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

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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 – lipoygenase-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* (Nematogorgani 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, apiole, 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, ursitcin) (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, quercetin, 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 alpha (TNF α) (Manjeet and Ghosh 1999), interleukins (IL-1β, 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 8th Gen 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.** BIOVIA Draw 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 = 22, y = 24, z = 24; 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 Å for AKBA and 1.987 Å 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-
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.
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 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.

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), carboxyl 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.
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

<table>
<thead>
<tr>
<th>Table 1. Docking results of the studied substances into the LOX-5 active site inhibitor.</th>
</tr>
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<tbody>
<tr>
<td><strong>Ligand</strong></td>
</tr>
<tr>
<td>AKBA reference ligand</td>
</tr>
<tr>
<td>Anethole cis-/ trans- isomers</td>
</tr>
<tr>
<td>Apiol</td>
</tr>
<tr>
<td>Carvone R/S</td>
</tr>
<tr>
<td>Petroselinic acid</td>
</tr>
<tr>
<td>Coumarin</td>
</tr>
<tr>
<td>Umbelliferone</td>
</tr>
<tr>
<td>Gallic acid</td>
</tr>
<tr>
<td>Ellagic acid</td>
</tr>
<tr>
<td>Quercetin</td>
</tr>
<tr>
<td>Gallotannin</td>
</tr>
<tr>
<td>Urticin A</td>
</tr>
<tr>
<td>Urticin B</td>
</tr>
<tr>
<td>Sitosterine</td>
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<tr>
<td>Inulin</td>
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</tbody>
</table>

* Amino acid residues not interacting with AKBA in the experiment are indicated
# The amount of links is in parentheses
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).
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-hydroxphenyl fragment and leucine carbonyl (Leu338). Four terminal hydroxyls with alanine (Ala513) and valine (Val335) residues, as well as bidentate bonds with isopropyl fragments of valine (Val509) and leucine (Leu338). The obtained results predict a high probability of ellagic acid to inhibit COX-2.

Apiole 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 apiole 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 studied natural compounds into the COX-2 inhibitor active site. 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 occurred in the area of celecoxib benzensulphonamide 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

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

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Binding energy kcal/mol</th>
<th>Hydrophobic interactions</th>
<th>Hydrogen bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anethole, cys-/trans-isomers</td>
<td>-6.3/-6.2</td>
<td>Val509(2), His75*, Leu338</td>
<td>–</td>
</tr>
<tr>
<td>Apiole</td>
<td>-7.5</td>
<td>Val335(3), Ala513, Val509(2), Trp341, Tyr371, Trp373, Phe504*, Leu338, Ala513, Leu517</td>
<td>Ser516</td>
</tr>
<tr>
<td>Carvone R/S</td>
<td>-6.7/-6.8</td>
<td>Val509(2), Ala513, Leu338(3), Val335, Tyr371, Trp373, Phe504*</td>
<td>–</td>
</tr>
<tr>
<td>Petrosemlenic acid</td>
<td>-7.0</td>
<td>Ala513, Val509, Tyr341</td>
<td>Arg499, Gln178, Leu338</td>
</tr>
<tr>
<td>Coumarin</td>
<td>-7.2</td>
<td>Val509(2), Leu338(2), Ser339(2), Leu338, Ala502*</td>
<td>–</td>
</tr>
<tr>
<td>Umbelliferone</td>
<td>-7.2</td>
<td>Val509(2), Leu338 (2), Ser339(2), Leu338, Ala502*</td>
<td>–</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>-6.3</td>
<td>Ser339, Val509 (2)</td>
<td>–</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>-8.6</td>
<td>Val335(3), Val509(4), Ala513(3), Leu338 (3)</td>
<td>Tyr341, Ser516, Leu338</td>
</tr>
<tr>
<td>Quercetin</td>
<td>-9.8</td>
<td>Leu338(2), Phe504, Val509(2), Val335</td>
<td>Tyr341(2), Phe504, Ser516</td>
</tr>
<tr>
<td>Gallotannin</td>
<td>-8.6</td>
<td>Val509(3), Ala513, Phe191*, Leu520*, Leu338</td>
<td>Arg106, Tyr341(2), Leu338, Ala513, Phe19*</td>
</tr>
<tr>
<td>Urticin A</td>
<td>-8.5</td>
<td>Val335, Leu338, Ala513(2), Val509, Val335, Leu517</td>
<td>Tyr341(3), His75, Ser339, Ser516*, Gln178*, Ser516*</td>
</tr>
<tr>
<td>Urticin B</td>
<td>-5.0</td>
<td>Ala513(2), Tyr341, Val335 Leu338, Val509</td>
<td>Arg106, Gln178, Tyr341</td>
</tr>
<tr>
<td>Sitostereine</td>
<td>-6.6</td>
<td>Val335(3), Val509(3), Ala513(6), Leu338(2), Leu517, Leu345, Val74*, Leu78(2)<em>, Val102(2)</em>, Tyr101(2)*, Tyr341(4), Phe504</td>
<td>–</td>
</tr>
</tbody>
</table>

* Amino acid residues not interacting with AKBA in the experiment are indicated
* The amount of links is in parentheses
Figure 7. 3D visualization of (A) allocation and interaction with amino acid residues of the active site of COX-2 native celecoxib 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-
taining 26 hydrophobic interactions (Fig. 12A). Analysis of amino acid residues and compatible conformation with the native ligand has resulted that 5-ethyl-6-methyl-heptane 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-
mained. 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, galloantannin, 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.

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