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Prediction of drug candidate from *Rosmarinus officinalis* L to inhibit IL-6R, IL-1R1, and TNF-α: In silico study

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Abstract

Tooth pain is a manifestation of pulpitis caused by dental caries. Toothache can be treated using eugenol. However, eugenol has several disadvantages, including its toxic effects on fibroblast pulp tissue in a dose-dependent manner. This research assesses the binding affinity of drug candidates, predicting physicochemical properties, pharmacokinetics, drug-likeness, LD50, and toxicity. Molecular docking results show that Rosmarinic acid, Carnosic acid, Carnosol, Ursolic acid can bind strongly to IL-6R and IL-1R1. Meanwhile, only the compounds Carnosic acid and Ursolic acid bind strongly to TNFR-1. Pharmacokinetic predictions of drug candidates show that only the Carnosol compound is able to penetrate the blood brain barrier and the human gastrointestinal tract. Drug-likeness prediction showed that all compounds met Lipinski's rule of five. However, the Ursolic acid compound has an MLOGP > 4.15. Toxicity prediction shows that Ursolic acid and Rosmarinic acid have a better LD50 than Eugenol.

Keywords: Binding affinity; IL-6R; IL-1R1; TNFR-1

1. Introduction

Dental caries is one of the most common dental problems in the world. In 2017, an estimated 3.5 million people in the world suffered from oral cavity diseases, with the most common disease being dental caries [1]. The World Health Organization (WHO) stated that dental caries may affect 60-90% of children globally, especially in developing countries [2]. In 2018, Indonesian Ministry of Health released a report regarding caries epidemiology. This report showed that at least 88.8% of Indonesians suffered from dental caries. This number is estimated to increase throughout the years.

Dental caries is a multifactorial disease which is mainly caused by the accumulation of substrate on the surface of the tooth, and triggers infection by cariogenic bacteria, resulting in progressive demineralization of the hard dental tissue. If the involved teeth do not receive immediate treatment, progressive development of carious lesions may occur, causing inflammation of the dental pulp, called pulpitis. The process of inflammation can be marked by several symptoms, such as increasing sensitivity towards external stimuli, including thermal or chemical stimuli, which may provoke onset of pain on the teeth [3,4].

Dental pain may occur as a result of both injury of the neurons inside the dental pulp, or as the consequences of pulpal inflammation. Toxins released by gram-negative cariogenic bacteria (e.g. Lipopolysaccharide / LPS), are able to bind

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onto Toll-like receptor-4 (TLR-4), and triggers NF-kB pathway, resulting in release of pro-nociceptive and pro-inflammatory mediators [5]. On the other hand, injury of the neurons or sensory nerve fibers, triggers the release of pain-related neuropeptide, Substance P (SP), and at the same time also induce the over-expression of its receptor, Neurokinin 1-receptor (NK1-R) [6]. The interactions between SP and NK1-R induces further release of pro-inflammatory and pro-nociceptive biomarkers, aggravating the pain and inflammation. NF-kB pathway and SP-NK1R bond, triggers the release of Cyclooxygenase-2 (COX-2) and phospholipase A2 (PLA2), respectively. This condition increases metabolism of free arachidonic acid, resulting in increased prostaglandin E2 (PGE2) synthesis, which causes increased pain and production of pro-inflammatory cytokines, such as IL-17, IL-6, TNF-α, IL-1β [7].

To overcome dental pain problems, dental practitioners often use medications placed intra-cavity, such as eugenol. Eugenol is an ingredient that is widely used in the field of dentistry as a temporary filling material, root canal filling mixture, and pulp sedative agent [8]. Eugenol has antibacterial, anti-inflammatory and anti-pain effects [9].

Although it has been widely used in dentistry, eugenol has several disadvantages, including its toxic effects on fibroblast pulp tissue in a dose-dependent manner [8,9]. In addition, the use of eugenol in the oral cavity carries the risk of eugenol intake and intoxication due to swallowing, inhalation, or absorption (Escobar-García et al., 2016). Apart from that, dental materials containing eugenol are also known to trigger local irritation of the skin and mucosa, ulcers, allergic contact dermatitis, and contact urticaria [10]. Some of the limitations of these materials indicate that there is still a need for alternative materials in the field of dentistry to reduce dental pain and inflammation, one of which is utilizing herbal medicine such as rosemary or *Rosmarinus officinalis* L(RO).

RO is an aromatic plant from the *Lamiaceae* family, and originally grew as a typical plant from the Mediterranean region. RO leaves, both fresh and dry, are widely used by people throughout the world, as a cooking spice and herbal drink mixture [11]. RO contains active phytochemical compounds such as alkaloids, flavonoids, and terpenoids, as well as carnosic acid (CA), rosmarinic acid (RA), carnosol (CO), and ursolic acid (UA) [12]. These active compounds are known to have anti-inflammatory, antinociceptive and neuroprotective effects [12]. These effects can be observed from several in vivo studies in experimental animals which induced sole edema, colitis, and inflammation in the hippocampus [11]. Even though it is thought to be able to reduce inflammation and pain, RO is still rarely used in the field of dentistry, for this reason, further research is still needed regarding the potential of RO in reducing pain due to pulpitis, especially as an alternative to eugenol.

2. Material and methods

In silico research using an Aspire E11 2GB 500GB HDD laptop, PyRx, PyMol, Biovia Discovery Studio 21 software, and several web servers (methods). The ligands Rosmarinic acid, carnosic acid, carnosol, ursolic acid, and eugenol as well as the proteins IL-6R, IL-1R1, and TNFR-1 are the material for in silico research.

The ligands used in this research were downloaded via the web server https://pubchem.ncbi.nlm.nih.gov/ in pdb format. Canonical SMILE and compound ID (CID) data were recorded in this study. We screened proteins related to inflammation and pain via the web server http://swisstargetprediction.ch/. Proteins were mapped using the web server https://string-db.org/ and three proteins IL-6R, IL-1R1, and TNFR-1 were selected. FASTA protein was obtained from the web server https://www.ncbi.nlm.nih.gov/. Protein modeling uses the web server https://swissmodel.expasy.org/ by inputting FASTA protein. Protein models are downloaded in pdb format. Proteins and ligands were docked using PyRx software. Binding affinity and RMSD data are taken from this process. Docking results are saved in pdb format. Combination of docked proteins and ligands using PyMol software. The molecules that have been combined will then be visualized. Ligand and protein interactions are assessed by the type of bond formed. The hydrogen bond distance between the ligand and the protein was analyzed from the visualization process using Biovia Discovery Studio 21 software.

3. Results and discussion

3.1. Molecular Docking Drug Candidate with IL-6R, IL-1R1, and TNFR-1

The results of molecular docking of drug candidate compounds against the IL6 receptor are shown in table 1. Binding affinity data shows that compounds Rosmarinic acid (-9.3 kcal/mol), Carnosic acid (-8.2 kcal/mol), Carnosol (-9.3 kcal/mol), Ursolic acid (-8.4 kcal/mol) are more negative than the control compound Eugenol (-6.1 kcal/mol). With the root mean square deviation (RMSD) value 0.000 for all drug candidate.

Table 1 Data binding affinity of drug candidates against IL-6R

Drug Candidates	Binding Affinity	RMSD	Hydrogen bond distance	Hydrogen bond
Rosmarinic acid [a]	-9.3 kcal/mol	0.000	2.84557 Å	I:ALA11:O
			1.99751 Å	R:SER93:O
			2.28655 Å	R:SER93:O
			3.10405 Å	N:UNK1:O
Carnosic acid [a]	-8.2 kcal/mol	0.000	3.25259 Å	N:UNK1:O
			2.80366 Å	N:UNK1:O
			2.00634 Å	R:VAL212:O
			2.63059 Å	A:ASN10:O
Carnosol [a]	-9.3 kcal/mol	0.000	2.96905 Å	N:UNK1:O
			2.53192 Å	I:ASN10:O
			2.27632 Å	R:VAL212:O
Ursolic acid [a]	-8.4 kcal/mol	0.000	3.11742 Å	N:UNK1:O
			2.36046 Å	I:GLU8:OE1
Eugenol [b]	-6.1 kcal/mol	0.000	3.08601 Å	N:UNK1:O
			2.19808 Å	I:ASN10:O
			2.58168 Å	I:TYR13:O

[a] Drug candidate compounds that have a more negative binding affinity than eugenol; [b] Comparator compound (standard drug)

Table 2 Data binding affinity of drug candidates against IL-1R1

Drug Candidates	Binding Affinity	RMSD	Hydrogen bond distance	Hydrogen bond
Rosmarinic acid [a]	-6.1 kcal/mol [b]	0.000	2.51216 Å	I:ASN10:O
			2.52583 Å	I:TYR13:O
Carnosic acid [a]	-9.3 kcal/mol [a]	0.000	2.96795 Å	N:UNK1:O
			1.92046 Å	R:VAL212:O
Carnosol [a]	-9.2 kcal/mol [a]	0.000	3.05838 Å	N:UNK1:O
			3.23248 Å	N:UNK1:O
			1.80283 Å	R:ILE13:O
			2.73816 Å	R:ILE92:O
			2.56981 Å	R:SER93:O
			1.99640 Å	R:GLU11:OE1
			2.88255 Å	R:GLU217:OE1
Ursolic acid [a]	-8.4 kcal/mol [a]	0.000	3.14883 Å	N:UNK1:O
			2.79549 Å	I:GLU8:OE1
Eugenol [b]	-8.2 kcal/mol [c]	0.000	1.86566 Å	R:VAL212:O
			2.26427 Å	I:ASN10:O
			3.25615 Å	IN:UNK1:O

[a] Drug candidate compounds that have a more negative binding affinity than eugenol; [b] Drug candidate compounds that have a less negative binding affinity than eugenol; [c] Comparator compound (standard drug)

The results of molecular docking of drug candidate compounds against the IL-1R1 receptor are shown in table 2. Binding affinity data shows that compounds Carnosic acid (-9.3 kcal/mol), Carnosol (-9.2 kcal/mol), Ursolic acid (-8.4 kcal/mol) are more negative than the control compound Eugenol (-8.2 kcal/mol) but Rosmarinic acid (-6.1 kcal/mol) are less negative than the control. With the root mean square deviation (RMSD) value 0.000 for all drug candidates.

The results of molecular docking of drug candidate compounds against the TNFR-1 receptor are shown in table 3. Binding affinity data shows that compounds Carnosic acid (-7.2 kcal/mol) and Ursolic acid (-7.8 kcal/mol) more negative than Eugenol (-6.8 kcal/mol), but Rosmarinic acid (-5.2 kcal/mol) and Carnosol (-6.6 kcal/mol) less negative than Eugenol (-6.8 kcal/mol).

Table 3 Binding affinity of drug candidates against TNFR-1

Drug Candidates	Binding Affinity	RMSD	Hydrogen bond distance	Hydrogen bond
Rosmarinic acid [a]	-5.2 kcal/mol [b]	0.000	3.07596 Å	A: LYS372
			2.95446 Å	A: LYS372
			2.58724 Å	A: PRO368
			2.06487 Å	A: LEU396
Carnosic acid [a]	-7.2 kcal/mol [a]	0.000	2.09771 Å	A: GLU389
Carnosol [a]	-6.6 kcal/mol [b]	0.000	2.00457 Å	A: LEU369
			3.04742 Å	A: ALA399
Ursolic acid [a]	-7.8 kcal/mol [a]	0.000	2.32501 Å	A: GLU438
Eugenol [b]	-6.8 kcal/mol [c]	0.000	2.41442 Å	A: PRO368
			2.57635 Å	A: LEU369
			2.28131 Å	A: PRO368

[a] Drug candidate compounds that have a more negative binding affinity than eugenol; [b] Drug candidate compounds that have a less negative binding affinity than eugenol; [c] Comparator compound (standard drug)

The binding affinity values of Rosmarinic acid, Carnosic acid, Carnosol and Ursolic acid towards IL-6R and IL-1R1 were more negative than the control compound Eugenol (Table 1 and Table 2). Meanwhile, only Carnosic acid and Ursolic acid compounds showed binding affinity values for TNFR-1 that were more negative than Eugenol (Table 3). Binding affinity shows the value of Gibbs energy (ΔG) which is a free enthalpy that can be used by a thermodynamic system [13]. If ΔG shows an increasingly negative value, the strength of the interaction between the ligand and the target protein is stronger [14]. From this research data, it shows that the compounds Rosmarinic acid, Carnosic acid, Carnosol and Ursolic acid can bind strongly to IL-6R and IL-1R1. In TNFR-1, only the ligands Carnosic acid and Ursolic acid can bind strongly to TNFR-1. The RMSD value for each docking result shows a value of less than 2.00 Å (Table 1, Table 2, and Table 3). RMSD values are used to assess the effectiveness of ligands in inhibiting target proteins [15].

The docking visualization results show that there is interaction between the ligand and the protein showing hydrogen bonds. The hydrogen bond distance formed is less than 2.70 Å in the compounds Rosmarinic acid, Carnosic acid, Carnosol, and Ursolic acid (Table 1, Table 2, and Table 3). If the hydrogen bond distance between the ligand and the protein is less than 2.70 Å, a strong bond will be formed [16]. Based on the results of this research, the compounds Rosmarinic acid, Carnosic acid, Carnosol, and Ursolic acid will bind strongly to IL-6R, IL-1R1, and TNFR-1.

3.2. Prediction of Physicochemical and Pharmacokinetic Properties of Drug Candidates

Physicochemical properties of the compounds Rosmarinic acid (RA), Carnosic acid (CA), Carnosol (CO), Ursolic acid (UA), and Eugenol (EU) can be seen in Figure 1. The physicochemical properties analyzed in this research include flexibility (FLEX), lipophilicity (LIP), size, polarity (POL), insolubility (INSOLU), in-saturation (INSATU). The red zone is a zone that has physicochemical properties in accordance with oral bioavailability. Radar bioavailability of active compounds is seen from six parameters, namely flexibility (FLEX), lipophilicity (LIP), size, polarity (POL), insolubility (INSOLU), in-saturation (INSATU). Each parameter has a different indicator value. The indicator values for each active compound can be seen in Table 4.

The molecular weight values (SIZE) shown in Table 4 show that CO, CA, RA and UA compounds meet the Lipinksi rule of five criteria. The same thing was also shown for the EU compound. The Lipinksi rule of five provides molecular weight requirements for drug candidates. A drug candidate must have a molecular weight smaller than 500 g/mol [17].

The polarity (POLAR) shown in Table 4 interprets the Topology Polar Surface Area (TPSA) values. From this data, it was found that the compounds RA, CA, CO, and UA had TPSA values in the range of 20 – 130 Å. The same thing is also shown by the EU compound's TPSA score. The TPSA value indicates the ability of a drug candidate to enter cells. The TPSA value must meet the range 20 – 130 Å. If a drug candidate compound has a TPSA value in the range of 20 – 130 Å then the drug candidate compound has the ability to enter cells [17].

Insolubility (INSOLU) was analyzed from the log S score. Table 4 shows that UA is included in the poor class, CA and CO are classified as moderately, and RA is classified as soluble, similar to EU. The insolubility or solubility of a compound depends on the solvent used, temperature and environmental pressure. The extent of solubility is expressed as the saturation concentration where adding more solute does not increase its concentration in solution [18]. A drug candidate is considered highly soluble if it can dissolve in 250 mL at a pH range of 1-7.5. Two topological approaches are included in Swiss ADME to predict solubility in water, the first is the application of the ESOL model by looking at the logS value and classifying it into five classes, namely insoluble < -10, poorly < -6, moderately < -4, soluble < - 2, and very soluble < 0. Another predictor is Swiss ADME developed by SILICOS-IT with the same class classification [17]. All predicted values are the decimal logarithm of molar solubility in water (log S). Swiss ADME also provides solubility in mol/l and mg/ml as well as qualitative solubility classes [18].

In-saturation parameters (INSATU) were analyzed using the Csp3 fraction score. Table 4 shows that the Csp3 fraction score for UA compounds is higher compared to other compounds. Meanwhile, CA and CO compounds have the same Csp3 fraction score. The EU comparison compound has a Csp3 fraction score close to the RA compound. The Csp3 fraction score is the ratio of sp3 hybridized carbons to the total number of carbons of a molecule [19].

The data in Table 4 also shows the number of rotatable bonds for each compound. This data shows that CA, CO, and UA compounds as drug candidates are more flexible than EU. The number of rotatable bonds is a measure of molecular flexibility and is important in determining the bioavailability of oral drug candidates [19].

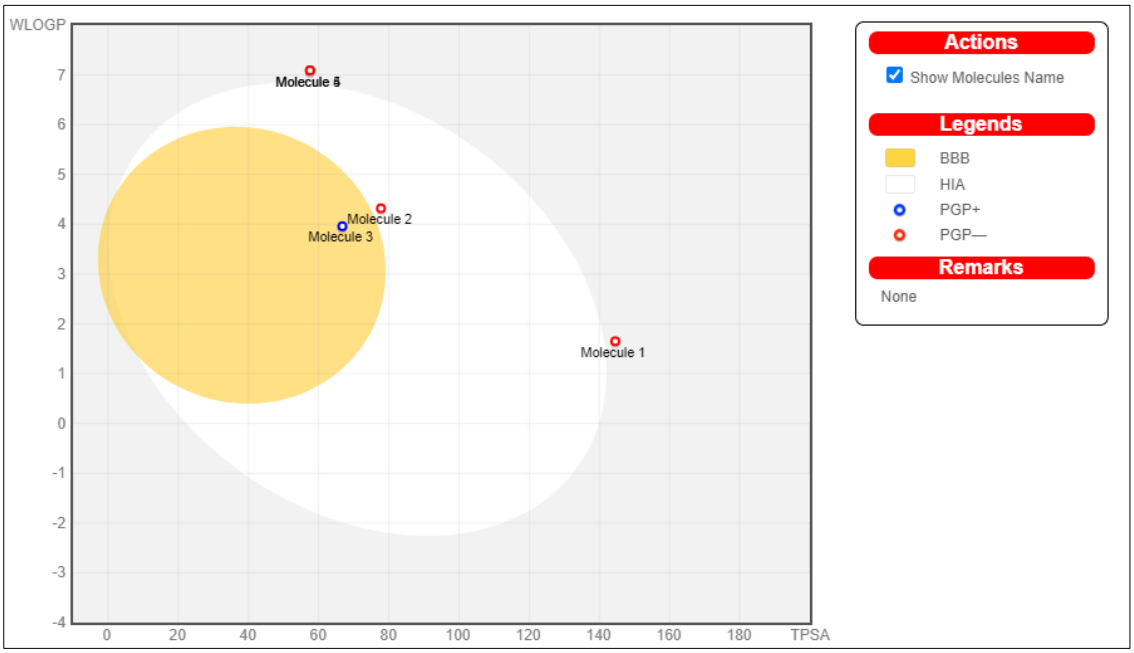

Figure 1 BOILED EGG model, the white area is the area that has human GI absorption and the yellow area is the area that has BBB permeation properties

The HGI absorption parameters were interpreted using the BOILED-Egg model by comparing the respective ALOGP and TPSA values. The BOILED-Egg model produces a fast, spontaneous, and efficient prediction method that is useful for drug discovery and development [20]. The white area is the space for molecules that have the greatest absorption in the gastrointestinal tract, while the yellow area is the space that is most likely to penetrate the BBB [17].

Table 4 Physicochemical properties of the compounds *Rosmarinus officinalis* L

No.	Active compounds	Bioavailability Indicator Score					
		LIPO	SIZE (g/mol)	POLAR (Å)	INSOLU	INSATU	FLEX
1.	Rosmarinic acid	+2.36	360.31	144.52	-3.44	0.11	7
2.	Carnosic acid	+4.89	332.43	77.76	-5.03	0.65	2
3.	Carnosol	+4.38	330.42	66.76	-4.77	0.65	1
4.	Ursolic acid	+7.34	456.70	57.53	-7.23	0.90	1
5.	Eugenol	+2.27	164.20	29.46	-2.46	0.20	3

P-gp substrate is widely distributed in the intestinal epithelium pumping xenobiotics back into the intestinal lumen and from brain capillary endothelial cells back into the capillaries [21,22]. Swiss ADME adopts vector machine algorithm (SVM) for known substrate/non-substrate or inhibitor/non-inhibitor datasets for binary classification. The resulting molecules will give a "Yes" or "No" answer if the analyzed molecule is expected to be a substrate for P-gp and CYP. The cytochrome p450 (CYP) isoenzyme bio-transformations more than 50-90% of therapeutic molecules from its five main isoforms (CYP1A2, CYP3A4, CYP2C9, CYP2C19, CYP2D6) [22].

3.3. Prediction of Drug-Likeness of Drug Candidates

The results of drug-likeness analysis of the active compounds Rosmarinic acid (RA), Carnosic acid (CA), Carnosol (CO), Ursolic acid (UA), and Eugenol (EU) are shown in Table 5. The compounds RA, CA, CO and UA fulfill the Lipinski rule of five. The EU compound also shows that it meets the Lipinski rule of five. However, the UA compound has an MLOGP value > 4.15 which violates the Lipinski rule of five.

Table 5 Drug-likeness of *Rosmarinus officinalis* L. compounds

No.	Active compounds	Lipinski	Value of Bioavailability
1.	Rosmarinic acid	Yes	0.56
2.	Carnosic acid	Yes	0.56
3.	Carnosol	Yes	0.55
4.	Ursolic acid	Yes[a]	0.85
5.	Eugenol	Yes	0.55

[a]There are physicochemical properties that violate Lipinski's rule of five

Testing of drug-likeness of candidate drug compounds using Swiss ADME shown in Table 5 identified all candidate drug compounds as meeting the Lipinski rule of five criteria. The basis for determining the Lipinski rule of five criteria is by filtering a chemical compound database to exclude molecules with characteristics that do not comply with an acceptable pharmacokinetic profile with five filter elements. The Lipinski filter is a five-character rule that characterizes small molecules based on a physicochemical property profile which includes Molecular Weight (MW) less than 500, MLOGP ≤ 4.15; number of N or O atoms ≤ 10, NH or OH ≤ 5. Lipinski strictly considers all nitrogen and oxygen as H-bond acceptors and all nitrogen and oxygen with at least one hydrogen as an H-bond donor. In addition, aliphatic fluorine is an acceptor and nitrogen alanine neither a donor nor an acceptor [23].

3.4. Prediction of Toxicity of Drug Candidates

The results of the toxicity analysis of the active compounds Rosmarinic acid (RA), Carnosic acid (CA), Carnosol (CO), Ursolic acid (UA), and Eugenol (EU) are shown in Table 6. The predicted Lethal Dose 50 (LD50) value shows that RA, CO and UA compounds have LD50 values of 5,000 mg/kg, 1,500 mg/kg and 2,000 mg/kg, respectively. Meanwhile, CA has an LD50 value of 287 mg/kg. The EU compound used as a comparison has an LD50 value of 1,930 mg/kg. Prediction of toxicity classes for drug candidate compounds shows that CO and UA are in toxicity class 4, the same as EU. Meanwhile, the RA compound is in toxicity class 5. The CA compound is in toxicity class 3. Prediction of the toxicity of

candidate compounds to organs shows that RA, CA, and CO are inactive in the prediction of hepatotoxicity and carcinogenicity. Meanwhile, UA compounds are active in hepatotoxicity and carcinogenicity. All drug candidates showed active activity in immunogenicity prediction. The prediction of mutagenicity and cytotoxicity shows that all drug candidates are inactive against potential mutagenicity and cytotoxicity.

The prediction results for acute toxicity and target toxicity shown in Table 6 show CA has an LD50 of 287 mg/kg and is classified into class III toxicity. This shows that CA will be toxic if swallowed. Other compounds, namely CO and UA, show LD50 values of 1500 mg/kg and 2000 mg/kg, respectively. This value is similar to the comparison, namely EU, which is 1930 mg/kg. If the LD50 value is more than equal to 300 mg/kg but less than equal to 2000 mg/kg, it is included in class IV. In class IV, a compound is predicted to be dangerous if swallowed [24]. The RA compound has an LD50 value of 5000 mg/kg which is included in toxicity class V. This means that RA is categorized as potentially dangerous if swallowed [24]. Toxicity to organs such as hepatotoxicity, carcinogenicity, immunogenicity, mutagenicity, and cytotoxicity. The prediction results show that toxicity to the liver (hepatotoxicity) is only active in the UA drug candidate. Meanwhile, RA, CA and CO are inactive. This also happens to EU compounds. Drug-induced hepatotoxicity is a significant cause of acute liver failure and one of the main reasons for drug withdrawal from the market [25]. The carcinogenicity parameter shows that it is active in all drug candidates. Different things happen to the EU, which shows inactivity in carcinogenicity properties. A chemical compound that can induce or increase tumor growth is called a carcinogen [24]. Data for carcinogenicity prediction were collected from the Carcinogenic Potency Database (CPD) [26]. RA, CA, CO, and UA compounds showed active immunogenicity effects. This result is different from the EU comparison compound which showed potential inactivity. The effect of xenobiotics on the immune system is called immunotoxicity. Immunotoxicity prediction is based on immune cell cytotoxicity data [27]. Mutagenicity and cytotoxicity parameters showed inactivity for all drug candidates including the EU comparator compound. Chemical compounds that cause abnormal genetic mutations such as changes in cell DNA are called mutagens [24]. These changes can cause damage to cells and result in certain diseases such as cancer. Prediction of cytotoxicity is related to mutagenicity. These two predictions are important for the development of drug candidate compounds that can cause cell damage and result in triggering malignancy [28].

Table 6 Prediction LD50 and toxicity drug candidates

	Rosmarinic acid	**Carnosic acid**	**Carnosol**	**Ursolic acid**	**Eugenol**
LD50 (mg/kg)	5.000	287	1.500	2.000	1.930
Toxicity Classification	5	3	4	4	4
Hepatotoxicity	inactive	inactive	inactive	active	inactive
Carcinogenicity	inactive	inactive	inactive	active	inactive
Immunogenicity	active	active	active	active	inactive
Mutagenicity	inactive	inactive	inactive	inactive	inactive
Cytotoxicity	inactive	inactive	inactive	inactive	inactive

4. Conclusion

Molecular docking results show that Rosmarinic acid, Carnosic acid, Carnosol, Ursolic acid can bind strongly to IL-6R and IL-1R1. Meanwhile, only the compounds Carnosic acid and Ursolic acid bind strongly to TNFR-1. Pharmacokinetic predictions of drug candidates show that only the Carnosol compound is able to penetrate the blood brain barrier and the human gastrointestinal tract. Drug-likeness prediction showed that all compounds met Lipinski's rule of five. However, the Ursolic acid compound has an MLOGP > 4.15. Toxicity prediction shows that Ursolic acid and Rosmarinic acid have a better LD50 than Eugenol.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors report no conflicts of interest to declare.

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