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Screening Phytochemical and Study Insilico of Family *Zingiberaceae* as Anti-inflammatory

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ABSTRACT

Traditional medicine employs many ingredients that have been used for generations for treatment and are based on society's norms. These ingredients are referred to as jamu. The research aim was to determine the types and uses of medicinal plants, the active compound content, and the potential of traditional herbal medicine as a fever-lowering bio-computation. The traditional herbal formula was determined using a direct interview method combined with a purposive sampling technique in this study. We used the software for phytochemical screening and molecular docking. COX-2 was used to analyse proteins, and six ligands were used: Quercetin, Curcuminoid, Zingerone, Heyneanone, Zerumbone, and Sabinene. This study discovered 22 different types of medicinal plants in Bangselok village, East Java, Indonesia. The Zingiberaceae family is frequently used as an ingredient in herbal medicine. Ginger, Lempuyang, Bangle, Temu Mangga, Temu Putih, and Temu Giring contain flavonoid compounds. The docking results showed that Quercetin, Zingerone, Heyneanone, Zerumbone, and Sabinene ligands were attached to domain A by the control of natural COX-2 ligands, namely NAG and EDO, predicting that they could be used as an anti-inflammatory and the combination of active compounds recommended as herbal medicine.

Introduction

Jamu is a traditional treatment in Indonesia based on the knowledge and wisdom of local communities, one of the people of Bangselok, Madura. Some plants are used as formulations of jamu making for traditional medicine (Saepudin et al., 2016). Combination of plant use on the making of Jamu based on disease type or patient complaints (Handayani, 2008). Jamu is used by the community to relieve some diseases like fever, cough, pain and to maintain body stamina. Steroids, tannins and saponins found in plants have a role in the traditional treatment success.

Inflammation is the body's protective response caused by physical trauma, chemical substances and microbiologic substances that damage the tissues (Martel et al., 2003), (Agustina et al.,2015). Inflammation causes

headaches, fever, pain, allergies, swelling, arthritis (Ehlers & Kaufmann, 2010). Inflammatory mediators include prostaglandins, leukotrienes, histamine, nitric oxide, cytokines (IL-1, Tumor Necrosis Factor (TNF), interferon (INF)-C, IL-6, IL-12, and IL-18), bradykinin, and serotonin produce inflations (Abbas et al., 2014), (Harborne, 1998), (Nile & Park, 2013). Prostaglandin synthesis forms from arachidonic acid with the help of cyclofluorine enzyme (COX); in addition, Nitric Oxidase and COX^{-2} stimulate the synthesis of pro-inflammatory mediators of interleukin-1 β (IL-1 β) and tumor necrosis factor (TNF) α. Cyclooxygenase-2 (COX-2), a key enzyme in fatty acid metabolism, is upregulated during both inflammation and cancer. COX-2 ıs induced by proinflammatory cytokines at the site of inflammation and enhanced COX-2-induced synthesis of prostaglandins stimulates cancer cell proliferation, promotes angiogenesis, inhibits apoptosis and increases metastatic potential.

Fever is a response to an inflammatory process caused by infection with microorganisms, one of which is $SARCoV^{-2}$ (Wu et al., 2020). Inflammatory signals can increase ACE^2 receptor expression in the lungs and lower the immune system, thereby increasing the risk of contracting COVID-19 (Smith et al., 2020). Virtual molecular screening analysis in several studies focused on the interaction of one active compound against COX^2. This study uses a combination of several active compounds found in several plants that have a role in inhibiting the synthesis of inflammatory mediators. Molecular Docking provides an overview of the interaction, bonding, and affinity of ligands with substrates. In addition, it can be used to predict whether a compound has activity or not.

The contribution of this study is complementary to the research data of medicinal plants, especially the Zingiberaceae family and their use and predicting new combinations of active compounds used as traditional herbal formulations as inflammatory agents.

Materials and Methods

Ethnobotany Observation

This study was carried out in Bangselok district in Sumenep Region of the Republic of Indonesia. Data was collected through semi-structured interviews with informants who knew or used plants as medicine (figure 1). This technique is commonly used in ethnobotanical studies (Pieroni et al., 2007). Interviews were conducted with selected informants, including about 10% of the total heads of family units (32 informants), to determine and explore traditional knowledge regarding medicinal plant species utilisation, usefulness, the utilised part, mode of preparation, or method of processing the plants. Respondents are traditional herbal medicine makers, age of the informants ranged from 50 to more than 65 years. The interview activities were carried out in their entirety using a questionnaire. Informant selection is based on the Snowball Sampling technique by determining the critical person. A key-person possesses strong power within society. The direction of the previous respondents determines the subsequent informants.

Figure 1. Ethnobotany Studi of Herbal Medicine Area

Screening Phytochemical Medicinal Plants

a. Plant Preparation

Fresh plants were gathered from Bangselok, Madura. Then, plants were well cleaned and washed with water, then cut and dried using an oven 70ºC temperature. After this period, the plant's rhizome has been grinded and transformed to powder by a grinder. The powders were preserved in clean plastic containers, kept away from light, heat and moisture until use.

b. Extraction Herbal Plant

1g of powdered rhizome was blended with 50 ml 70% ethanol solvents and agitation at room temperature until 1 hour. A 1 gram powdered rhizome was mixed with 50 mL 70% ethanol solvents and stirred at room temperature for 1 hour. The extracts were then collected and filtered using a 0.45-micron filter paper. The extracts were then concentrated at 40°C under decreased pressure in a rotating evaporator. The extracts were then weighted and stored at -20°C until they were used in the various assays (Zhang et al., 2018).

c. Test for Flavonoid

The test for flavonoid adopted is reported by Bone (2013) and Harborne (1998). Each sample (0.30 g) weighed into a beaker was extracted with 30 cm^3 of distilled water for 2 hours and filtered with Whatman filter paper number 42 (125 mm). To 10 cm^3 of the aqueous filtrate of each wood, the extract was added 5 cm^3 of 1.0 M dilute ammonia solution, followed by the addition of 5 cm^3 of concentrated tetraoxosulphate (VI) acid. The appearance of yellow colouration, which disappeared on standing, shows the presence of flavonoids.

d. Test for Steroid

The analytical method used is according to (Bone, 2013). Each sample (0.30 g) weighed into a beaker was mixed with 20 cm^3 of ethanol; the component was extracted for 2 hours. The ethanolic extract of each sample (5 cm^3) was added to 2 cm^3 acetic anhydride followed by 2 cm^3 of concentrated tetraoxosulphate (VI) acid. A violet to blue or green colour change in a sample(s) indicates the presence of steroids.

e. Test for Tannins

The analysis used was the method reported (Bone, 2013). Each wood powder sample (0.30 g) was weighed into a test tube and boiled for 10 minutes in a water bath containing 30 cm^3 of water. Filtration was carried out after boiling using number 42 (125 mm) Whatman filter paper. To 5 cm^3 of the filtrate were added, three drops of 0.1% ferric chloride. A brownish-green or a blue-black colouration showed a positive test.

f. Test for Saponin

The methodology is as reported (Bone, 2013). Distilled water (30 cm3) was added to wood powder samples (0.30 g) and boiled for 10 minutes in the water bath, and filtered using Whatman filter paper number 42 (125 mm). A mixture of distilled water (5 cm^3) and the filtrate (10 cm^3) was agitated vigorously for a stable, persistent froth. The formation of emulsion with three drops of olive oil showed a positive result.

g. Prepare Protein and Ligand

The proteins used in the 3D structure of COX-2 with 5F19 code are downloaded from the RCSB database (www.rcsb.org/). In contrast, the flavonoids ligands (Curcuminoid PubChem ID: 1550234, Quercetin PubChem ID: 5280343, Gingerol PubChem ID: 168115, Heyneanone PubChem ID: 71578079, Sabinene PubChem ID: 18818), Steroid ligand (Stigmasterol PubChem ID: 5280794), Saponin ligands (Ginsenosides PubChem ID: 3086007), ligands tannins (Tannins PubChem ID: 7115), native ligan (NAG: PubChem ID 24139 and EDO: PubChem ID 439174) are downloaded on PubChem database (www.pubchem.ncbi.nlm.nih.gov/). Protein preparation uses the Discovery Studio program by eliminating water molecules and ligands. The preparation of ligands uses the PyRx program to minimize free energy and converts the compound into AutoDock.

h. Docking and visualization of Protein-Ligan Interaction Results

The docking process is done with the AutoDock Vina 1.1 program on the PyRx. Result of visualized docking by using PyMol v 2.3.2.1 program, Discovery Studio 2019 Client code.

Results and Discussion

1. Inventory of Medicinal Plants in Bangselok Village

Based on the interviews, the inventory of medicinal plants used as traditional herbal medicine by the people of Bangladesh is 22 plant species (Table 1). The plants used as medicinal plants are the *Zingiberaceae* family. Several medicinal plants were found around the yard, such as *Oxalidaceae*, *Euphorbiaceae*, *Rutaceae*, *Menispermaceae*, *Lamiaceae*, *Moraceae*, *Malvaceae*. The parts of the plants that are usually used in traditional herbal medicine are the leaves, stems, roots, flowers, fruits, and rhizomes. Leaf parts are often used as a traditional medicine because they have a water content of 70-80%, the accumulation of photosynthesis is thought to have substances that can cure the disease (Purwaningsih, 2014). Leaves are part of the readily available plant and are easy to mix (Hamzari, 2017). The people of Bangselok often use the rhizome part for herbal medicine because the rhizome contains many ingredients, including flavonoids, saponins, and essential oils (Muharrami et al., 2017).

Table 1. Inventory of Names of Medicinal Plants in Bangselok Village, Madura

No	Local Name	Family	Species	Part of Plant	Use
1	Chabi Jhamo	Piperaceae	*Piper retrofractum* Vahl	Fruits	Aphrodisiacs, contraceptives, herbal medicine, lumbago, fever, stomach ulcers
2	Konceh	Zingiberaceae	*Boesenbergia pandurata* (Roxb.) Schlecht.	Rhizome, Leaves	Sari rapet, fertilizer womb, herbal medicine, gout, puerperal fever, digestion, thrush
4	Konyi' pote	Zingiberaceae	*Curcuma zedoaria* (Berg.) Roscoe.	Rhizome	Whitish, smooth digestion, cancer
5	Lampojang	Zingiberaceae	*Zingiber zerumbet* (L.) J. E. Smith	Rhizome	Appetite, fever
6	Laos	Zingiberaceae	*Alpinia galanga* (L.) Swartz	Rhizome	Aprodisiac, Smooth blood, Rheumatism, contraception,
7	Bangle/ Pandhiang	Zingiberaceae	*Zingiber purpureum* Roxb.	Rhizome	Overweight, worms, fever
8	Temo Celleng	Zingiberaceae	*Curcuma aeruginosa* Roxb.	Rhizome	Leucorrhoea, intestinal worms, appetite enhancer
9	Temo Giring	Zingiberaceae	*Curcuma heyneana* Val. & v. Zijp.	Rhizome	Appetite enhancer
10	Temo Pao	Zingiberaceae	*Curcuma mangga* Val.	Rhizome	Fever, cancer, vaginal discharge, indigestion
11	Blimbing Buluh	Oxalidaceae	*Averrhoa carambola* L.	Fruits	Cough
12	Brotowali	Menispermacea e	*Tinospora crispa* (L.) Miers ex Hook. f. & Thoms.	Leaves, Stem	Diabetes, rheumatis, itching, diarrhea.
13	Cermeh	Euphorbiaccae	*Phyllanthus acidus* (L.) Skeels	Leaves	Streamlining breast milk, cough medicine
14	Kapoh	Sterculiaceae	*Ceiba pentandra* (L.) Gaertn.	Leaves	Cough medicine, asthma
15	Jheruk Pecel	Rutaceae	*Citrus aurantifolia* (Christm. & Panz) Swingle	Skin Fruits	Cough medicine

Tabel 1. Continue

16	Mengkudu/ Koddhu'	Rutaceae	*Morinda citrifolia* L.	Leaves, Fruits	Smoothen breast milk, diabetes, gout, rheumatism, osteoporosis
17	Kates Rambei	Caricaceae	*Carica papaya* L.	Leaves, Root	Streamlining breast milk, appetite
18	Sage	Papilionaceae	*Abrus precatorius* L.	Leaves	Asthma, appetite
19	Jeih	Zingiberaceae	*Zingiber officinale* Roxb	Rhizome	Aphrodisiac, fever
20	Kemangih	Lamiaceae	*Ocimum sanctum* L.	Leaves	Antiseptic, thrush, fever
21	Bunga sepatu/ merebheng	*Malvaceae*	*Hibiscus rosa-sinensis* L.	Leaves	Lung disease
22	Meniran	Euphorbiaceae	*Phylanthus urinaria* L	Leaves	Fitness and health

Table 2. Medicinal plants used by the people of Bangselok as ingredients for traditional herbal medicine

No	Local name	Species	Compound Content	Reference
1	Jahe	*Zingiber officinale* Roxb	Gingerols, shogaols, paradols, quercetin, zingerone, gingerenone-A, and 6-dehydrogingerdione, β-bisabolene, α-curcumene, zingiberene, α-farnesene, and β-sesquiphellandrene	(Purwaningsih, 2014), (Ravindran & Babu, 2016)
2	Lempuyang	*Zingiber zerumbet* (L.) J. E. Smith	Zerumbone, kaempferol, *α*-pinene, *β*-pinene, Δ3-carene, camphor, *β*-caryophyllene, ar-curcumene, humulene oxide, humulene dioxide, linalool, borneol, *α*-terpineol, flavone	(Zakaria et al., 2011)
3	Bangle	*Zingiber purpureum Roxb*	Saponin, flavonoid, minyak atsiri, tanin, steroid, triterpenoid, sabinene, terpinen-4-ol, Cassumunarin A B C, zerumbone, butadiene, limonene	(Chanwitheesuk et al., 2005)
4	Temu Pao/Mangga	*Curcuma mangga* Val.	Alkaloid, flavonoid, tanin, kurkuminoid dan terpenoid, zerumin A, β-sitosterol, curcumin, demethoxycurcumin and bisdemethoxycurcumin	(Malek et al., 2011)
5	Temu Putih	*Curcuma zedoaria* (Berg.) Roscoe.	Kurkumin, minyak atsiri dan flavonoid, terpenoid, tanin, saponin, alkaloid, terpinoid, dan steroid, Epicurzerene, monoterpenoids, curcumenene, curzerenone, 1,8-cineole, debromofiliforminol	(Dosoky & Setzer, 2018), (Tariq et al., 2016)
6	Temu Giring	*Curcuma heyneana* Val. & v. Zijp.	flavonoid, kurkumin, fenolik, minyak atsiri, steroid, terpenoid, saponin dan tannin, curcumin, 1,8-cineole/limonene, isocurcumenol, curcumanolides A, B	(Xu & Chang, 2007)

According to the findings of the interview, the plants used as ingredients for traditional herbal medicine by the people of Bangselok included Ginger (*Zingiber*

officinale Roxb), Lempuyang (*Zingiber zerumbet (L.)* JE Smith), Bangle (*Zingiber purpureum Roxb*), Temu Mangga/Pao (*Curcuma mango Val*), Temu Putih (*Curcuma zedoaria* B (*Curcuma heyneana Val. & V. Zijp*). Ginger (*Zingiber officinale Roxb*) Ginger rhizome contains essential oils, gingerol, shogaol and zingiberen (Table 2) (Ravindran & Babu, 2016). Gingerol is a phenolic compound that functions as an anti-inflammatory. Gingerol can inhibit cyclooxygenase and lipoxygenase activity in arachidonic acid, causing a decrease in the number of prostaglandins and leukotrienes (Bone, 2013). Lempuyang (*Zingiber zerumbet (L.)* J. E. Smith) Lempuyang rhizome contains active compounds including kaempferol, quercetin, curcumin and essential oils (Zakaria et al., 2011). Lempuyang rhizome has anti-inflammatory activity, and Zingiber zerumbet extract can inhibit the action of the enzymes cyclooxygenase, lipoxygenase, myeloperoxidase and nitric oxide synthase (Jyothilakshmi, 2016).

Bangle (*Zingiber purpureum Roxb*) Bangle rhizome has properties to reduce fever, pain and constipation. Bangle rhizome contains saponins, flavonoids, essential oils, tannins, steroids, triterpenoids, antioxidants and phenolic compounds (Chanwitheesuk et al., 2005). The phenolic compounds contained in bangle rhizomes can inhibit the activity of inhibiting inflammation by inhibiting the cyclooxygenase (COX) and lipoxygenase enzymes and inhibiting the release of histamine (Xu & Chang, 2007). Temu Mangga/Pao (*Curcuma mangga Val.*) Curcuma manga rhizome contains essential oils, alkaloids, flavonoids, tannins, and terpenoids (Table 2). The phenolic compounds in mango ginger can induce glutathione-S-transferase (GST) activity, an enzyme that plays a role in the detoxification of foreign compounds in the body, and can suppress oxidative stress (Robert et al., 2012).

Temu Putih (*Curcuma zedoaria (Berg) Roscoe*) Temu Putih rhizome contains curcumin compounds, essential oils and flavonoids, terpenoids, tannins, saponins, alkaloids, terpenoids, and steroids (Tariq et al., 2016). The activity of curcumin as an anti-inflammatory is by inhibiting the production of prostaglandins by inhibiting the activity of the cyclooxygenase enzyme (Robert et al., 2012). Rhizoma C. zedoaria showed significant p <0.001 anti-inflammatory, when compared with controls with standard drugs (Indomethacin 10 mg/kg. i.p and Rumalaya forte 200 mg/kg). Petroleum ether extract 200 and chloroform 400 mg/kg of C. zedoaria extract showed maximum anti-inflammatory activity at 2 to 6 hours (Kaushik & Jalalpure, 2011). Temu Giring (*Curcuma heyneana Val. & V. Zijp*) The rhizome of temu giring functions to improve blood circulation. Temu giring rhizome contains flavonoids, curcumin, phenolics, essential oils, steroids, terpenoids, saponins and tannins (Xu & Chang, 2007). Temu giring rhizome contains dihydrosuberenol, and demethoxycurcumin compounds that have antioxidant activity (Rahayu et al, 2018) and zedoarindiol compounds have anti-inflammatory effects and inhibit iNOS COX-2 pro-inflammatory cytokines (Yue et al., 2010).

2. Phytochemical Screening of Medicinal Plants used by The Community

The efficacy of traditional herbal medicine depends on the processing method and the active compound content of traditional herbal medicine. The results of phytochemical screening of plants that make up traditional herbs to determine the content of flavonoids, steroids, tannins and saponins are presented in Table 3.

a. Flavonoid

Zingiber officinale Roxb, Zingiber zerumbet (L.) J. E. Smith, Zingiber purpureum Roxb, Curcuma mango Val, Curcuma zedoaria (Berg.) Roscoe, Curcuma heyneana Val. & *v. Zijp* contains flavonoids; it is shown that the reaction results in red, yellow or orange colour (Harborne, 1998).

Flavonoid compounds are polar compounds because they have unsubstituted hydroxyl (-OH) groups to form hydrogen bonds. Besides that, flavonoids, which are polyphenolic compounds, can donate hydrogen atoms to free radical compounds, so the antioxidant activity of polyphenol compounds can be generated in neutralization reactions of free radicals or the termination of chain reactions that occur (Robert et al., 2012). In the extraction process, the active

compounds in a plant are easily dissolved or bound by solvents according to their polarity so that a polar ethanol solution will more easily extract the flavonoids in the plant. Plant tissue Flavonoid compounds also reduce pain, antimicrobial, anti-bleeding, sedative, heart disease drugs, anti-diabetes, anti-bleeding, wound medicine, and anti-inflammatory (Ravindran & Babu, 2016).

b. Stereoid

The reaction of triterpenoids with Liebermann's reagent produces a red-purple colour, while steroids give a green-blue colour. Based on the ability of triterpenoid compounds and steroids to form colour by H^2SO^4 in an anhydrous acetic acid solvent. The difference in colour produced by triterpenoids and steroids is due to different groups on the C-4 atom (Saleh & Mariana, 2011). Several steroid compounds include glucocorticoids as anti-inflammatory, allergies, fever, leukaemia and hypertension and cardenolide is a cardiac glucoside steroid used as a diuretic and heart-strengthening drug (Coutinho & Chapman, 2011). In the test of steroid compounds on herbal plants as raw material for traditional herbal medicine, it was found that Ginger (*Zingiber officinale Roxb*), Lempuyang (*Zingiber zerumbet (L.) JE Smith*), Temu Mangga/Pao (*Curcuma mangga Val*), Temu Putih (*Curcuma zedoaria (Berg.) Roscoe*), Temu Giring (*Curcuma heyneana Val. & V. Zijp*) contains steroid compounds.

c. Tanin

The test results showed that the herbal plants contained tannin compounds but the *Curcuma mango Val* and *Curcuma zedoaria (Berg.) Roscoe* did not show any tannin compounds. Tannin is a class of polyphenolic compounds commonly found in plants. Tannins can be defined as polyphenolic compounds with a molecular weight of more than 1000 g/mol and can form complex compounds with protein. Tannins have a sizeable biological role because they function as a protein depositor and metal gel. Therefore, tannins are predicted to act as biological antioxidants. (Redondo et al., 2014).

d. Saponin

Tanam Ginger (*Zingiber officinale Roxb*), Bangle (*Zingiber purpureum Roxb*), Temu Mangga/Pao (*Curcuma mangga Val*), Temu Giring (*Curcuma heyneana Val. & V. Zijp*) showed positive results for saponins. Saponins are generally in the form of glycosides, so they tend to be polar. The emergence of foam in the saponin test shows that saponins can become glucose and other compounds (Harborne, 1998). Saponin compounds can reduce superoxide by forming hydroperoxide intermediates, thereby preventing biomolecular damage by free radicals (Baev, 2013). Saponin compounds can provide antitussive and expectorant effects that can cure coughs. Saponins also have anti-inflammatory activity because they have been shown to inhibit the release of pro-inflammatory substances stimulated by lipopolysaccharides (Miladiyah et al., 2018).

Table 3. Phytochemical Screening Results of Traditional Herbal Medicine Ingredients for The People of Bangselok, Madura

No	Anti-Inflammatory Plants	Flavonoid	Steroid	Tanin	Saponin
1	*Zingiber officinale* Roxb	+	+	+	+
2	*Zingiber zerumbet* (L.) J. E. Smith	+	+	+	-
3	*Zingiber purpureum Roxb*	+	-	+	+
4	*Curcuma mangga* Val.	+	+	-	+
5	*Curcuma zedoaria* (Berg.) Roscoe.	+	+	-	-
6	*Curcuma heyneana* Val. & v. Zijp.	+	+	+	+

3. Results of Docking Complex COX^2 (Cyclooxygenase-2) with Native Ligand (NAG and EDO)

COX^2 protein has 5 natural ligands including 1,2-ethanediol (EDO), acrylic acid (AKR), 2-acetamido-2-deoxy-beta-d-glucopyranose (NAG), octyl beta-d-glucopyranoside (BOG), protoporphyrin IX containing CO (COH). The NAG and EDO ligands have a role in inflammation. The

results of the interaction of NAG and EDO ligands are presented in Figure 2.

The NAG native ligand has a molecular weight of 221.1 g/mol, which requires an -6.7 kcal/mol energy to interact with COX^2 (domain B), while the native EDO ligand has a molecular weight of -3.4 kcal/mol (domain A). The NAG ligand native interaction occurs in the B domain (green colour), while the native EDO ligand occurs in the A domain (yellow colour) on the COX2 protein. The NAG ligand native carboxyl group forms hydrogen bonds with three amino acid residues (GLY45, CYS41, GLN461) with a 2.1–2.3 Å. Strong bonds have a length of 2.2 Å - 2.5 Å, whereas most electrostatic bonds have a distance of 2.5 Å - 3.2 Å, and weak electrostatic that are scattered have a distance of more than 3.2 Å (Fouzia & Salim, 2019). In general, the NAG native ligand binds to polar amino acid residues to be hydrophilic. Meanwhile, the native carboxyl group ligand EDO forms conventional hydrogen and hydrogen bonds with four amino acid residues (THR129, THR149, ASP125, ARG150) with a range of 2.2-3.0 Å. The EDO native ligand binds to polar or hydrophilic amino acid residues.

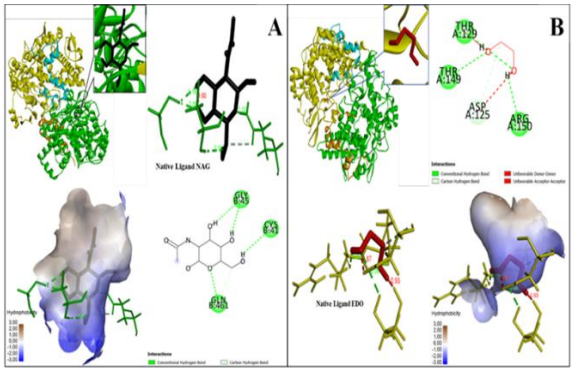

Figure 2. The results of the interaction of the COX*2* protein complex with NAG (a) and EDO (b) ligands.

4. Results of Docking Complex COX^2 (Cyclooxygenase-2) with Flavonoids Compound

Classes of flavonoid compounds that will interact with COX^2 protein include quercetin, curcuminoid, gingerol, sabinene, heyneanone. The results of the interaction between the flavonoid compound and COX^2 protein showed that curcuminoid, quercetin, gingerol, sabinene, native ligand NAG bind to the B domain (green colour) (figure 4a) with bond energies including -10.2 kcal/mol, -9, 7 kcal/mol, -6.2 kcal/mol, -5.9 kcal/mol, -6.8 kcal/mol (Table 5). The heyneanone compound and the EDO native ligand bind to the COX^2 protein in the A domain (yellow) with a bond energy of -7.8 kcal/mol, -3.2 kcal/mol. Small binding affinity values predicted the best binding position (Shashank, 2013). The curcuminoid and quercetin binding sites overlap with the NAG native ligand on the amino acid residues of CYS41, GLN461, GLY45, which form hydrogen bonds and van der Waals forces (Table 5). The similarity of the amino acid residues of the bonds formed indicates possible anti-inflammatory activity (Miladiyah et al., 2018). It can be concluded that curcuminoid and quercetin can inhibit the performance of the NAG native ligand, thereby reducing prostaglandin synthesis as an inflammatory mediator. According to Fouzia & Salim (2019), natural molecules from thyme essential oil and flavonoids (Apigenin, Luteolin, Thymol, Carvacrol, Naringenine, and Chlorogenique) are highly recommended to treat inflammation by inhibition of the responsible enzyme. Heyneanone interacts with the native EDO ligand on the amino acid residues of ASP125 (Table 4) in the A domain.

Table 4. Bond energies, amino acid residues, types of combination bonds between the flavonoid ligand and COX^2.

Goup compound	Ligand	Binding Afinity (kcal/mol)	Domain Site	Amino acid residues	Bond Type
	Curcuminoid	-10,2	B	GLU465, CYS47, ASN34	Hidrogen bond
				CYS41, **GLN461**, GLY135, TYR130, LYS468, ARG44, VAL46, **GLY45**, ARG469, ASN43, GLN42, MET48, TRP323,TYR136	Van der waals

Table 4. Continue

Goup compound	Ligand	Binding Afinity (kcal/mol)	Domain Site	Amino acid residues	Bond Type
Flavonoid	Quercetin	-9,7	B	LEU152, HIS39, PRO153, CYS36, PRO156	Pi-Alkyl
				GLY135, **GLY45**, TYR130, ASP125	Hidrogen bond
				TYR136, ARG469, ALA151, LEU152, CYS47, **CYS41**, CYS36, HIS39, **GLN461**, PRO40	Van der waals
	Heyneanone	-7,8	A	PRO153, VAL46, ARG44	Pi-Alkyl
				GLN370, SER126	Hidrogen bond
				GLN372, SER121, HIS122, TYR373, ARG44, **ASP125**, ILE124, PBO542, SER541, PRO127, ALA543	Van der waals
	Gingerol	-6,2	A; B	LYS532, PHE371	Pi-Alkyl
				ASN375, ARG376, HIS226	Hidrogen bond
				LEU145, GLN374, GLY225, GLY227, GLY536, TYR373, ASN537, VAL538, PRO128, SER143	Van der waals
	Sabinene	-5,9	B	PHE142	Pi-Alkyl
				-	Hidrogen bond
				HIS386, GLN203, TYR385, THR206, TRP387, PHE210	Van der waals
	Native ligand NAG	-6,8	B	HIS388, LEU391, LEU390, HIS207, ALA199, ALA202	Pi-Alkyl
				GLY45, **CYS41**, **GLN461**	Hidrogen bond
	Native ligand EDO	-3,2	A	THR120, THR149, **ASP125**, ARG150	Hidrogen bond

The carbon (C) atoms in the COX^2 alkyl group form hydrophobic bonds with curcuminoid ligands, quercetin, gingerol, heyneanone, and sabinene (Figure 3). The bond position shows that the curcuminoid, quercetin, gingerol, heyneanone, and sabinene ligands not only bind to the non-polar (hydrophobic) amino acid residues shown in the light brown area (positive hydrophobicity) but also bind to the polar amino acid residues (hydrophilic) which shown in the blue area (negative hydrophobicity) (Figure 3). The native NAG ligand forms hydrophobic bonds with non-polar and polar amino acid residues, while the native EDO ligand forms hydrophobic bonds with polar (hydrophilic) amino acid residues (Figure 4b). A hydrophobic bond is a weak non-covalent bond that will occur at a distance above 3.7 Å. This distance causes the hydrophobic bond to become a fragile bond among other bonds, with a maximum distance of 3.5 Å as one factor that indicates a weak to a strong bond group.

5. Results of Docking Complex COX^2 (Cyclooxygenase-2) with Flavonoid-Saponin Combination

The COX^2 alkyl group forms hydrophobic bonds with the Curcuminoid, Quercetin, heyneanone, gingerol, sabinene, native ligand EDO and NAG ligand. The bond positions of curcuminoid, Quercetin, heyneanone, gingerol, sabinene, native EDO and NAG ligands tend to bind to non-polar (hydrophobic) amino acid residues shown in

white / brown areas (positive hydrophobicity) (Figure 4). However, at the native end of the NAG, ligands bind. Polaric (hydrophilic) amino acid residues are shown in blue areas (negative hydrophobicity). Solubility plays a significant role in the therapeutic efficacy of flavonoids. The low solubility of flavonoid aglycones in water, coupled with its short residence time in the intestine and its lower absorption, does not allow humans to suffer acute toxic effects from the consumption of flavonoids, exception of a rare occurrence of allergy (Yunta, 2016).

The docking result of the combination of flavonoid compounds (Quercetin, curcuminoid, gingerol, heyneanone, sabinene) and saponins (ginsenosides) with COX^2 shows that the flavonoid compound (curcuminoid, Quercetin, heyneanone, gingerol) interacts in domain B (green) (Figure 4).) with a bond energy of -10.2 kcal/mol, -9.7 kcal / mol, -7.6 kcal/mol, -7.1 kcal/mol (Table 5). Interaction of curcuminoid, Quercetin, overlapping with native ligand NAG on amino acid residues of CYS41, GLN461, GLY45, while native EDO ligand overlaps with Quercetin on amino acid residues of ASP125 in domain B.

The flavonols are one such group with different compounds such as quercetin, kaempferol, myricetin, fisetin, and morin, exhibiting beneficial effects such as anti-inflammatory and antioxidants, antiallergic, antiviral, as well as anticancer activity (Yunta, 2016). The saponins can inhibit the mediators of inflammation, such as histamine, serotonin and prostaglandins, along with their antioxidant property, which inhibits ROS formation and plays a significant role in inflammation (Murthy, 2006). Sabinene compounds interact in the A domain (yellow colour) with an -6.4 kcal/mol (Table 5). The binding affinity ginsenosides value of -9.6 kcal/mol is smaller than the native ligand EDO -3.3 kcal/mol, causing the native EDO ligand to move from domain A to domain B.

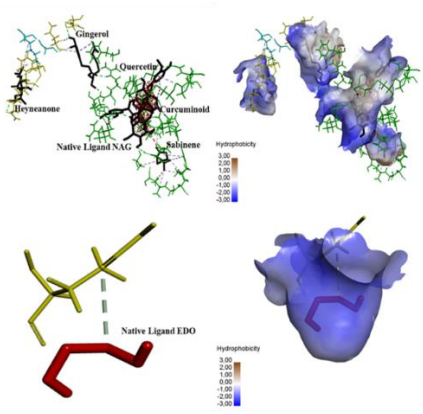

Figure 3. The results of the interaction of COX^2 with flavonoids

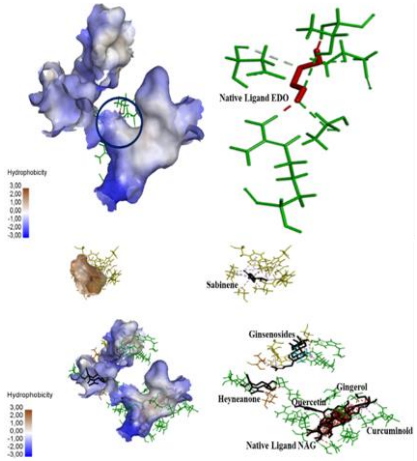

Figure 4. The results of the interaction of COX^2 with a combination of Flavonoids-Saponins

Table 5. Bond energy, amino acid residues, type of flavonoid-Saponin ligand combination bond with COX^2

Goup compound	Ligand	Binding Afinity (kcal/mol)	Domain Site	Amino acid residues	Bond type
	Curcuminoid	-10,2	B	GLU465, CYS47, ASN34	Hidrogen bond
				CYS41, **GLN461**, GLY135, TYR130, LYS468, ARG44, VAL46, GLY45, ARG469, ASN43, GLN42, MET48, TRP323,TYR136	Van der waals
				LEU152, HIS39, PRO153, CYS36, PRO156	Pi-Alkyl

Table 5. Continue

Goup compound	Ligand	Binding Afinity (kcal/mol)	Domain Site	Amino acid residues	Bond type
Flavonoid-Saponin	Quercetin	-9,7	B	GLY135, **GLY45**, TYR130, **ASP125**	Hidrogen bond
				TYR136, ARG469, ALA151, LEU152, CYS47, CYS41, CYS36, HIS39, GLN461, PRO40	Van der waals
				PRO153, VAL46, ARG44	Pi-Alkyl
	Heyneanone	-7,6	B	GLN370, SER126	Hidrogen bond
				GLN372, SER121, HIS122, TYR373, ARG44, ASP125, ILE124, PBO542, SER541, PRO127, ALA543	Van der waals
				LYS532, PHE371	Pi-Alkyl
	Gingerol	-7,1	B	ASN375, ARG376, HIS226	Hidrogen bond
				LEU145, GLN374, GLY225, GLY227, GLY536, TYR373, ASN537, VAL538, PRO128, SER143	Van der waals
				PHE142	Pi-Alkyl
	Sabinene	-6,4	A	-	Hidrogen bond
				HIS386, GLN203, TYR385, THR206, TRP387, PHE210	Van der waals
				HIS388, LEU391, LEU390, HIS207, ALA199, ALA202	Pi-Alkyl
	Ginsenosides	**-9,6**	**A; B**	VAL538	Hidrogen bond
				TYR373, ASN537, GLN374, ASN375, GLY536, GLY227, GLY225, HIS226, ARG376	Van der waals
				TRP139, VAL538, PHE142, LEU145	Pi-Alkyl
	Native ligand NAG	-6,9	B	**GLY45**, CYS41, **GLN461**	Hidrogen bond
	Native ligand EDO	-3,3	B	ALA151, THR149, THR129, **ASP125**	Hidrogen bond

6. Results of Docking Complex COX^2 ($Cyclooxygenase^{-2}$) with Combination of Flavonoids-Steroids

The results of the interaction of the combination of flavonoids (curcuminoid, quercetin, heyneanone, gingerol, sabinene) and steroids (Stigmasterol) with COX^2 show that the interaction of flavonoids (curcuminoid, quercetin, gingerol) occurs in domain B (Figure 5) with a bond energy of -10.2 kcal/mol, -9.7 kcal/mol, -7.2 kcal/mol (Table 7). The interaction of flavonoid groups (curcuminoid, quercetin) overlapping with the native ligand NAG on the amino acid residues of CYS41, GLN461, GLY45 in domain B by forming hydrogen bonds and van der Waals forces. The interaction of stigmasterol, heyneanone, sabinene with COX^2 occurred in domain A (Figure 5) with bond energies of -8.5 kcal/mol, -7.8 kcal/mol, -6.3 kcal/mol (Table 6). The difference between curcuminoid and quercetin amino acid residues with the native ligand EDO amino acid residues (PRO218, GLN454, THR212) causes the native EDO ligand to be unable to interact with curcuminoids and

quercetin in domain B. The transfer of the native EDO ligand domain from domain A to domain B is caused by the difference between binding affinity and stigmasterol (Table 6).

Table 6. Bond energy, amino acid residues, type of flavonoid-steroid combination ligand bond with COX^2

Goup compound	Ligand	Binding Afinity (kcal/mol)	Domain Site	Amino acid residues	Bond Type
Flavonoid-Steroid	Curcuminoid	-10,2	B	GLU465, CYS47, ASN34	Hidrogen bond
				CYS41, **GLN461**, GLY135, TYR130, LYS468, ARG44, VAL46, **GLY45**, ARG469, ASN43, GLN42, MET48, TRP323,TYR136	Van der waals
				LEU152, HIS39, PRO153, CYS36, PRO156	Pi-Alkyl
	Quercetin	-9,7	B	GLY135, **GLY45**, TYR130, ASP125	Hidrogen bond
				TYR136, ARG469, ALA151, LEU152, CYS47, **CYS41**, CYS36, HIS39, GLN461, PRO40	Van der waals
				PRO153, VAL46, ARG44	Pi-Alkyl
	Heyneanone	-7,8	A	GLN370, SER126	Hidrogen bond
				GLN372, SER121, HIS122, TYR373, ARG44, ASP125, ILE124, PBO542, SER541, PRO127, ALA543	Van der waals
				LYS532, PHE371	Pi-Alkyl
	Gingerol	-7,2	B	ASN375, ARG376, HIS226	Hidrogen bond
				LEU145, GLN374, GLY225, GLY227, GLY536, TYR373, ASN537, VAL538, PRO128, SER143	Van der waals
				PHE142	Pi-Alkyl
	Sabinene	-6,3	A	-	Hidrogen bond
				HIS386, GLN203, TYR385, THR206, TRP387, PHE210	Van der waals
				HIS388, LEU391, LEU390, HIS207, ALA199, ALA202	Pi-Alkyl
	Stigmasterol	**-8,5**	**A**	-	Hidrogen bond
				ARG120, SER119, PRO84	Van der waals
				VAL116, TYR115, PHE99, LEU93, PHE96, ILE112, TRP100, ILE92, VAL89	Pi-Alkyl
	Native ligand NAG	-6,9	B	**GLY45**, **CYS41**, **GLN461**	Hidrogen bond
	Native ligand EDO	-3,2	B	PRO218, GLN454, THR212	Hidrogen bond

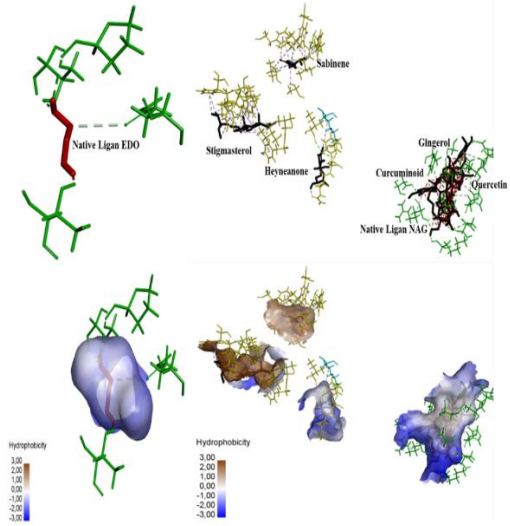

Figure 5. The results of interaction of COX^2 with a combination of Flavonoids-Steroids (a), Hydrophobicity (b)

The carbon (C) atom in the COX^2 alkyl group forms hydrophobic interactions with curcuminoids, quercetin which overlaps with the NAG native ligand because it has the same amino acid residue bonds. The interaction position of non-polar (hydrophobic) amino acid residues binds to curcumonoids, quercetin, and the NAG native ligand shown in white / brown areas (Figure 5). Sabinene, stigmasterol binds to non-polar (hydrophobic) amino acid residues which are shown in brown areas (Figure 5), whereas native EDO ligands tend to bind to polar (hydrophilic) amino acid residues which are shown in blue areas. The similarity of interactions of amino acid residues with ligands is related to the characteristics, solubility and boiling point of the ligand complex (Them et al., 2019).

7. Results of Docking Complex COX^2 (Cyclooxygenase^{-2}) with the Combination of Flavonoids-Tannin

The carbon (C) atom in the COX^2 alkyl group forms hydrophobic interactions with curcuminoids, quercetin which overlaps with the NAG native ligand because it has the same amino acid residue bonds shown in the white / brown area (Figure 6). Sabinene and tannins bind to non-polar (hydrophobic) amino acid residues that are shown in brown areas (Figure 6).

The interaction results of the combination of flavonoids (curcuminoid, quercetin, gingerol, heyneanone, sabinene) and tannins with COX2 show that the interaction of curcuminoid, quercetin, overlap with native NAG ligands on the amino acid residues GLY45, CYS41, GLN461 in the B domain with a bond energy of -10 kcal/mol, -9.7 kcal/mol, -6.8 kcal/mol (Table 7). Tanning overlapping interactions with sabinene on the amino acid residues TRY385, TRP387 in domain A with bond energies of -9.0 kcal/mol, -6.4 kcal/mol. The interaction of heyneanone, gingerol, and native EDO ligand with COX2 occurred in domain A with bond energies of -7.8 kcal.mol, 7.2 kcal/mol, -3.4 kcal/mol (Table 7).

Table 7. Bonding energies, amino acid residues, types of combination bonds between the Flavonoid-Tannin ligand and COX^2

Goup compound	Ligand	Binding Afinity (kcal/mol)	Domain Site	Amino acid residues	Bond Type
Flavonoid-Tannin	Curcuminoid	-10	B	GLU465, CYS47, ASN34	Hidrogen bond
				CYS41, **GLN461**, GLY135, TYR130, LYS468, ARG44, VAL46, **GLY45**, ARG469, ASN43, GLN42, MET48, TRP323,TYR136	Van der waals
				LEU152, HIS39, PRO153, CYS36, PRO156	Pi-Alkyl
	Quercetin	-9,7	B	GLY135, **GLY45**, TYR130, ASP125	Hidrogen bond
				TYR136, ARG469, ALA151, LEU152, CYS47, **CYS41**, CYS36, HIS39, GLN461, PRO40	Van der waals

Table 7. Continue

Goup compound	Ligand	Binding Afinity (kcal/mol)	Domain Site	Amino acid residues	Bond Type
				PRO153, VAL46, ARG44	Pi-Alkyl
	Heyneanone	-7,8	A;B	GLN370, SER126	Hidrogen bond
				GLN372, SER121, HIS122, TYR373, ARG44, ASP125, ILE124, PBO542, SER541, PRO127, ALA543	Van der waals
				LYS532, PHE371	Pi-Alkyl
	Gingerol	-7,2	A	ASN375, ARG376, HIS226	Hidrogen bond
				LEU145, GLN374, GLY225, GLY227, GLY536, TYR373, ASN537, VAL538, PRO128, SER143	Van der waals
				PHE142	Pi-Alkyl
	Sabinene	-6,4	A	-	Hidrogen bond
				HIS386, GLN203, **TYR385**, THR206, **TRP387**, PHE210	Van der waals
				HIS388, LEU391, LEU390, HIS207, ALA199, ALA202	Pi-Alkyl
	Tannin	**-9,0**	**A**	-	Hidrogen bond
				PHE381, **TYR385**, OAS530, ARG120, TYR355, VAL523, LEU352, **TRP387**, LEU384	Van der waals
				VAL349, ALA527, LEU359, VAL116	Pi-Alkyl
	Native ligand NAG	-6,8	B	**GLY45**, **CYS41**, **GLN461**	Hidrogen bond
	Native ligand EDO	-3,4	A	ASP125, ALA151	Hidrogen bond

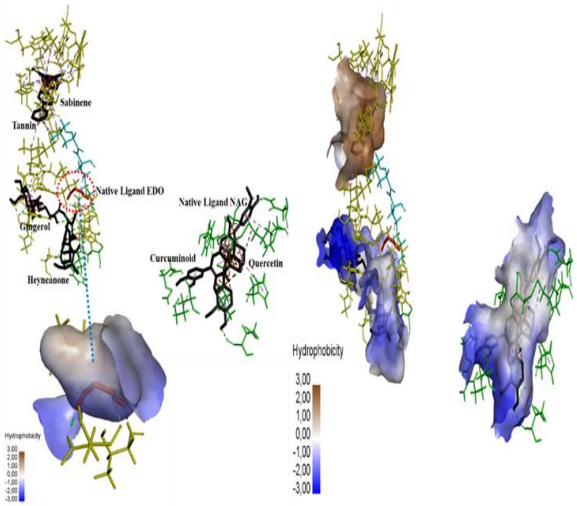

Figure 6. The results of the interaction of COX^2 with the combination of Flavonoids-Tannin (a), Hydrophobicity (b)

8. Results of Docking Complex COX^2 (Cyclooxygenase^{-2}) with a combination of flavonoids, steroids, tannins, saponins

The results of the interaction of the combination of flavonoids, steroids, tannins, saponins with COX^2 indicate that curcuminoids, quercetin, and tannins interact in the B domain (green) with a bond energy of -10.2 kcal/mol, -9.7 kcal/mol, -8, 7 kcal/mol (Table 9). they overlap between stigmasterol and ginsenosides in domains A and B with a bond energy of -9.7 kcal/mol and -9.6 kcal/mol, overlapping between the native ligand NAG and gingerol in domain A with a bond energy of -6.9 kcal/mol, 7.1 kcal/mol. These interactions among molecules of a homogeneous substance are responsible for determining their bulk properties: melting

point, boiling point, viscosity, surface tension, etc (table 8, figure 7). The interactions between molecules of two different substances significantly influence the rates of chemical reactions, the effectiveness of chromatographic separations, and molecular recognition in biological processes (Murthy, 2006).

Table 8. Bond energy, amino acid residues, type of ligand combination bond flavonoids, Tannins, Steroids, Saponins with COX^2

Goup compound	Ligand	Binding Afinity (kcal/mol)	Domain Site	Amino acid residues	Bond Type
Flavonoid, Saponin, Tannin, Steroid	Curcuminoid	-10,2	B	GLU465, CYS47, ASN34	Hidrogen bond
				CYS41, GLN461, GLY135, TYR130, LYS468, ARG44, VAL46, GLY45, ARG469, ASN43, GLN42, MET48, TRP323,TYR136	Van der waals
				LEU152, HIS39, PRO153, CYS36, PRO156	Pi-Alkyl
	Quercetin	-9,7	B	GLY135, GLY45, TYR130, ASP125	Hidrogen bond
				TYR136, ARG469, ALA151, LEU152, CYS47, CYS41, CYS36, HIS39, GLN461, PRO40	Van der waals
				PRO153, VAL46, ARG44	Pi-Alkyl
	Heyneanone	-7,6	B	GLN370, SER126	Hidrogen bond
				GLN372, SER121, HIS122, TYR373, ARG44, ASP125, ILE124, PBO542, SER541, PRO127, ALA543	Van der waals
				LYS532, PHE371	Pi-Alkyl
	Gingerol	-7,1	A	**GLN461**, ASN34	Hidrogen bond
				-	Van der waals
				PRO156, LYS468	Pi-Alkyl
	Sabinene	-6,4	A	-	Hidrogen bond
				HIS386, GLN203, TYR385, THR206, TRP387, PHE210	Van der waals
				HIS388, LEU391, LEU390, HIS207, ALA199, ALA202	Pi-Alkyl
	Ginsenosides	**-9,6**	**A**	VAL538	Hidrogen bond
				TYR373, ASN537, GLN374, ASN375, GLY536, GLY227, GLY225, HIS226, ARG376	Van der waals
				TRP139, VAL538, PHE142, LEU145	Pi-Alkyl

Table 8. Continue

Goup compound	Ligand	Binding Afinity (kcal/mol)	Domain Site	Amino acid residues	Bond Type
	Tannin	**-8,6**	**B**	-	Hidrogen bond
				PHE381, TYR385, OAS530, ARG120, TYR355, VAL523, LEU352, TRP387, LEU384	Van der waals
				VAL349, ALA527, LEU359, VAL116	Pi-Alkyl
	Stigmasterol	**-9,7**	**A**	-	Hidrogen bond
				ARG120, SER119, PRO84	Van der waals
				VAL116, TYR115, PHE99, LEU93, PHE96, ILE112, TRP100, ILE92, VAL89	Pi-Alkyl
	Native ligand NAG	-6,9	A	GLY45, CYS41, **GLN461**	Hidrogen bond
	Native ligand EDO	-3,3	A	ASP125, ARG150, THR129	Hidrogen bond

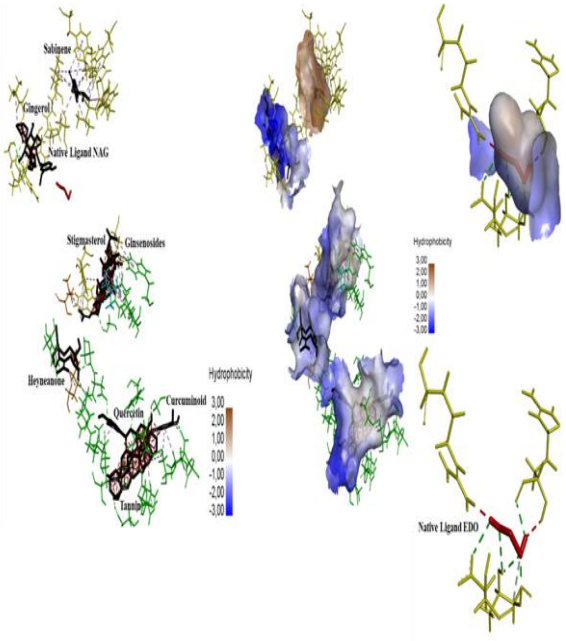

Figure 7. The results of the interaction of COX^2 with a combination of flavonoids, steroids, tannins, saponins (a), hydrophobicity (b)

The virtual molecular screening analysis carried out in this study was based on a combination of active compounds present in medicinal plants as ingredients for traditional herbal medicine. Based on the results of molecular screening analysis, it can be predicted that the combination of plant formulations is anti-inflammatory, among others, formulation of 1 ginger-temu manga, two ginger-bangle, three temu giring-bangle, temu giring-temu mango.

Traditional herbal medicine is made from one or a mixture of medicinal plants. Traditional herbal medicine is Indonesian society's local wisdom, which is used to maintain stamina or relieve symptoms of diseases caused by microorganism infection or malfunctioning of the body's metabolism, such as coughing, fever, joint pain, blood circulation, anemia, etc. The active compounds found in plants as ingredients for herbal medicine have an essential role in relieving disease symptoms. The right combination of active compounds can relieve symptoms of a disease or inhibit the synthesis of specific proteins. The combination of flavonoids-tannins, flavonoids-steroids, flavonoids-saponins can inhibit prostaglandin synthesis as an inflammatory mediator through the arachidonic acid pathway. This research needs to be followed up on other proteins as inflammatory mediators, such as

leukotrienes, histamine, nitric oxide, cytokines (IL-1, Tumor Necrosis Factor (TNF), interferon (INF) -C, IL-6, IL-12, and IL-18, bradykinin, serotonin or pro-inflammatory agents, such as interleukin-1 β (IL-1 β) and tumor necrosis factor (TNF) α.

Besides inhibiting the synthesis of inflammatory mediators, herbal medicine also acts as an antioxidant. According to Them (Them et al., 2019), that the combination of alkaloids, saponins and flavonoids in the leaves of Launaea sarmentosa has a high antioxidant activity of 24.15 µg / mL compared to vitamin C of 6.45 µg / mL in the DPPH test. Based on the antioxidant activity, bioinorganic complexes (transition metal active compounds) have more potent free radical scavenging activity than the combination of active compounds or single compounds (Jabeen et al, 2017). The transition metals Fe, Mn, and Cu, are types of transition metals that naturally bond with flavonoids in the form of complex compounds (Kasprzak et al,. 2015). In order to comprehensively determine the efficacy of traditional herbal medicine, it is necessary to carry out a detailed characterization in terms of its crystal structure, bioinorganic complexes, the presence of functional groups of inactive compounds in traditional herbal medicine.

Conclusion

Based on the findings and discussions, it is possible to conclude that there are 22 medicinal plant inventories in Bangselok Village. In the Zingiberaceae family, phytochemical analysis revealed that the flavonoid group was dominant over steroids, tannins, and saponins. Based on the active compound content, the virtual screening results of traditional herbal formulation combinations revealed that the combination of flavonoids-steroids, flavonoids-tannins, and flavonoids-saponins is more recommended as an anti-inflammatory because it has similar interactions with amino acid residues, binding affinity values, and hydrophobicity bonds with the native NAG and EDO ligands.

Acknowledgment

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