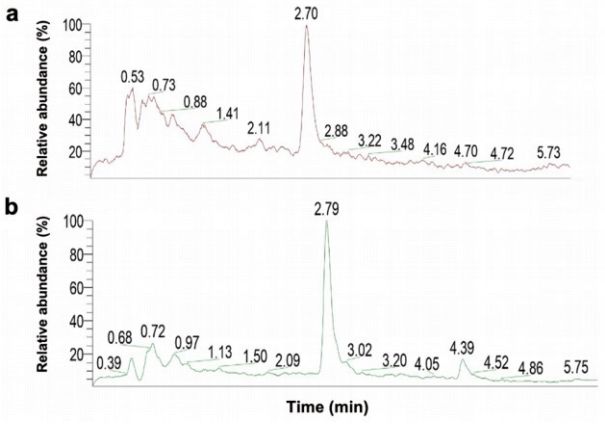

Figure 2. The LC-MS results from *Cosmos caudatus K* leaves extracts: (a) quercetin; (b) kaempferol.

The interaction of both non- and encapsulated ligands to COX-2 enzyme is shown in Figure 4, and the type of chemical bonds formed is listed in Table 1. There were four hydrogen bonds and nine hydrophobic bonds, with amino acid residues involving His207, Gln454, His214, Val 291, Val447, His386, and His388 in the interactions of non-encapsulated thymoquinone +

quercetin to COX-2 (Figure 4a1). In the interactions of non-encapsulated thymoquinone + kaempferol to COX-2, three hydrogen bonds and ten hydrophobic bonds with the amino acid residues involved His388, Gln203, His386, Leu294, Val291, Val447, His207, and His214 (Figure 4b1). Figure 4c1 (quercetin + alpha-hederin) showed seven hydrogen bonds and six hydrophobic bonds with amino acid residues involving His351, Ser581, Ser579 Asp347, Gly354, Glu346, Lys358, Pro514, His95, and His356 (Figure 4c1). Four hydrogen bonds, two electrostatic bonds, and six hydrophobic bonds with the amino acid residues involved Gln370, His122, Ser126, Arg61, Pro127, Lys137, and Trp139, the interaction of non-encapsulated kaempferol + alpha-hederin interacted with COX-2 (Figure 4d1). The binding energies of encapsulated thymoquinone + quercetin, thymoquinone + kaempferol, quercetin + alpha-hederin, and kaempferol + alpha-hederin to COX-2 enzyme were -331.10 cal/mol, -283.03 cal/mol, -418.20 cal/mol, and -402.92 cal/mol, respectively.

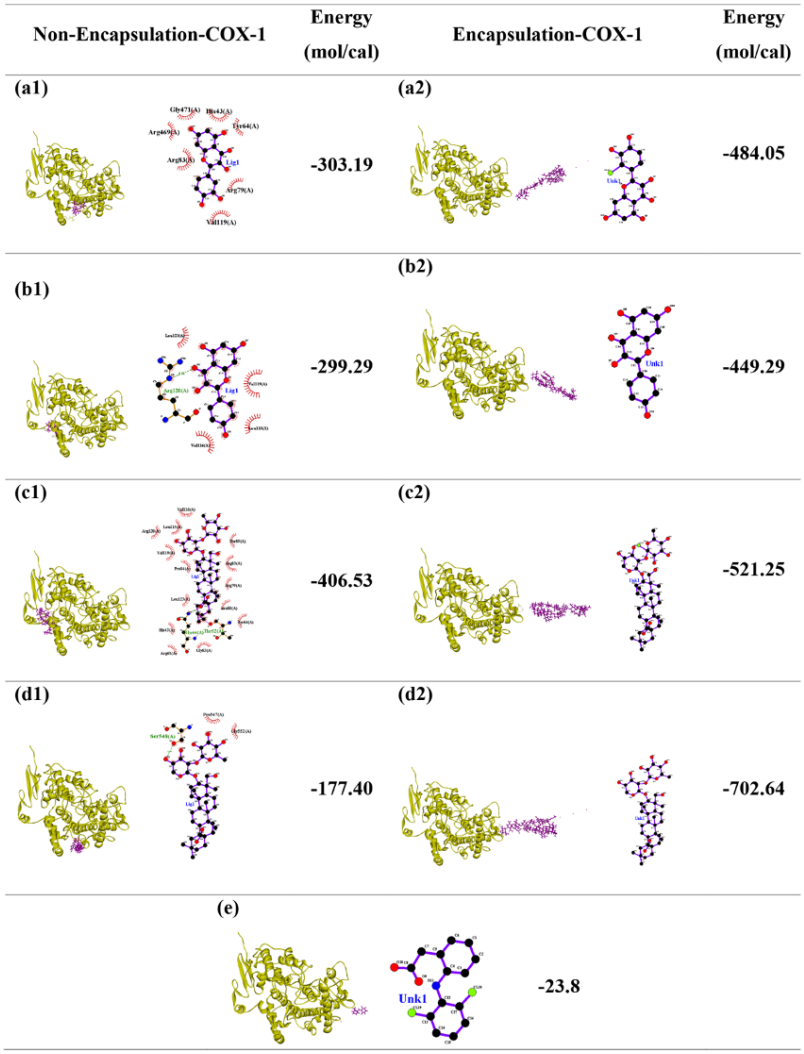

Figure 3. Molecular interaction between COX-1 non-encapsulated (1) and encapsulated (2) ligand combination from: (a) thymoquinone + quercetin; (b) thymoquinone + kaempferol; (c) quercetin + alpha-hederin; (d) kaempferol + alpha-hederin; and (e) diclofenac sodium. Encapsulated ligands are when ligands are docked to chitosan and Na-TPP, then docked to enzyme target.

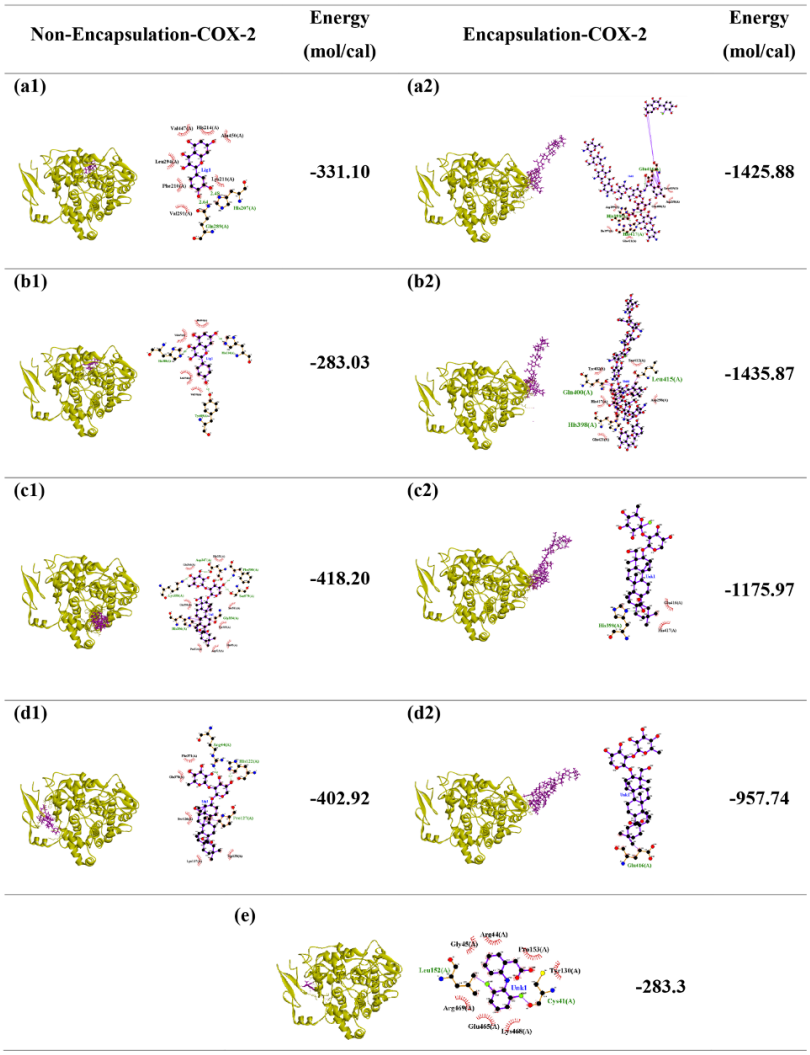

Figure 4. Molecular interaction between COX-2 non-encapsulated (1) and encapsulated (2) ligand combination from: (a) thymoquinone + quercetin; (b) thymoquinone + kaempferol; (c) quercetin + alpha-hederin; (d) kaempferol + alpha-hederin; and (e) diclofenac sodium. Encapsulated ligands are when ligands are docked to chitosan and Na-TPP, then docked to enzyme target.

In the encapsulated ligands against COX-2, as shown in Figures 4a2-4d2, lower binding energies (more negative) than those in the interaction between non-encapsulated ligands and COX-2. This means that encapsulated ligands interacted stronger with COX-2 than non-encapsulated ligands to COX-2.

There were seven hydrogen bonds, two electrostatic bonds, and other bonds (Pi-lone pair type), with amino acid residues involving Asp258, Asp399, Glu416, Leu415, and His282 in the encapsulated thymoquinone + quercetin docked to COX-2 (-1425.88 cal/mol). There were two hydrogen bonds, four electrostatic bonds, and two hydrophobic bonds with amino acid residues involving Glu516, Glu424, His417, Tyr402, Glu416, and His398 in the interactions of encapsulated thymoquinone + kaempferol to COX-2, with -1435.87 cal/mol binding energy (Figure 4b1). When encapsulated quercetin + alpha hederin bound COX-2, the binding energy was -1175.97 cal/mol, resulted in seven hydrogen bonds, five electrostatic bonds, and one hydrophobic bond with the amino acid residues involved Asp399, Glu416, Glu424, Gln400, Leu415, Asp258, Thr420, and His417 (Figure 4c2). In Figure 4d2, encapsulated kaempferol + alpha hederin had formed three hydrogen bonds, two electrostatic bonds, one hydrophobic bond, and two metal-acceptor type bonds to the amino acid residues involving Glu416, His398, Asp399, Val295, Phe200, and Ile413, and had -957.74 cal/mol binding energy.

Interaction between a control ligand, diclofenac sodium, and COX-2 resulted in 6 hydrophobic bonds and two metal-acceptor bonds in the amino acid residues Ser121, Ile124, Arg469, Cys41, Arg44, Leu152, Arg469, and Leu152. Intriguingly, the binding energy of diclofenac sodium to COX-2 was higher than those of all combinations of encapsulated ligands at -283.3 cal/mol, suggesting that encapsulated ligands have more interactions to COX-2 than diclofenac sodium. Nevertheless, sodium diclofenac is one of the nonselective NSAIDs with the utmost COX-2 selectivity [18].

Table 1. Ligand interaction of COX-2 with non-encapsulated (1) and encapsulated (2) ligand combination of: (a) thymoquinone + quercetin; (b) thymoquinone + kaempferol; (c) quercetin-alpha hederin; (d) kaempferol + alpha-hederin; and (e) diclofenac sodium.

Label	Interaction	Chemistry bond	Label	Interaction	Chemistry bond
(a1)	A:HIS207:HD1 - :LIG:O	Hydrogen bond	(a2)	:UNK1:P6 - A:ASP258:OD2	Electrostatic
	A:GLN454:HE22 - :LIG1:O	Hydrogen bond		:UNK1:P10 - A:ASP258:OD2	Electrostatic
	A:HIS207:CE1 - :LIG1:O	Hydrogen bond		:UNK1:H - A:ASP399:OD2	Hydrogen bond
	A:HIS214:CE1 - :LIG1:O	Hydrogen bond		:UNK1:H - A:ASP258:OD2	Hydrogen bond
	A:VAL291:CG1 - :LIG1	Hydrophobic		:UNK1:H - A:GLU416:OE2	Hydrogen bond
	A:VAL291:CG2 - :LIG1	Hydrophobic		:UNK1:H - A:LEU415:O	Hydrogen bond
	:LIG1:C – a:VAL447	Hydrophobic		:UNK1:O91 - A:HIS282	Other bond (Pi-lone pair type)
	A:HIS207 - :LIG1:C	Hydrophobic			
	A:HIS214 - :LIG1:C	Hydrophobic			
	A:HIS386 - :LIG1:C	Hydrophobic			
	A:HIS388 - :LIG1:C	Hydrophobic			
	:LIG1 – A:VAL447	Hydrophobic			
(b1)	:LIG:H – A:HIS388:NE2:B	Hydrogen bond	(b2)	:UNK1:H - A:HIS417:O	Hydrogen bond
	A:GLN203:HE21 - :LIG1:O	Hydrogen bond		:UNK1:H - A:TYR402:OH	Hydrogen bond
	A:HIS386:CD2 - :LIG1:O	Hydrogen bond		:UNK1:NA15 - A:GLU416:OE2	Electrostatic
	A;LEU294:CD2 - :LIG1	Hydrophobic		:UNK1:NA17 - A:GLU424:OE2	Electrostatic
	:LIG1:C – A:VAL291	Hydrophobic		A:GLU416:OE1 - :UNK1	Electrostatic
	:LIG1 - A:VAL447	Hydrophobic		A:GLU416:OE2 - :UNK1	Electrostatic
	:LIG1 - a:VAL291	Hydrophobic		:UNK1:H - A:HIS417	Hydrophobic
	A:HIS207 - :LIG1:C	Hydrophobic		A:HIS398 - UNK1:C1	Hydrophobic
	A:HIS214 - :LIG1:C	Hydrophobic			
	A:HIS386 - :LIG1:C	Hydrophobic			
	A:HIS388 - :LIG1:C	Hydrophobic			
(c1)	A:HIS351:HN - :LIG1:O50	Hydrogen bond	(c2)	A:GLN400:HE22 - :UNK1:O	Hydrogen bond
	A:HIS351:HD1 - :LIG1:O	Hydrogen bond		:UNK1:H - A:LEU415:O	Hydrogen bond
	A:SER581:HG - :LIG1:O	Hydrogen bond		:UNK1:H - A:ASP399:OD1	Hydrogen bond
	:LIG1:H - A:SER579:OG	Hydrogen bond		:UNK1:H - A:ASP258:OD2	Hydrogen bond
	:LIG1:H - A:ASP347:O	Hydrogen bond		:UNK1:N95 - A:HIS417	Electrostatic
	A:GLY354:CA - :LIG1:O	Hydrogen bond		:UNK1:NA15 - A:ASP399:OD2	Electrostatic
	:LIGH57 - A:GLU346:O	Hydrogen bond		:UNK1:NA15 - A:GLU416:OE2	Electrostatic
	:LIG1C1 - A:LYS358	Hydrophobic		:UNK1:NA16 - A:ASP399:OD1	Electrostatic
	:LIG1:C41 - A:PRO514	Hydrophobic		:UNK1:NA17 - A:GLU424:OE1	Electrostatic
	A:HIS95 - :LIG1:C42	Hydrophobic		A:HIS417 - :UNK1:C41	Hydrophobic
	A:HIS356 - :LIG1	Hydrophobic			
	A:HIS356 - :LIG1:C48	Hydrophobic			
(d1)	:LIG1:H58 - A:GLN370:O	Hydrogen bond	(d2)	A:HIS398:HD1 - :UNK1:O24	Hydrogen bond
	:LIG1:H59 - A:GLN370:O	Hydrogen bond		:UNK1:H - A:GLU416:OE1	Hydrogen bond
	:LIG1:H63 - A:HIS122:O	Hydrogen bond		:UNK1:H - A:ASP399:OD2	Hydrogen bond
	:LIG1:H77 - A:SER126:O	Hydrogen bond		:UNK1:NA15 - A:GLU416:OE2	Electrostatic
	A:ARG61:NH2 - :LIG1	Electrostatic		:UNK1:NA16 - A:GLU416:OE1	Electrostatic
	A:PRO127 - :LIG1	Hydrophobic		:UNK1:C1 - A:ILE413	Hydrophobic
	:LIG1C41 - A:LYS137	Hydrophobic		:UNK1:Na17 – A:VAL295:O	Other bond (Metal- acceptor type)
	:LIG1:C47 - A:PRO127	Hydrophobic		:UNK1:Na18 – A:PHE200:O	Other bond (Metal- acceptor type)
	A:TRP139 - :LIG1:C42	Hydrophobic			
(e)	:UNK:CL18 – A:ARG469	Hydrophobic			
	:UNK:CL19 – A:CYS41	Hydrophobic			
	:UNK1 – A:ARG44	Hydrophobic			
	:UNK1 – A:LEU152	Hydrophobic			
	:UNK1 – A:ARG469	hydrophobic			
	:UNK1:NA20 – A:SER121:O	Other bond (metal-acceptor type)			
	:UNK1:NA20 – A:ILE124:O	Other bonds (metal-acceptor type)			

Molecular docking is a widely applied, rapid, and cost-effective computational tool for predicting in silico the binding models and affinities of molecular identification events [26, 27, 28]. Receptor-ligand

docking represents a predominantly important technique because of its importance in the current drug discovery development [29]. An in-depth understanding of the nature of molecular recognition is also of great significance in facilitating the discovery, design, and development of novel drugs. In the current study, through *in silico* molecular docking, encapsulation of ligand combination from *N. sativa* and *C. caudatus* K. extracts is proposed to have selective anti-inflammatory activity toward COX-2 enzyme.

There are some possible explanations for these results. First, the active sites in COX-2 are larger than COX-1. Hence the extension in ligand sizes, as in the case of encapsulated ligands, can increase the selectivity toward COX-2 enzyme [30]. Another thing is that since the encapsulated ligands are bulky and more complex, as a result, those ligands have more hydrophobic characteristics. Ligands with hydrophobic interactions contribute significantly to the binding affinity of the docked complex and are entropy-driven interactions [28].

In this current work, encapsulated ligands had lower binding energy than non-encapsulated ligands when interacting with the COX-2 enzyme, indicating a stronger interaction with the enzyme [20]. The binding affinity value comes from the interaction between the ligands and the target compounds and can occur from interaction within ligands and the solvent's influence during the docking process [26]. The total energy produced by dockings ligands-receptor can be influenced by some of the existing energies such as energy in the Van Der Waals forces, electrostatic bonds, or energy that occurs between ligands and complex with solvents, with the binding energy calculation derived from ΔG = G complex - [G receptor + G ligand] [31]. Nevertheless, the molecular docking results should be investigated further through *in vitro* and *in vivo* approaches.

4. Conclusion

Thymoquinone and alpha-hederin are suggested to be the primary compounds in *N. sativa* extract. Moreover, *C. caudatus* K. extract compounds were mainly quercetin and kaempferol. The *in silico* molecular docking on the combination of compounds in *N. sativa* and *C. caudatus* K. showed that encapsulated ligands possess specific anti-inflammatory through inhibition of COX-2 enzyme. In addition, encapsulated ligands had a stronger ligand bond with the receptor target, as indicated by the smaller energy produced in the encapsulation results. This suggests that encapsulated active compounds in *N. sativa* and *C. caudatus* K can be proposed for selective anti-inflammatory drugs.

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