PHARMACIA

Pharmacia 69(3): 733–744
DOI 10.3897/pharmacia.69.e89400

Research Article

Molecular docking investigation of anti-inflammatory herbal compounds as potential LOX-5 and COX-2 inhibitors

Liliia Vyshnevska[1], Hanna I. Severina[1], Yuliya Prokopenko[1], Alexander Shmalko[1]

1 *National University of Pharmacy, Kharkiv, Ukraine*

Corresponding author: Liliia Vyshnevska (realmanutd.ua@gmail.com)

Received 22 June 2022 ♦ **Accepted** 24 July 2022 ♦ **Published** 5 August 2022

Citation: Vyshnevska L, Severina HI, Prokopenko Y, Shmalko A (2022) Molecular docking investigation of anti-inflammatory herbal compounds as potential LOX-5 and COX-2 inhibitors. Pharmacia 69(3): 733–744. https://doi.org/10.3897/pharmacia.69.e89400

Abstract

According to the World Journal of Gastroenterology, more than 5 million people worldwide suffer from inflammatory bowel disease. The use of phytotherapeutic remedies in treatment of chronic inflammatory processes can be an effective alternative in patient's therapy. The advantage of herbal medicines is the ability to influence various links of pathogenesis, lack of addiction, and the absence of withdrawal syndrome with long-term use in chronic pathology. In order to develop a new combined remedy with anti-inflammatory activity for the treatment of colitis, thirteen herbs, which are used in official or traditional medicine in inflammatory processes, were selected among the Ukrainian flora members. To select the most promising drugs and optimize further pharmacological research, molecular docking of the main active substances of the selected herbs to the fundamental pro-inflammatory enzymes – lipoxygenase-5 (LOX-5) and cyclooxygenase-2 (COX-2) – was carried out. Native inhibitors AKBA and celecoxib, respectively, were used as the reference ligands. The selection of candidate structures for *in silico* research was carried out according to the bibliosemantic research and logical-structural analysis concerning anti-inflammatory effect of the substances, which are part of chemical composition of the selected herbs. Molecular docking results have shown a high affinity level for the active site of the LOX-5 inhibitor gallotannin, quercetin, inulin, sitosterine, and moderate for ellagic acid. High affinity level for the active site of the COX-2 inhibitor was found for inulin, quercetin, gallotannin, ellagic acid and urticin A, moderate one – for gallic acid. For the further pharmacological *in vitro* and *in vivo* studies for anti-inflammatory activity, medicinal herbs with the highest content of the mentioned compounds were selected: *Inula helenium*, *Cichorium intybus*, *Capsella bursa-pastoris*, *Foeniculum vulgare*, *Equisetum arvense*, *Veronica officinalis*. Besides, it is recommended to use aqueous extracts of the selected herbs for the further pharmacological studies.

Keywords

molecular docking, herbal remedy, anti-inflammatory, LOX-5, COX-2

Introduction

According to the World Journal of Gastroenterology, more than 5 million people worldwide suffer from inflammatory bowel disease. The choice of drugs with pronounced pharmacological activity, minimal side effects and the possibility of long-term use, including in chronic diseases, is one of the most important problems in therapy (Burger and Travis 2011; Rogler et al. 2013; Fakhoury et al. 2014). The use of phytotherapeutic remedies in treatment of chronic inflammatory processes can be an effective alternative in patient's therapy (Langhorst et al. 2015; Cheifetz et al. 2017). The advantage of herbal medicines is the ability to influence pathogenesis, lack of addiction, and the

absence of withdrawal syndrome after long-term use in chronic pathology (Nguyen et al. 2016; Yoon et al. 2019). Herbs are the source of micronutrients, vitamins, amino acids, proteins, polyphenols, unsaturated fatty acids, tannins, essential oils, and others (Singh et al. 2011). Herbal remedies are widely used in the world as part of both official and traditional medicine, so the expansion and updating of the range of phytotherapeutic remedies is possible through the implementation of traditional herbs into scientific medicine (Ekor 2014; El-Dahiyat et al. 2020). Therefore, development of multifaceted action drug containing herbs applied for the treatment of inflammatory bowel disease and nonspecific ulcerative colitis has become relevant.

At the beginning of the study, literature sources concerning the use of medicinal herbs in treatment of inflammatory bowel disease were analyzed. It was found that *Foeniculum vulgare* (Badgujar et al. 2014), *Acorus calamus*, *Potentilla palustris*, *Petroselinum crispum* (Motley 1994), *Urtica dioica* (Rauf et al. 2021), *Inula helenium*, *Bergenia crassifolia*, *Veronica officinalis* (Joo 2014), *Carum carvi*, *Levisticum officinale* (Nematgorgani et al. 2020), *Cichorium intybus* (Lee et al. 2019), *Capsella bursa-pastoris* (Oguntibeju 2018), and *Equisetum arvense* are used both in traditional and in official medicine of Ukraine and other countries due to wide spectra of their pharmacological activity, e.g.: anti-inflammatory, regenerative, absorptive, immunomodulatory, etc (Keshavarz et al. 2013; Rizvi et al. 2014). Pharmacological activity of the mentioned herbs is mostly associated with the content of polysaccharides (inulin), phenolic compounds and polyphenols, such as flavonoids (quercetin) (Lee et al. 2019; Sato and Mukai 2020), terpene compounds – components of essential oils (anethole, apiol, carvone) (Sim et al. 2015), fatty acids (petrolselenic acid), coumarins (coumarine, umbelliferone) (Germoush et al. 2018), phenolic acids (gallic acid, ellagic acid), hydrolyzable tannins (gallotanin) (Oguntibeju 2018; Zhang et al. 2018), as well as steroid and phenolic glycosides (sitosterine, urticin) (Oguntibeju 2018; Lee et al. 2019).

Structural features and biological activity of natural polysaccharides determine their efficiency for the treatment of inflammatory bowel disease. These compounds have positive effects on the intestinal microbiota; they regulate formation of cytokines involved in inflammation development, restore the intestinal epithelial barrier (Wang et al. 2021), reduce neutrophil infiltration (Yang et al. 2022) and, consequently, regulate oxidative stress (Nie et al. 2017; Li et al. 2021). The lack of toxic effects on human body and the availability of natural sources make polysaccharides promising objects for scientific studies. However, there is still no data concerning polysaccharides' specific mechanism of action on the links of intestinal diseases pathogenesis.

Although etiology and pathogenesis of inflammatory bowel disease remain unknown, therapy includes the use of anti-inflammatory drugs together with an anti-inflammatory diet (Triantafyllidi et al. 2015; El-Dahiyat et al. 2020). Therefore, the given research was aimed to analyze the effect of the mentioned groups of herbal substances on the inflammatory process triggers.

Anti-inflammatory effect of herbal polyphenols has long been known (Yoon and Baek 2005). The main groups of polyphenols – flavonoids, stilbenes, lignans and phenolic acids, can affect various parts of the inflammatory process. Polyphenols can incorporate into arachidonic acid metabolism by inhibiting various pro-inflammatory enzymes – cyclooxygenase (COX-1,2), lipoxygenase (LOX-5,12,15), and phospholipase A2 (PLA2). Thus, quercetin and kaempferol inhibit COX-2 (Welton et al. 1986; Lee et al. 2010), while catechin can inhibit COX-2 only at very high concentrations (Noreen et al. 1998). Besides, flavonols such as kaempferol, quercetine, morin, and myricetin are thought to be better LOX inhibitors than flavones.

Molecular anti-inflammatory activity of flavonoids is realized by transcription factors inhibition, such as nuclear factor-kappa B NF-kB (NF-kB) and activating protein-1 (AP-1), which control immune and inflammatory response expression (Serafini et al. 2008). It has also been found that some flavonoids may reduce expression of proinflammatory cytokines, in particular tumor necrosis factor α (TNF α) (Manjeet and Ghosh 1999), interleukins (IL-1b, IL-6, IL-8, IL-10) and monocytic chemoattractant protein-1(Santangelo et al. 2007; Lee et al. 2010). Realization of anti-inflammatory activity of polyphenols, in particular flavonols, is possible due to inhibition of nitric oxide synthase (inducible NO synthase iNOS) and corresponding inhibition of nitric oxide production, which is one of the directions in treatment of ulcerative colitis and Crohn's disease (Li et al. 2016; Asakura and Kitahora 2018).

Besides, the role of natural polyphenols in inflammatory bowel disease correction is recognized and characterized by many molecular mechanisms of anti-inflammatory effect, including the above mentioned (Arya et al. 2020). Possibility of multifactorial effects of polyphenols on different biotargets in some cases does not allow to understand mechanism of pharmacological action and to determine necessary parameters for their action selectivity. Only scientific achievements of recent decades in molecular technology and macromolecules isolation; estimation of active sites structure with ligand conformational placement features in receptor or enzyme; developed arsenal of *in silico* methods for predicting the affinity of the ligand to the receptor allow predicting the possibility of a particular impact on the biotarget and pharmacological response. Scientists consider virtual screening as a promising tool for developing new drugs and determining the role of individual compounds in the implementation of various types of pharmacological activity. In addition, methods of molecular modelling of pharmacological activity, docking for example, can meet many clinical needs under development, reduce time and costs, and keep laboratory animals alive (Umesh et al. 2019; Sabe et al. 2021; Mateev et al. 2021).

In order to develop a new combined remedy with anti-inflammatory activity for treatment of colitis, the ***aim*** of the

given research was molecular docking of the main active ingredients of herbal material to biotargets – lipoxygenase-5 (LOX-5) and cyclooxygenase-2 (COX-2), as fundamental proinflammatory enzymes. The study will optimize further pharmacological screening and predict any possible mechanism of pharmacological action. In addition, the obtained results of molecular docking can substantiate the choice of markers for standardization of the finished product or herbal material, explain pharmacological features and optimize the complex herbal remedy composition.

Undoubtedly, therapeutic effect of herbs is achieved through the synergism of different groups of natural compounds, but the problem of insufficient information about the pharmacological mechanisms of individual substances remains relevant (Caesar and Cech 2019). Therefore, determination of the pharmacological potential of every individual compound remains interesting from both practical and scientific point of view.

Material and methods

13 herbal species from Ukrainian flora were selected for the study. According to the results of bibliosemantic research, the selected herbs are used in official or traditional medicine.

The hardware used is the ASUS VivoBook X530UN S15 with an Intel Core i7 8550U 8thGen and Windows 10, 64-bit operating system. Flexible molecular docking was carried out using Vina and AutoDockTools 1.5.6 software (Forli et al. 2016). Biotarget macromolecule was selected from PDB (Protein Data Bank): PDB ID – 6NCF, PDB ID 3LN1.

Ligands preparation. BIOVIADraw 2017R2 tool was used for ligand structures base construction. The structures were saved as .mol files, were optimized by Chem3D software using molecular-mechanic MM2 algorithm and then saved in .pdb format. Then, AutoDockTools-1.5.6 was used to convert the files in .pdbqt format (Trott and Olson 2010).

Proteins preparation. Discovery Studio Visualizer 2017/R2 was used to remove the solvent and native ligand from the crystal. The proteins were saved as .pdb files. Proteins were saved as .pdb files. AutoDockTools-1.5.6. was used to add polar hydrogen to the protein structure and to save the data as .pdbqt files.

Grid box size, as well as its center, were determined according to the native ligand.

Lipoxygenase-5 (LOX-5) (PDB ID 6NCF) x = 11.6, y = -23.38, z = -18.01; size x = 30, y = 28, z = 26; Cyclooxygenase-2 5 (PDB ID 3LN1) x = 18,84, y = -52,89, z = -53,81; size x = 22, y = 24, z = 24.

AutoDock Vina software was used for molecular docking.

To validate the docking method, the reference ligands – AKBA and Celecoxib – were extracted and then reused for the redocking process after given charged, set torque and saved as .pdbqt. RMSD value is the quantitative characteristic of the technique's validity, which characterizes higher probability of a successful docking result. RMSD was calculated using ProFit Results outsource. It was 2.023 A for AKBA and 1.987 A for celecoxib between experimental and reference conformation of the substances.

Visualization and analysis of the obtained results of the docking studies were carried out using Discovery Studio V17.2.0.16349.

Results and discussion

The general algorithm of research on rationalization of development of phytotherapeutic remedy with anti-inflammatory activity is shown in Fig. 1. The algorithm based on results of the literary data analysis concerning the mentioned pathology. The literature data were summarized, and the most promising herbs were selected, according to such criteria as information on the use of herbs for treatment of inflammatory bowel disease by official or traditional medicine; the ability of active herbal compounds to influence the implementation of anti-inflammatory activity, as well as the presence of herbs in Ukrainian flora. On it basis, 13 herbs from 9 botanical families were selected, 13 active substances were chosen, and scientific methodology was substantiated.

The selection of candidate structures for *in silico* research was carried out according to the bibliosemantic research and logical-structural analysis concerning anti-inflammatory effect of the substances, which are part of chemical composition of the selected herbs. The structures of the main biologically active substances from the groups of terpene compounds, polyphenols and polysaccharides are shown in Fig. 2.

5-LOX enzyme was chosen as the main biotarget for the search of the herbal compounds' anti-inflammatory action, due to the mentioned compound initiates biosynthesis of powerful inflammatory mediators – leukotrienes. Therefore, the search for selective inhibitors of this enzyme continues and remains relevant. Although the human enzyme LOX-5 macromolecule was isolated in 2011 (Gilbert et al. 2011), only in 2020 it became possible to detect and isolate it in conformation with a selective inhibitor in the active site (Gilbert et al. 2020). Peculiarities of conformational arrangement of ligands, which specify their selectivity for anti-inflammatory response, were determined. LOX-5 macromolecule (PDB ID 6NCF) with its natural non-competitive inhibitor – pentacyclic triterpene acid (3α,8α,17α,18α-3-acetyloxy-11-oxours-12-en-23-oic acid – AKBA) in the active site – was used for bioactive substances docking, a multicomponent remedy constituents. AKBA is a bioactive substance of frankincense with well-studied and experimentally proven anti-inflammatory properties (Stürner et al. 2018). AKBA performs allosteric modulation by being fixed at the binding site between the membrane-binding (amino terminal) and catalytic domains of one of the two monomers by macromolecules, causing conformational changes in the enzyme's distal structural fragments. The cavity of the active site has a U-shaped rather narrow shape and is "corked" by lateral residues of phenylalanine (Phe177) and tyros-

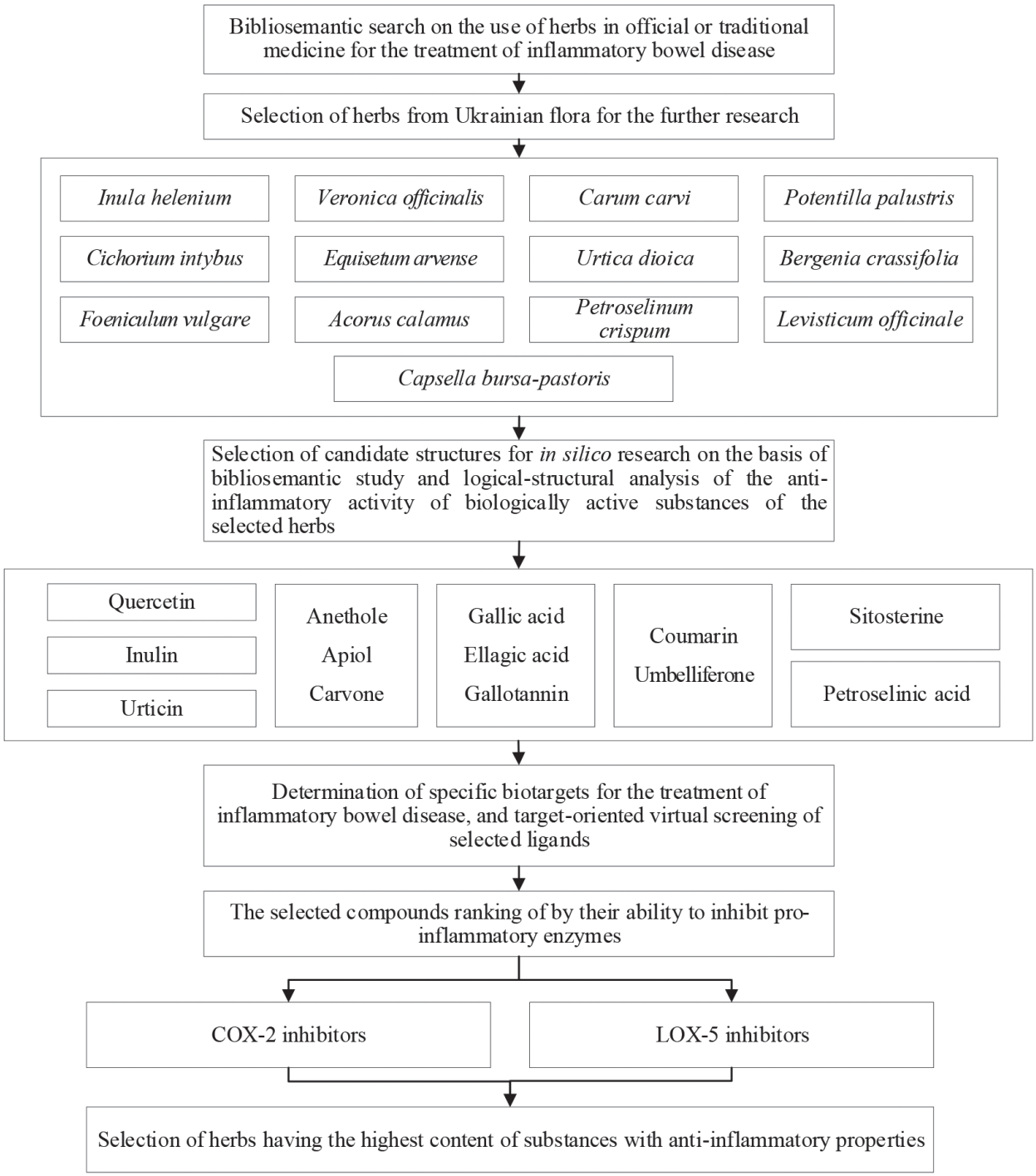

Figure 1. General algorithm of the study.

ine (Tyr181) (Gilbert et al. 2011). Residues of amino acids forming hydrophobic pocket are:

catalytic domain: Ile126, His130, Lys133, Glu134, Thr137, Arg138; amino terminal domain: Arg68, Leu66, Glu108, Val110, Val109, Arg101.

To evaluate the efficiency of the used methodology and the docking parameters in the experimental data reproduction, the native AKBA ligand was docked into the LOX-5 allosteric site, and the affinity was – 9.1 kcal/mol. Reproducibility of placement into the active site, as well as amino acid residues interactions, is shown in Fig. 3A and in Table 1.

All necessary native interactions and one additional connection with imidazole cycle of histidine (His125) are visualized. However, the presence of interaction with isoleucine residue (Ile126) testifies the identity of the conformational arrangement relative to the native position. Docking results of the mentioned biologically active substances were evaluated according to the binding energy parameter (kcal/mol) relative to the reference ligand, according to the type and amount of interaction with amino acid residues of the active site, and according to the spatial position in the hydrophobic pocket cavity.

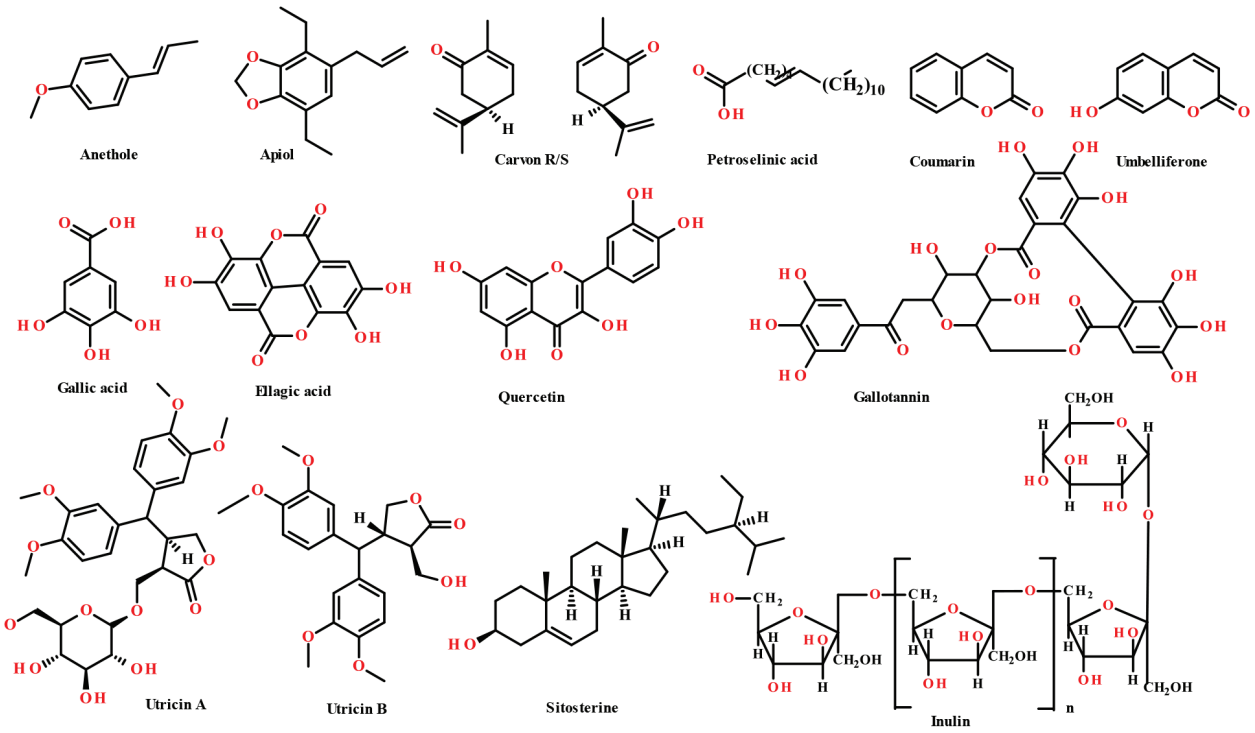

Figure 2. Biologically active compounds of the studied herbs.

Compounds that are part of the essential oils – anethole, apiole, carvone – also have shown high scoring function values of about -5.0 to -5.9 kcal/mol, as well as unfavorable conformational arrangement for allosteric modulation: fixation of "small" ligands occurs at the entrance to hydrophobic pocket (interaction with Arg101, Arg138), obvious inability of deeper immersion, and majority of the interactions happen with adjacent amino acid residues that are not part of the active site (Table 1, Fig. 3B on apiol example).

The inability to penetrate the allosteric LOX-5 site was predicted for petroselinic acid: high scoring function -4.8 kcal/mol and no hydrophobic interactions were discovered. Coumarin and umbelliferone docking has resulted almost in the same manner. Despite the large number of hydrophobic interactions (Table 1), their specificity with relatively to the amino acids of the active site was insufficient, which has shown in the values of scoring functions: -5.9 and -6.4, respectively.

Phenolic acids have shown a moderate inhibitory effect probability on LOX-5, in particular gallic acid having scoring function of -6.8 kcal/mol. For ellagic acid, although formation of hydrogen bonds trihedral network with threonine (Thr137) hydroxy group and histidine carboxyl group remains possible (Table 1), but hydrophobic fixation with only three ligaments and depth of immersion in the pocket (Fig. 4A) may turn out to be insufficient to block the enzyme.

Quercetin shows its spatial arrangement similar to ellagic acid with fixation in the front of the pocket (Fig. 4B). However, the scoring function is higher (-8.2 kcal/mol) due to stronger hydrophobic interactions and additional conformation stabilization by hydrogen bonds between quercetin hydroxyl groups with carboxyl groups of aspartic (Asp166) and glutamic acids (Glu134), carbonyl and arginine's guanidine residue (Arg138), and others.

Gallotannin has shown the highest affinity for the AKBA inhibitor site – the scoring function value was -9.4, versus -10.0 kcal/mol for the reference ligand. In terms of interactions with amino acids, a large amount of hydrogen ones, additionally stabilizing all fragments position of gallotannin molecule, has been discovered together with the hydrophobic bonds. Concerning co-location with the native ligand, a high affinity for the AKBA site becomes apparent, as the molecule is completely and deeply immersed into the active site, occupying almost identical to the native ligand spatial position (Fig. 5A).

Utricin A and B glycosides have shown quite a significant affinity level for LOX-5 inhibitor site – scoring function was -8.1 and -7.9, relatively. For both compounds, interaction with amino acid residues of the active site was predicted, but in conformation with the native ligand, inability to dive into the narrow pocket and pass beyond it, with fixation due to interaction with adjacent amino acid residues, becomes apparent (Fig. 5B).

A high affinity level was predicted for LOX-5 allosteric site in sitosterine: its' binding energy was -8.3 kcal/mol. Compatible conformation with the native ligand (Fig. 6A) shows the similarity of their fixation with the possibility of rather deep immersion into the hydrophobic cavity. An extensive network of hydrophobic strong bonds (Table 1), fixing all molecule fragments and indicating a high probability of allosteric effects on the macromolecule, is obvious.

Table 1. Docking results of the studied substances into the LOX-5 active site inhibitor.

Ligand	Biotarget – LOX-5 enzyme			
	Binding energy kcal/mol	Hydrophobic interactions	Hydrogen bonds	Other interactions
AKBA reference ligand	-10.0	Val110 (3), Lys133 Ile126, His125*, His130(3)	Val110, Arg138(2), Val109	Arg101(2)Pi-Cation
Anethole *cys*-/*trans*- isomers	-5.0/-5.0	Lys133(2), Glu134, His130	Arg101	–
Apiol	-5.9	Pro164*, Val107*, Tyr142, Arg138	Arg101, Arg138, Glu134, Thr137	Arg101(2) Pi-Cation
Carvone R/S	-5.0/-5.1	Val107*, Pro164*, Arg165*, Arg101	Arg165*, Asp166	–
Petroselinic acid	-3.8	–	Arg138(2)	–
Coumarin	-5.9	Ala388(4)*, Val389(3)*, Leu111, Pro98*, Arg101(2)	Lys394*, Arg101	Glu134 Pi-Anion
Umbelliferone	-6.4	Ala388(3)*, Val389(3)*, Leu111, Pro98*, Arg101	Lys394*, Arg101	Glu134 Pi-Anion
Gallic acid	-6.8	Ala388*, Arg112*, Val389*	Lys394(2)*, Arg112(2)*, Glu134(2), His130,	–
Ellagic acid	-7.8	Thr137, Val107 (2)	His130, Thr137(4)	–
Quercetin	-8.2	Val107*, Val110(2), Thr137	His130(2), Glu134(2), Arg138, Asp166*	Arg101(2) Pi-Cation
Gallotannin	-9.4	Thr137, Val110(2)	Arg68, Arg101, His130, Glu108, Thr137	Arg101(2) Pi-Cation
Urticin A	-8.0	Val109(2)*, Arg138	Thr104*, Thr137, Arg138, Glu134, His130, Trp102*, Asp166*, Gln141*	–
Urticin B	-7.9	Val109(2), Arg138	Thr137(3), Arg138, Tyr142*, Trp102*	Asp166 Pi-Anion
Sitosterine	-8.3	Val110 (4), Lys133, Leu66, Arg68, Val107(2)*, Lys133, His130	–	–
Inulin	-8.9	Val110	Arg68, Arg101, Thr104*, Val110, His130(2), Thr137(2), Trp102(2)*, Glu134(2), Asp166(2)*	–

* Amino acid residues not interacting with AKBA in the experiment are indicated
# The amount of links is in parentheses

High affinity level was also predicted for inulin (-8.9 kcal/mol) with an extensive hydrogen bonds network of all fragments of the molecule with amino acids of the active site (Fig. 6B). Only two amino acids – threonine and tryptophan (Thr 104, Trp102) – were not experimentally determined residues, but they were visualized in the immediate vicinity of the native ligand. Obtained results indicate a high probability of the inhibitory ability of inulin in relation to LOX-5.

Cyclooxygenase-2 (COX-2) became the second bio-target for predicting possible mechanism of anti-inflammatory effect realization by complex herbal preparation components. The enzyme COX-2 homotetrameric macromolecule consists of two homodimers, each of which contains a binding site of a high-affinity selective inhibitor – celecoxib (Wang et al. 2010). Hydrophobic binding pocket of celecoxib was formed by aromatic (Phe504, Trp373, Tyr341), non-polar (Ala513, Leu338, 345, 370, 517, Val335, 509) and polar amino acids (Ser339, Met508, Gln178), as well as two polar positively charged arginine residues (Arg106, 499). To estimate the used docking parameters efficiency for the experimental data reproducing, the native ligand, celecoxib, a COX-2 enzyme inhibitor, was docked. The affinity was -12.5 kcal/mol, which corresponds to the high celecoxib affinity for the enzyme. Reproducibility of allocation into the active site and interactions with amino acid residues is shown in Fig. 7A and in Table. 2. All experimentally established connections can be visualized by X-ray diffraction analysis (Wang et al. 2010). Evaluation of docking results for the herbal compounds was carried out by the binding energy parameter (kcal/mol) in relation to the reference ligand, by the type and amount of interaction with amino acid residues of the active site and by spatial position in the cavity of hydrophobic pocket.

Binding energy values for all studied ligands after docking into the active site of the COX-2 inhibitor were significantly higher than the reference ligand: from -5.0 kcal/mol in urticin B to -9.8 kcal/mol in quercetin (Table 2). The highest quercetin affinity to COX-2 inhibitor can be explained by the analysis of conformational allocation in the active site cavity (Fig. 7B). For quercetin, a complete immersion into the hydrophobic pocket and formation of strong and short hydrophobic interactions with the main amino acids for inhibitory activity manifestation were predicted: chromene cycle was fixed by interaction with phenylalanine (Phe504) phenyl ring, methyl radicals of valine and leucine (Val335, Leu338). Dihydroxyphenyl radical of quercetin was fixed with methyl groups of valine and leucine (Val335, Leu338). Hydrogen bonds formed with the amide group of glutamine (Gln178), phenylalanine (Phe504) amino group, hydroxyphenyl tyrosine (Tyr341) and carbonyl serine (Ser516) promote stabilization of the ligand-enzyme conformation. Detailing of co-location with celecoxib demonstrates the absolute identity of position in the pocket and the overlap of all parts of both molecules, which proves the highest

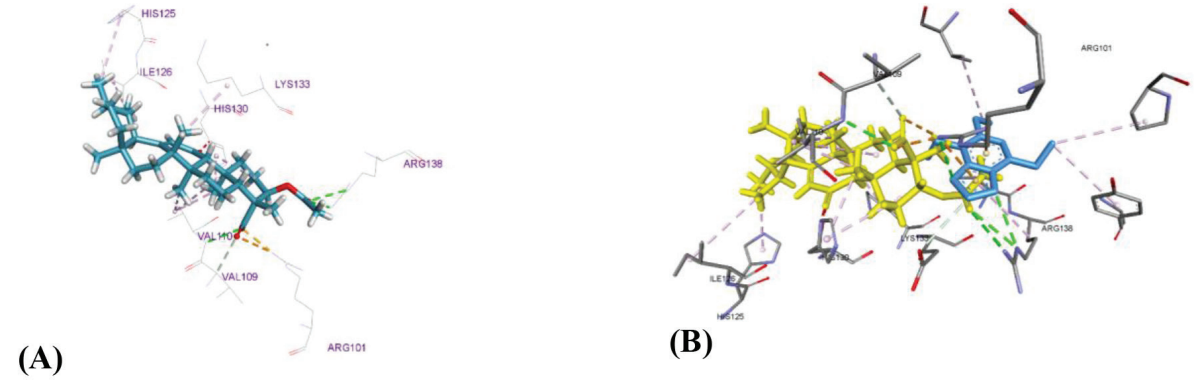

Figure 3. 3D visualization of interaction with amino acid residues of the LOX-5 active site (**A**) reference ligand of AKBA; (B) native AKBA ligand compatible conformation (yellow molecule) with apiol (blue molecule).

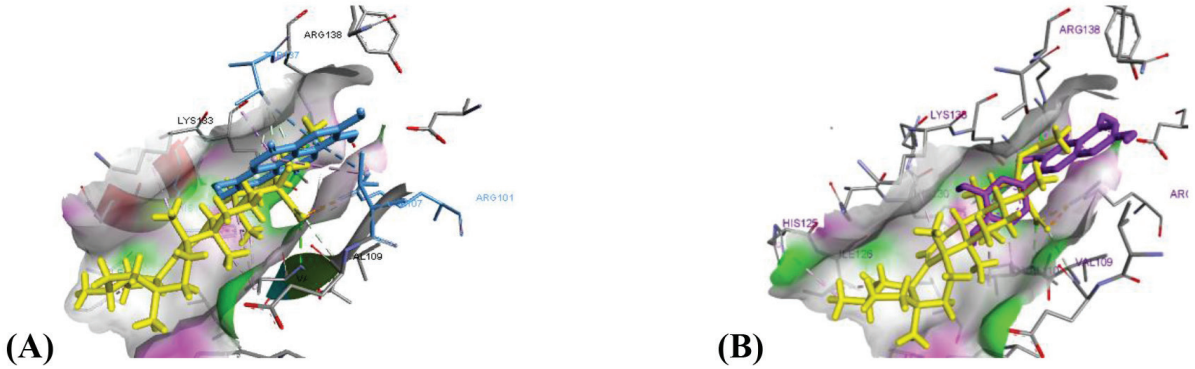

Figure 4. 3D visualization of the native AKBA ligand compatible conformation (yellow molecule) with (**A**) ellagic acid (blue molecule) and (**B**) quercetine (purple molecule).

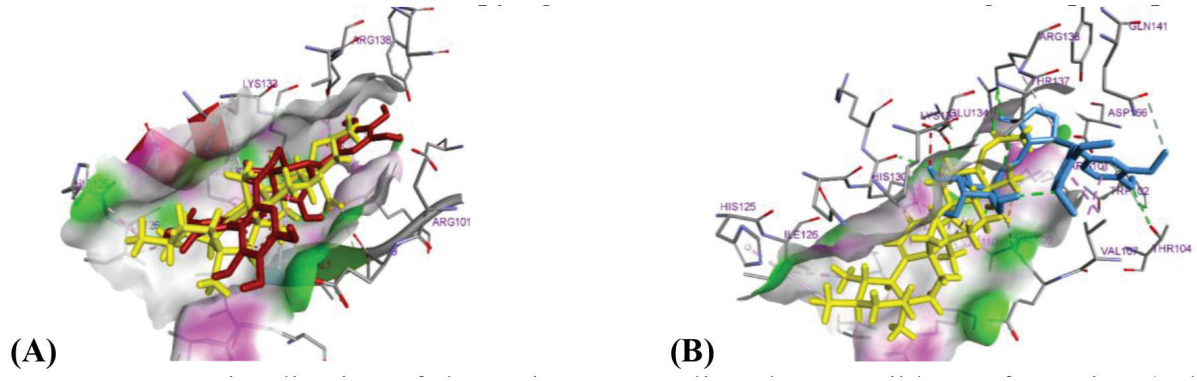

Figure 5. 3D visualization of the native AKBA ligand compatible conformation (yellow molecule) with (**A**) gallotannin (red molecule) and (**B**) urticin A (blue molecule).

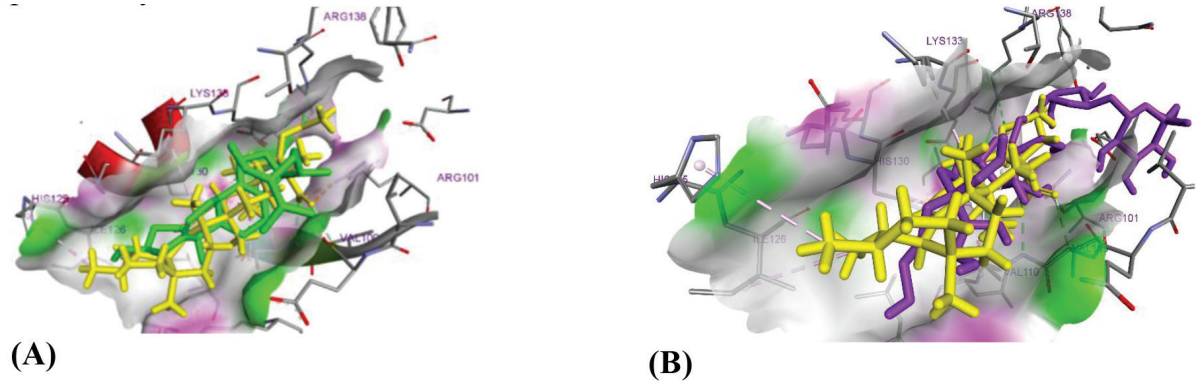

Figure 6. **(A)** 3D visualization of the native AKBA ligand compatible conformation (yellow molecule) with sitosterine (green molecule); **(B)** AKBA ligand compatible conformation (yellow molecule) with inuline (purple molecule).

Table 2. Docking results for the studied natural compounds into the COX-2 inhibitor active site.

Ligand	Biotarget – COX-2 enzyme		
	Binding energy kcal/mol	Hydrophobic interactions	Hydrogen bonds
Celecoxib reference ligand	-12.1	Val335(2), Ser339, Val509(2), Leu370, Leu345, Leu517, Tyr371, Tyr341, Trp373, Ala513(2)	Arg106, Arg499, Gln178, Leu338, Ser339
Anethole, *cys*-/*trans*-isomers	-6.3/-6.2	Val509(2), His75*, Leu338	–
Apiol	-7.5	Val335(3), Ala513, Val509(2), Tyr341, Tyr371, Trp373, Phe504*, Leu338, Ala513, Leu517	Ser516
Carvone R/S	-6.7/-6.8	Val509(2), Ala513, Leu338(3), Val335, Tyr371, Trp373, Phe504*	–
Petroselinic acid	-7.0	Ala513, Val509, Tyr341	Arg499, GLn178, Leu338
Coumarin	-7.2	Val509(2), Leu338(2), Ser339(2), Leu338(2), Leu338, Ala502*	–
Umbelliferone	-7.2	Val509(2), Leu338 (2), Ser339(2), Leu338, Ala502*	–
Gallic acid	-6.3	Ser339, Val509 (2)	Tyr341, Arg499, His75*, Ser339
Ellagic acid	-8.6	Val335(3), Val509(4), Ala513(3), Leu338 (3)	Tyr341, Ser516, Leu338
Quercetin	-9.8	Leu338(2), Phe504, Val509(2), Val335	Tyr341(2), Phe504, Ser516
Gallotannin	-8.6	Val509(3), Ala513, Phe191*, Leu520*, Leu338	Arg106, Tyr341(2), Leu338, Ala513, Phe195*
Urticin A	-8.5	Val335, Leu338, Ala513(2), Val509, Val335, Leu517	Tyr341(3), His75, Ser339, Ser516*, Gln178*, Ser516*
Urticin B	-5.0	Ala513(2), Tyr341, Val335 Leu338, Val509	Arg106, Gln178, Tyr341
Sitosterine	-6.6	Val335(3), Val509(3), Ala513(6), Leu338(2), Leu517, Leu345, Val74*, Leu78(2)*, Val102(2)*, Val102*, Tyr101(2)*, Tyr341(4), Phe504	–
Inulin	-6.2	–	His337*, Arg499, Gln178(3), Ser339(2), Asp333*, Gly340*, Leu338*, Pro500*, Asp501*

* Amino acid residues not interacting with AKBA in the experiment are indicated

The amount of links is in parentheses

probability of inhibitory activity of quercetin in relation to the enzyme COX-2.

Significant ability to inhibit COX-2 enzyme was predicted for gallotannin with its scoring function of -8.6 kcal/mol (Fig. 8A, Table 2). Seven hydrophobic interactions with the amino acids of the active site fixed the main part of gallotannin molecule with additional stabilization by hydrogen bonds with arginine (Arg106) guanidine fragment, tyrosine (Tyr341) 4-hydroxyphenyl fragment and leucine carbonyl (Leu338). Only 3,4,5-trihydroxybenzoic acid residue was firmly fixed outside the experimentally established active site, but its location was rather deep, and the size of gallotannin molecule made possible to occupy completely celecoxib active site and, consequently, demonstrated a high probability of COX-2 inhibition.

Apiol and carvone have demonstrated fixation only at the entrance to the inhibitor binding pocket, although with many hydrophobic interactions (Fig. 8B). This type of allocation and values of scoring functions, -7.5 and -6.8 kcal/mol, respectively, has shown a poor ability of apiol and carvone to inhibit COX-2.

Coumarin and umbelliferone were similarly fixed into the cavity space of the active site, interacting with the same amino acid residues and overlapping in the visualization of benzopyranone cycles (Fig. 9A).

A fairly deep immersion into the pocket was predicted for the mentioned compounds, as evidenced by the fixation in the area of benzenesulfonamide fragment of celecoxib. However, that type of allocation lead to the "Unfavorable Acceptor-Acceptor" formation: interaction between the tertiary nitrogen atom of imidazole histidine (His75) and benzopyranone carbonyl (Fig. 9A, red bond), so the existence of such type of conformation considered to be improbable. Binding energy value was predicted for gallic acid: -6.3 kcal/mol, and only 3 hydrophobic and 4 hydrogen interactions (Fig. 9B). Nevertheless, like coumarin derivatives (Fig. 9A), immersion into the hydrophobic pocket of the gallic acid molecule was predicted to be quite deep, as its fixation occured in the area of celecoxib benzenesulfonamide fragment. Therefore, it is possible to assume moderate inhibitory ability of gallic acid in relation to COX.

Scoring function value for ellagic acid was -8.6 kcal/mol, and the possibility of forming a branched network containing 13 hydrophobic bonds with amino acids of the active site entirely (Fig. 10A) was calculated: dilactone cycle was fixed by two tetrahedral bonds networks with alanine (Ala513) and valine (Val335) residues, as well as bidentate bonds with isopropyl fragments of valine (Val509) and leucine (Leu338). Four terminal hydroxyls can additionally stabilize conformation due to hydrogen interactions, and compatible conformation with celecoxib indicates the possibility of fixing of ellagic acid in the correct area (Fig. 10B). The obtained results predict a high probability of ellagic acid to inhibit COX-2.

Urticin A and B glycosides have demonstrated ambiguous results: along with the scoring function of -8.5 kcal/mol for urticin A, -5.0 kcal/mol for urticin B was predicted. Hydrophobic fixation of all fragments was predicted for urticin A with additional stabilization of pyranoside

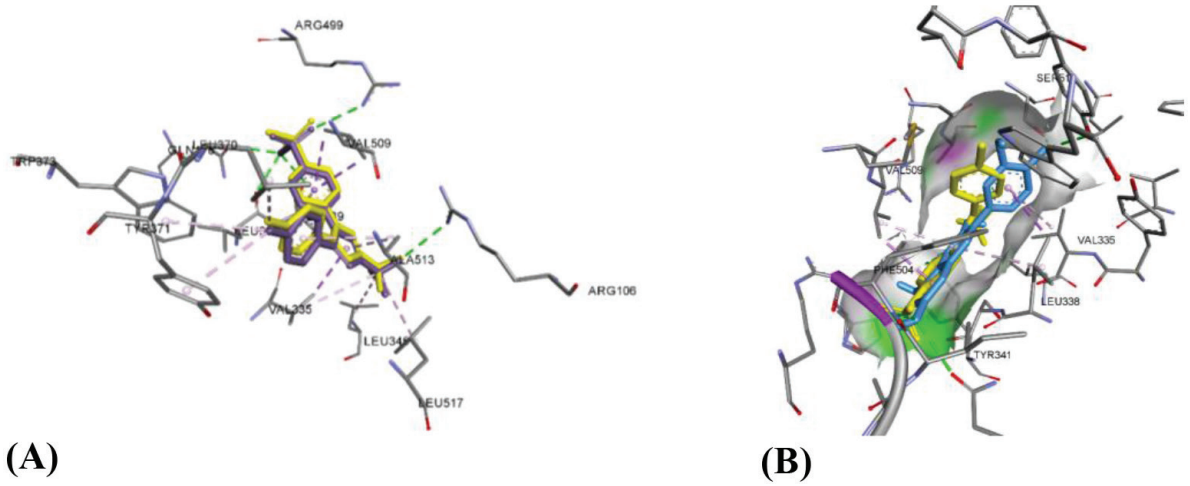

Figure 7. 3D visualization of (**A**) allocation and interaction with amino acid residues of the active site of COX-2 native celecoxib [51] and reference ligand (yellow molecule) (**B**) compatible conformation of the native celecoxib ligand (yellow molecule) with quercetin (blue molecule).

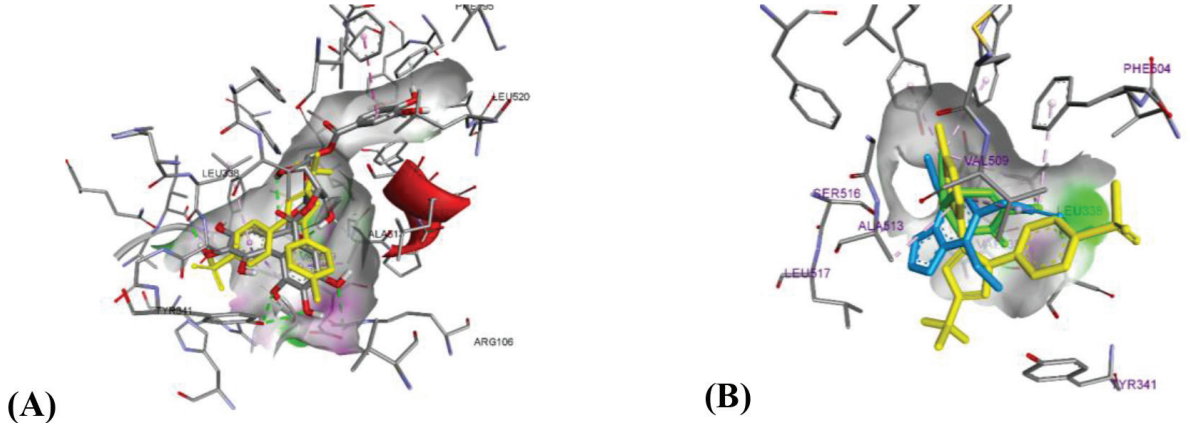

Figure 8. 3D visualization of (**A**) conformation of the native celecoxib ligand (yellow molecule) with gallotannin (grey molecule) (B) celecoxib compatible conformation with apiol (blue molecule) and carvone (green) in COX-2 active site.

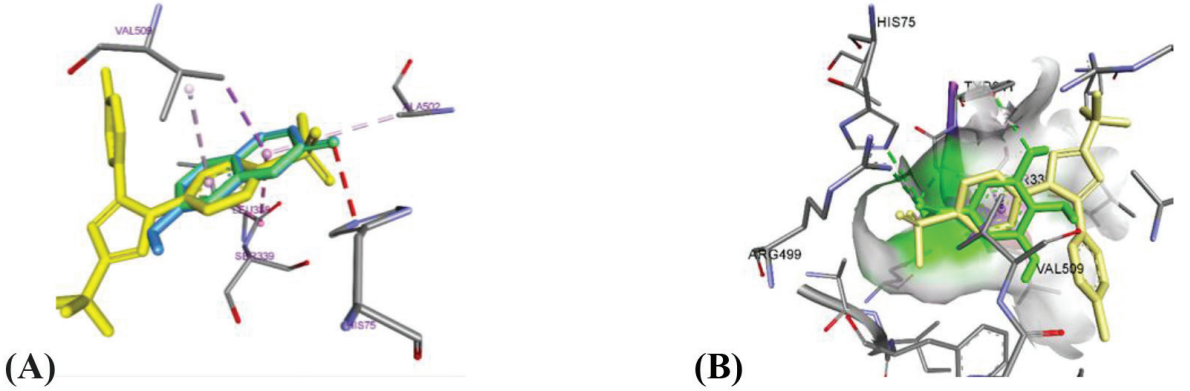

Figure 9. Visualization of celecoxib compatible conformation with (**A**) coumarin (blue) and umbelliferone (green) (**B**) gallic acid (green molecule) in the COX-2 active site.

fragment by hydrogen bonds (Fig. 11A), exclusively with amino acid residues of the active site.

The ligand was deeply immersed into the pocket, and its position made it possible to be fixed in the macromolecule in positions like celecoxib (Fig. 11B). Therefore, possibility of urticin A inhibitory activity on COX-2 is obviously feasible.

A low affinity level was predicted for the site of COX-2 inhibitor in sitosterine – the binding energy was -6.6 kcal/mol, despite the apparent branched network con-

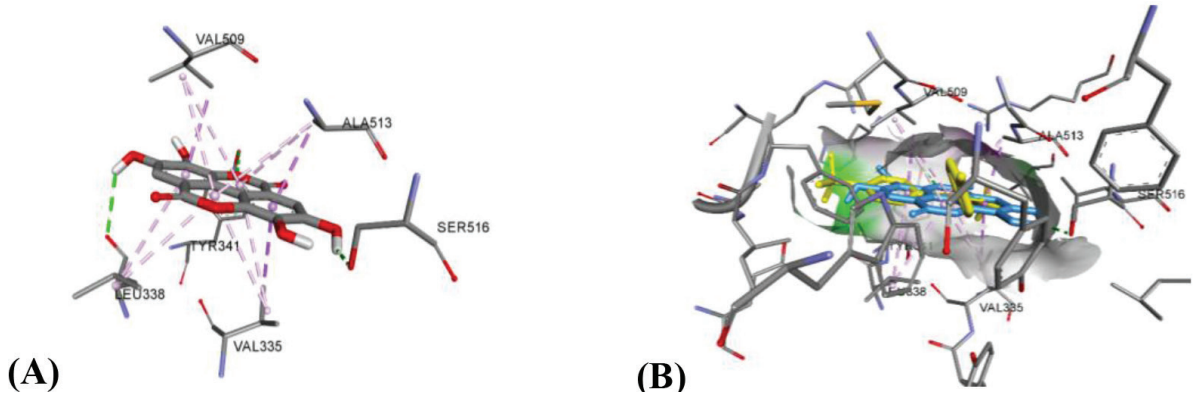

Figure 10. 3D visualization (**A**) interaction of ellagic acid with amino acids residues of the active site of COX-2 inhibitor and (**B**) celecoxib compatible conformation (yellow molecule) and ellagic acid (blue molecule).

Figure 11. 3D visualization of (**A**) interaction of urticin A with amino acids residues of the active site of COX-2 inhibitor and (**B**) celecoxib compatible conformation (yellow molecule) and urticin A (purple molecule)

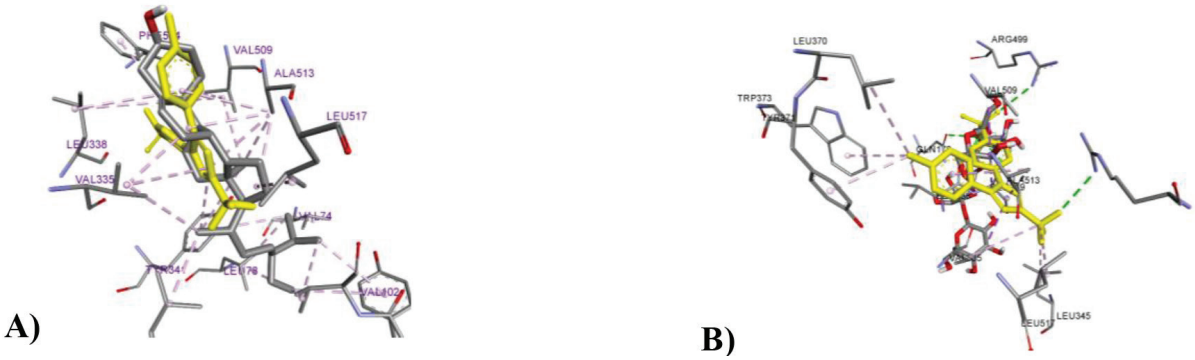

Figure 12. Visualization of the allocation into the COX-2 active site for celecoxib (yellow molecule) (**A**) sitosterine (grey molecule) and (**B**) inulin.

taining 26 hydrophobic interactions (Fig. 12A). Analysis of amino acid residues and compatible conformation with the native ligand has resulted that 5-ethyl-6-methylheptane fragment of sitosterine was fixed outside the active site, which explains its low affinity to COX-2. Hydrogen bonds exclusively, as well as only three residues of the active site were predicted after inulin placement into the COX-2 active site. This type of conformational location (Fig. 12B) and low affinity (-6.2 kcal / mol) indicate a low probability of enzyme inhibition.

Obtained docking results also point to the inability of anethole and petroselinic acids to have an inhibitory effect on COX-2 both in terms of scoring functions and due to the features of the interactions with amino acid residues and allocation into the active site.

Conclusion

Results have shown that some herbal substances have rather high level of affinity for the LOX-5 active site inhibitor. Therefore, possible manifestation of anti-inflammatory effect of the following biologically active substances: gallotannin, quercetin, inulin, and sitosterine was deter-

mined. Ellagic acid has a moderate probability of manifestation by the mentioned mechanism. Possibility of anti-inflammatory effect by inhibition mechanism of COX-2 enzyme was predicted for inulin, quercetin, gallotannin, ellagic acid and urticin A. Moderate activity was detected for gallic acid. Therefore, obtained results of *in silico* studies for the mentioned substances predict a significant anti-inflammatory potential by a multifactorial mechanism, which may be effective in the treatment of bowel inflammatory processes.

For the further pharmacological *in vitro* and *in vivo* studies, as well as a complex herbal remedy composition optimization, six medicinal herbs with the highest content of the mentioned compounds were selected: *Inula helenium*, *Cichorium intybus*, *Capsella bursa-pastoris*, *Foeniculum vulgare*, *Equisetum arvense*, *Veronica officinalis*. Due to hydrophilic nature of the most promising natural compounds of the selected herbs, it is recommended to use aqueous extracts of them.

References

Arya VS, Kanthlal SK, Geevarghese L (2020) The role of dietary polyphenols in inflammatory bowel disease: A possible clue on the molecular mechanisms involved in the prevention of immune and inflammatory reactions. Journal of Food Biochemistry 44(11): e13369. https://doi.org/10.1111/jfbc.13369

Asakura H, Kitahora T (2018) Chapter 23 - Antioxidants and polyphenols in inflammatory bowel disease: ulcerative colitis and crohn disease. Polyphenols: prevention and treatment of human disease (Second Edition) 279–292. https://doi.org/10.1016/B978-0-12-813008-7.00023-0

Badgujar SB, Patel VV, Bandivdekar AH (2014) Foeniculum vulgare Mill: a review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. BioMed research international 2014: e2842674. https://doi.org/10.1155/2014/842674

Burger D and Travis S (2011) Conventional medical management of inflammatory bowel disease. Gastroenterology 140(6): 1827–1837.e2. https://doi.org/10.1053/j.gastro.2011.02.045

Caesar LK, Cech NB (2019) Synergy and antagonism in natural product extracts: when 1 + 1 does not equal 2. Natural product reports 36(6): 869–888. https://doi.org/10.1039/c9np00011a

Cheifetz AS, Gianotti R, Luber R, Gibson PR (2017) Complementary and alternative medicines used by patients with inflammatory bowel diseases. Gastroenterology 152(2): 415–429.e15. https://doi.org/10.1053/j.gastro.2016.10.004

Ekor M (2014) The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Frontiers in pharmacology 4: 177. https://doi.org/10.3389/fphar.2013.00177

El-Dahiyat F, Rashrash M, Abuhamdah S, Abu Farha R, Babar ZU (2020) Herbal medicines: a cross-sectional study to evaluate the prevalence and predictors of use among. Jordanian adults. Journal of pharmaceutical policy and practice 13: 2. https://doi.org/10.1186/s40545-019-0200-3

Fakhoury M, Negrulj R, Mooranian A, Al-Salami H (2014) Inflammatory bowel disease: clinical aspects and treatments. Journal of Inflammation Research 7: 113–120. https://doi.org/10.2147/JIR.S65979

Forli S, Huey R, Pique ME, Sanner MF, Goodsell DS, Olson AJ (2016) Computational protein-ligand docking and virtual drug screening with the AutoDock suite. Nature protocols 11(5): 905–919. https://doi.org/10.1038/nprot.2016.051

Germoush MO, Othman SI, Al-Qaraawi MA, Al-Harbi HM, Hussein OE, Al-Basher G, Alotaibi MF, Elgebaly HA, Sandhu MA, Allam AA, Mahmoud AM (2018) Umbelliferone prevents oxidative stress, inflammation and hematological alterations, and modulates glutamate-nitric oxide-cGMP signaling in hyperammonemic rats. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 102: 392–402. https://doi.org/10.1016/j.biopha.2018.03.104

Gilbert NC, Bartlett SG, Waight MT, Neau DB, Boeglin WE, Brash AR, Newcomer ME (2011) The structure of human 5-lipoxygenase. Science (New York, N.Y.) 331(6014): 217–219. https://doi.org/10.1126/science.1197203

Gilbert NC, Gerstmeier J, Schexnaydre EE, Borner F, Garscha U, Neau DB, Werz O, Newcomer M (2020) Structural and mechanistic insights into 5-lipoxygenase inhibition by natural products. Nature chemical biology 16(7): 783–790. https://doi.org/10.1038/s41589-020-0544-7

Joo YE (2014) Natural product-derived drugs for the treatment of inflammatory bowel diseases. Intestinal research 12(2): 103–109. https://doi.org/10.5217/ir.2014.12.2.103

Keshavarz A, Minaiyan M, Ghannadi A, Mahzouni P (2013) Effects of *Carum carvi* L. (Caraway) extract and essential oil on TNBS-induced colitis in rats. Research in pharmaceutical sciences 8(1): 1–8.

Langhorst J, Wulfert H, Lauche R, Klose P, Cramer H, Dobos GJ, Korzenik J (2015) Systematic review of complementary and alternative medicine treatments in inflammatory bowel diseases. Journal of Crohn's and Colitis 9(1): 86–106. https://doi.org/10.1093/ecco-jcc/jju007

Lee J, Choi HS, Lee J, Park J, Kim SB, Shin MS, Lee S, Hwang GS, Koo BA, Kang KS (2019) Preparation of herbal formulation for inflammatory bowel disease based on in vitro screening and in vivo evaluation in a mouse model of experimental colitis. Molecules (Basel, Switzerland) 24(3): 464. https://doi.org/10.3390/molecules24030464.

Lee KM, Hwang MK, Lee DE, Lee KW, Lee HJ (2010) Protective effect of quercetin against arsenite-induced COX-2 expression by targeting PI3K in rat liver epithelial cells. Journal of agricultural and food chemistry 58(9): 5815–5820. https://doi.org/10.1021/jf903698s

Li C, Wu G, Zhao H, Dong N, Wu B, Chen Y, Lu Q (2021) Natural-derived polysaccharides from plants, mushrooms, and seaweeds for the treatment of inflammatory bowel disease. Frontiers in Pharmacology 12: 651813. https://doi.org/10.3389/fphar.2021.651813

Li Y, Yao J, Han C, Yang J, Chaudhry MT, Wang S, Liu H, Yin Y (2016) Quercetin, inflammation and immunity. Nutrients 8(3): 167–172. https://doi.org/10.3390/nu8030167

Manjeet KR, Ghosh B (1999) Quercetin inhibits LPS-induced nitric oxide and tumor necrosis factor-alpha production in murine macrophages. International journal of immunopharmacology 21(7): 435–443. https://doi.org/10.1016/s0192-0561(99)00024-7

Mateev E, Valkova I, Tzankova D, Georgieva M, Zlatkov A (2021) Optimizing molecular docking protocols of pyrrole containing mao-b in-

hibitors through correlation coefficients. Proceedings of CBU in Medicine and Pharmacy 2: 92–98. https://doi.org/10.12955/pmp.v2.179

Motley TJ (1994) The Ethnobotany of Sweet Flag, Acorus calamus (Araceae). Economic Botany 48(4): 397–412. https://doi.org/10.1007/BF02862235

Nematgorgani S, Agah S, Shidfar F, Janani L, Faghihi A, Hosseini S (2020) The effect of *Urtica dioica* leaf extract intake on serum TNF-α, stool calprotectin and erythrocyte sedimentation rate in patients with inflammatory bowel disease: A double-blind, placebo-controlled, randomized, clinical trial. 1: 75 – 87. https://doi.org/10.3233/MNM-190367

Nguyen GC, Croitoru K, Silverberg MS, Steinhart AH, Weizman AV (2016) Use of complementary and alternative medicine for inflammatory bowel disease is associated with worse adherence to conventional therapy. Inflammatory bowel diseases 22(6): 1412–1417. https://doi.org/10.1097/MIB.0000000000000773

Nie Y, Lin Q, Luo F (2017) Effects of non-starch polysaccharides on inflammatory bowel disease. International journal of molecular sciences 18(7): e1372. https://doi.org/10.3390/ijms18071372

Noreen Y, Serrano G, Perera P, Bohlin L (1998) Flavan-3-ols isolated from some medicinal plants inhibiting COX-1 and COX-2 catalysed prostaglandin biosynthesis. Planta medica 64(6): 520–524. https://doi.org/10.1055/s-2006-957506

Oguntibeju OO (2018) Medicinal plants with anti-inflammatory activities from selected countries and regions of Africa. Journal of inflammation research 11: 307–317. https://doi.org/10.2147/JIR.S167789

Protein Data Bank (2022) A structural view of biology. http://www.rcsb.org/pdb/home/home.do

Rauf A, Akram M, Semwal P, Mujawah A, Muhammad N, Riaz Z, Munir N, Piotrovsky D, Vdovina I, Bouyahya A, Adetunji CO, Shariati MA, Almarhoon ZM, Mabkhot YN, Khan H (2021) Antispasmodic potential of medicinal plants: a comprehensive review. Oxidative Medicine and Cellular Longevity 2021: e4889719. https://doi.org/10.1155/2021/4889719

Rizvi W, Fayazuddin M, Shariq S, Singh O, Moin S, Akhtar K, Kumar A (2014) Anti-inflammatory activity of roots of Cichorium intybus due to its inhibitory effect on various cytokines and antioxidant activity. Ancient science of life 34(1): 44–49. https://doi.org/10.4103/0257-7941.150780

Rogler G, Vavricka S, Schoepfer A, Lakatos PL (2013) Mucosal healing and deep remission: what does it mean?. World journal of gastroenterology 19 (43): 7552–7560. https://doi.org/10.3748/wjg.v19.i43.7552

Sabe VT, Ntombela T, Jhamba LA, Maguire G, Govender T, Naicker T, Kruger HG (2021) Current trends in computer aided drug design and a highlight of drugs discovered via computational techniques: A review. European Journal of Medicinal Chemistry. 224: e113705. https://doi.org/10.1016/j.ejmech.2021.113705

Santangelo C, Varì R, Scazzocchio B, Di Benedetto R, Filesi C, Masella R (2007) Polyphenols, intracellular signalling and inflammation. Annali dell'Istituto superiore di sanita 43(4): 394–405.

Sato S and Mukai Y (2020) Modulation of chronic inflammation by quercetin: the beneficial effects on obesity. Journal of inflammation research 13: 421–431. https://doi.org/10.2147/JIR.S228361

Serafini M, Peluso I, Raguzzini A (2010) Flavonoids as anti-inflammatory agents. The Proceedings of the Nutrition Society 69(3): 273–278. https://doi.org/10.1017/S002966511000162X

Sim MO, Lee HI, Ham JR, Seo KI, Kim MJ, Lee MK (2015) Anti-inflammatory and antioxidant effects of umbelliferone in chronic alcohol-fed rats. Nutrition research and practice 9(4): 364–369. https://doi.org/10.4162/nrp.2015.9.4.364

Singh J, Cumming E, Manoharan G, Kalasz H, Adeghate E (2011). Medicinal chemistry of the anti-diabetic effects of momordica charantia: active constituents and modes of actions. The open medicinal chemistry journal 5(2): 70–77. https://doi.org/10.2174/1874104501105010070

Stürner KH, Stellmann JP, Dörr J, Paul F, Friede T, Schammler S, Reinhardt S, Gellissen S, Weissflog G, Faizy TD, Werz O, Fleischer S, Vaas L, Herrmann F, Pless O, Martin R, Heesen C (2018) A standardised frankincense extract reduces disease activity in relapsing-remitting multiple sclerosis (the SABA phase IIa trial). Journal of neurology, neurosurgery, and psychiatry 89(4): 330–338. https://doi.org/10.1136/jnnp-2017-317101

Triantafyllidi A, Xanthos T, Papalois A, Triantafillidis JK (2015) Herbal and plant therapy in patients with inflammatory bowel disease. Annals of gastroenterology 28(2): 210–220.

Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry 31: 455–461. doi: 10.1002/jcc.21334.

Umesh HR, Ramesh KV, Devaraju KS (2020) Molecular docking studies of phytochemicals against trehalose–6–phosphate phosphatases of pathogenic microbes. Beni-Suef University Journal of Basic and Applied Sciences 9(1): 5–19. https://doi.org/10.1186/s43088-019-0028-6

Wang JL, Limburg D, Graneto MJ, Springer J, Hamper JR, Liao S, Pawlitz JL, Kurumbail RG, Maziasz T, Talley JJ, Kiefer JR, Carter J (2010) The novel benzopyran class of selective cyclooxygenase-2 inhibitors. Part 2: the second clinical candidate having a shorter and favorable human half-life. Bioorganic & medicinal chemistry letters 20(23): 7159–7163. https://doi.org/10.1016/j.bmcl.2010.07.054

Wang Y, Zhu H, Wang X, Yu Y, Xie J (2021) Natural food polysaccharides ameliorate inflammatory bowel disease and its mechanisms. Foods 10(6): 1288–1344. https://doi.org/10.3390/foods10061288

Welton AF, Tobias LD, Fiedler-Nagy C, Anderson W, Hope W, Meyers K, Coffey JW (1986) Effect of flavonoids on arachidonic acid metabolism. Progress in clinical and biological research 213: 231–242.

Yang W, Zhao P, Li X, Guo L, Gao W (2022) The potential roles of natural plant polysaccharides in inflammatory bowel disease: A review. Carbohydrate polymers 277: e118821. https://doi.org/10.1016/j.carbpol.2021.118821

Yoon JH, Baek SJ (2005) Molecular targets of dietary polyphenols with anti-inflammatory properties. Yonsei medical journal 46(5): 585–596. https://doi.org/10.3349/ymj.2005.46.5.585

Yoon SL, Grundmann O, Smith KF, Mason S R (2019) Dietary supplement and complementary and alternative medicine use are highly prevalent in patients with gastrointestinal disorders: results from an online survey. Journal of dietary supplements 16(6): 635–648. https://doi.org/10.1080/19390211.2018.1472712

Zhang S, Chen X, Devshilt I, Yun Q, Huang C, An L, He X (2018) Fennel main constituent, trans-anethole treatment against LPS-induced acute lung injury by regulation of Th17/Treg function. Molecular Medicine Reports 18: 1369–1376. https://doi.org/10.3892/mmr.2018.9149