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In silico Toxicity and Pharmaceutical Properties to Get Candidates for Antitumor Drug

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Drug metabolism is a crucial aspect of medical practice and pharmacology, involving the transformation of drugs by various bodily systems to create compounds that are more easily eliminated from the body. Sorafenib was reported as a useful adjuvant treatment in patients with hepatocellular carcinoma who underwent surgical resection. However, poor pharmacokinetic properties such as limited water solubility, rapid elimination and metabolism lead to low bioavailability, restricting its further clinical application. Rosmarinic acid, soluble in ethanol and found in Rosemary leaves, has demonstrated therapeutic benefits in conditions such as cancer, diabetes, inflammatory disorders, neurodegenerative disorders, and liver disease.
Method: *In silico* is a term for experiments or tests carried out using computer simulation methods. *In silico* testing has emerged as a valuable approach for initiating the exploration of novel drug

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compounds or enhancing the efficacy of existing ones. This method involves predicting, generating hypotheses, and uncovering potential breakthroughs in medicine and therapy through virtual simulations.

Results: Caco2 value of Sorafenib ligand as a comparison, namely 0.762. The highest Caco2 value is owned by Betulinic Acid and the value The lowest Caco2 is owned by Rosmarinic Acid. The highest sample HIA value was owned by Ursolic Acid and the lowest was owned by Rosmarinic Acid. Meanwhile, Sorafenib's HIA value is 85,494. ligands such as Carnosol and rosmanol have a high distribution volume, while the Sama carnosic league, ursolic acid and betulinic acid have a low distribution volume. The Rosmarinic acid ligand has a good distribution volume of 0.393, while the distribution volume value of Sorafenib is -0.009. The highest BBB sample value was owned by Carnosol and the lowest was owned by Rosmarinic acid. Meanwhile, the comparison ligand has a value of -1.473 and is considered less distributed in the brain. all ligands such as Carnosic Acid, Carnosol, Rosmanol, Ursolic Acid, Betulinic Acid, Rosmarinic acid do not have mutagenicity and Cytotoxic effects, but have an effect on immunity. The comparison ligand Sorafenib turned out to have effects on hepatotoxicity, immunity and cytotoxicity.

Conclusion: In pharmacokinetic research, the six phenolic acid compounds in Rosemary exhibited superior properties compared to the reference ligand Sorafenib.

Keywords: Rosemary; rosmarinic acid; insilico; pharmaceutical properties; antitumor.

1. INTRODUCTION

Drug metabolism, particularly in the liver, is a dynamic and complex process that significantly influences the efficacy and safety of pharmacological interventions. An understanding of these metabolic pathways is essential for the development, prescribing, and administration of drugs in clinical practice.

The overall process of biotransformation ensures that drugs are converted into more water-soluble forms, facilitating their removal from the body through processes like urine or bile excretion. The liver is a key organ in these transformations, housing enzymes responsible for many biotransformation reactions.

Understanding the different phases of biotransformation is crucial in predicting the fate of drugs in the body, optimizing dosing regimens, and managing potential drug interactions or adverse effects. It also contributes to the field of pharmacokinetics, which explores how the body handles drugs over time.

Inter-individual variability in drug response is partially attributed to differences in specific enzymes crucial for drug metabolism. For instance, Cytochrome P450 enzymes (CYPs) represent the most important phase I drug-metabolizing enzymes that contribute to approximately 75% of total drug metabolism. Human CYPs are membrane-associated proteins ubiquitously expressed in most tissues. They are generally highly expressed in the liver, but their distribution might differ from an enzyme to another. Notably, CYP families 1, 2 and 3 are the primary ones responsible for metabolizing drugs, xenobiotics and certain endogenous molecules [1].

Phase I reactions indeed involve changes to the chemical structure of the drug, and various enzymatic processes can contribute to these modifications. Here are some key points regarding Phase I modifications. Types of Phase I Reactions: Oxidation: Introduction of oxygen or removal of hydrogen from the drug molecule. Cytochrome P450 enzymes are crucial in oxidizing many drugs. Reduction: Addition of electrons or removal of oxygen, leading to a reduction in the drug molecule. Hydrolysis: Cleavage of chemical bonds through the addition of water. Cyclization/Deserialization: Formation of cyclic structures or breaking of cyclic structures. Removal of Hydrogen or addition of Oxygen: Alterations to the drug molecule involving hydrogen removal or oxygen addition. Conversion of Prodrugs: Phase I modifications can activate prodrugs, which are inactive forms of drugs administered to the body. The conversion of prodrugs to their active forms often occurs through Phase I reactions. Pharmacological Activity of Metabolites: Metabolites generated through Phase I modifications can retain pharmacological activity, and in some cases, they may contribute to the overall therapeutic effects of the drug. The example you provided with diazepam illustrates how metabolites produced through Phase I modification (desmethyldiazepam and

oxazepam) exhibit similar physiological and psychological effects as the parent drug. Individual Variation: The extent and nature of Phase I metabolism can vary among individuals, leading to differences in drug response and potential for side effects. Genetic factors, as well as factors such as age, gender, and concomitant drug use, can influence the activity of enzymes involved in Phase I reactions [2].

Phase II reactions involve the conjugation of drug molecules with endogenous substances, resulting in the formation of water-soluble and pharmacologically inactive compounds that are easily excreted. The primary goal of Phase II reactions is to increase the water solubility of the drug or its Phase I metabolites. Conjugation renders the compound more polar and less lipophilic, facilitating its elimination from the body. Understanding Phase II modifications is essential for comprehending the overall fate of drugs in the body. The combination of Phase I and Phase II reactions ensures that drugs are transformed into metabolites suitable for elimination, contributing to the body's ability to maintain homeostasis and prevent the accumulation of potentially toxic substances [3].

It also emphasizes Phase III metabolism, where transporter-mediated elimination plays a crucial role in removing drug conjugates and metabolites from cells. The classification of Phase III pathways includes ATP-binding cassette (ABC) transporters, such as P-glycoprotein, and solute carrier (SLC) transporters, which facilitate the transport of substances across membranes. The text underscores the significance of these processes in organs like the liver, intestines, kidneys, and lungs for effective drug elimination. Understanding the interactions between enzymatic catalysis and transporter-mediated elimination is essential for comprehending drug metabolism and its implications for individual responses to medications [4].

Sorafenib was reported as a useful adjuvant treatment in patients with hepatocellular carcinoma who underwent surgical resection. However, its therapeutic value remains controversial. meta-analysis examined the available data regarding the efficacy and safety of sorafenib in patients with hepatocellular carcinoma after radical surgery [5].

Sorafenib, a molecular targeted multi-kinase inhibitor, has received considerable interests in recent years due to its significant profiles of efficacy in cancer therapy. However, the poor pharmacokinetic properties significantly limited the further clinical application in cancer therapy [6].

Rosmarinic acid, present in the leaves of the Rosemary plant (*Rosmarinus officinalis* L.), is a naturally occurring compound that exhibits solubility in ethanol. Numerous studies have validated the therapeutic advantages of Rosmarinic acid (RA) across diverse conditions, encompassing cancer, diabetes, inflammatory disorders, neurodegenerative disorders, and liver disease. This bioactive phenolic compound is commonly found in plants belonging to the Lamiaceae and Boraginaceae families. The biosynthesis of Rosmarinic acid (RA) involves an enzyme-catalyzed reaction utilizing the amino acids tyrosine and phenylalanine.

The biosynthesis of Rosmarinic acid (RA), initially identified in Coleus blumei, is a intricate and non-linear enzymatic process. This process commences with the aromatic amino acids phenylalanine and tyrosine [7]. Phenylalanine undergoes deamination, catalyzed by the enzyme phenylalanine ammonia-lyase (PAL), leading to the formation of cinnamic acid within the lignin branch of the flavonoid biosynthetic pathway. Additionally, the benzene ring of cinnamic acid undergoes hydroxylation facilitated by cytochrome-P450 monooxygenase cinnamic-4 hydroxylase in the flavonoid pathway, resulting in the production of 4-coumaric acid [4].

2. METHODOLOGY

The research was carried out in silico to look for active compounds from the Rosemary plant for antitumor treatment. Insilico is a term for experiments or tests carried out using computer simulation methods. In silico testing has emerged as a valuable approach for initiating the exploration of novel drug compounds or enhancing the efficacy of existing ones. This method involves predicting, generating hypotheses, and uncovering potential breakthroughs in medicine and therapy through virtual simulations. The benefits of the in silico approach encompass error reduction, diminished reliance on animal testing, and a decrease in solvent usage [8].

2.1 Sample

The test material used was the two-dimensional structure of the Rosemary plant, namely phenolic

acid compounds, such as carnosol acid (CA), carnosol, romano, ursolic acid, Betulinic acid, and rosmarinic acid (RA), and netrin.

2.2 Tools and Materials

1. Hardware

The hardware used is ASUS VivoBook Flip 14 with Intel Celeron N4020 processor, up to 2.8 GHz, and Windows 2010 Ultimate 64-bit SP-1 operating system.

2. Software

The software used in this study is:

a. SWISS ADME to predict drug similarity. It can be accessed free of charge from the website (http://www.swissadme.ch/).
b. Prediction of the pharmacokinetic properties of Rosmarinic acid with the help of pkCSM (http://biosig.unimelb.edu.au/pkcsm/)
c. Lipinski's rule of five to evaluate drug similarity or determine whether a chemical compound with a certain pharmacological activity has chemical and physical properties that will make it an orally active drug in humans. It can be accessed free of charge from the website (http://www.scfbioiitd.res.in/software/drugdesign/lipinski.jsp).
d. Protox II to predict compound poisoning and LD50. It can be accessed for free from the website (http://tox.charite.de/protox_II/).

2.3 Procedures

1. Rosemary plant compound test ligand search downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/).
   Ligands are downloaded in SMILES format and stored according to their respective names.
2. Predict the pharmacokinetic properties of phenolic acid compounds in Rosemary with the help of pkCSM (http://biosig.unimelb.edu.au/pkcsm/) and compare the results with comparison ligands, by comparing absorption, distribution, metabolism, excretion.
3. The prediction of the drug's similarity with the ligand is tested first for its molecular properties concerning the Lipinski Rule of Five. Rosmarinic acid compound, downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/).
   Ligands are downloaded in smile format and saved according to their respective names. SWISS ADME and Lipinski rule of five to predict drug similarity. It can be accessed free of charge from the website (http://www.swissadme.ch/) and (http://www.scfbioiitd.res.in/software/drugdesign/lipinski.jsp). The results were compared with the Lipinski Rule of Five: molecular mass, lipophilicity, hydrogen bond donors, hydrogen bond acceptors, and molar endurance.
4. The toxicity of the candidate medicinal compound of the Rosemary plant, namely the Rosmarinic acid compound, was predicted for its toxicity using the Protox Web Server (http://tox.charite.de/protox_II/), and the observed results were predicted for the LD50 (lethal dose 50%) toxicity class, mean similarity, prediction accuracy, and toxicity model reports hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity, Aryl hydrocarbon Receptor (AhR), Androgen Receptor (AR), Androgen Receptor Ligand Binding Domain (AR-LBD), Aromatase, Estrogen Receptor Alpha (ER), Estrogen Receptor Ligand Binding Domain (ER-LBD), Peroxisome Proliferator-Activated Receptor Gamma (PPARGamma), Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE), Heat shock factor response element (HSE), Mitochondrial Membrane Potential (MMP), Phosphoprotein (Tumor Suppressor) p53 and ATPase family AAA domain-containing protein 5 (ATAD5).

3. RESULTS

3.1 PKCSM

Based on the results of predicting the HIA value of Caco2 using pkCSM, results were obtained as in the table above, all sample ligands and comparison ligands had HIA values above 30%.

Based on the results of predicting CNS values using pkCSM, results such as the table above were obtained, the ligand samples Carnosic Acid, Carnosol, Ursolic Acid and Betylonic Acid were deemed unable to penetrate the Central Nervous System. Samples that can penetrate the central nervous system are Rosmanol and Rosmarinic acid. Meanwhile, the comparison ligand which

has a value of -2.025 is also able to penetrate the Central Nervous System.

The table above shows that T. Pyroformis toxicity results greater than -0.5 are considered non-toxic. Almost all ligands above -0.3 are considered to be of low acute toxicity except Rosmanol. The reference ligand compound of -0.515 is considered low acute toxicity.

3.2 Drug Similarity Prediction

We showcase our Bioavailability Radar for quickly assessing drug suitability, as shown in Figure 1. This radar considers six physicochemical properties: lipophilicity, size, polarity, solubility, flexibility, and saturation. Each axis represents a physicochemical range, delineated by descriptors , and depicted as a pink area. For a molecule to be deemed drug-like, its radar plot must entirely fall within this pink area.

3.3 Toxicity of Drug Compound Candidates

The toxic dose is often given as the LD50 value in mg/kg body weight. LD50 is the median lethal dose which means the dose at which 50% of the test subjects die after exposure to a compound. Toxicity class is determined according to the globally harmonized chemical labeling classification system (GHS). The LD50 value is given in [mg/kg] [9]:

Class I: fatal if swallowed (LD50 ≤ 5)
Class II: fatal if swallowed (5 < LD50 ≤ 50)
Class III: toxic if swallowed (50 < LD50 ≤ 300)
Class IV: harmful if swallowed (300 < LD50 ≤ 2000)
Class V: may be harmful if swallowed (2000 < LD50 ≤ 5000)
Class VI: non-toxic (LD50 > 5000)

Based on the results of toxicity predictions using the Protox Web Server, results were obtained as in the table above, with a toxicity class prediction value of 5 for Betulinic Acid and Rosmarinic acid ligands. The predicted value of the toxicity class is 4 for the Carnosol, Rosmanol and Ursolic Acid ligands, while the predicted class 3 is only for the Carnosic Acid league. The reference ligand Sorafenib had a class 4 predictive value.

Table 1. Absorption results

No	Compound	Absorption		
		HIA (%) (30%)	Caco-2 cel (nm/sec) (>0.90)	Qualified/ No
	Carnosic Acid	99,03	0,803	No
	Carnosol	91,206	0,572	No
	Rosmanol	93,407	1,015	Qualifed
	Ursolat Acid	100	1,171	Qualifed
	Betulinic Acid	99,763	1,175	Qualifed
	Rosmarinic Acid	32,516	-0,937	No

Table 2. Distribution results

No	Compound	Distribution		
		VDss (Human) (>0,45	BBB Permeability (>0,3)	CNS Permeability (>-2)
	Carnosic Acid	-1,027	-0,545	-1,998
	Carnosol	0,819	-0,096	-1,816
	Rosmanol	0,653	-0,581	-2,101
	Ursolat Acid	-1,088	-0,141	-1,187
	Betulinic Acid	-1,18	-0,322	-1,343
	Rosmarinic Acid	0,393	-1,378	-3,347

Table 3. Metabolic results

No	Compound	Metabolism	
		CYP2D6	CYP3A4
	Carnosic Acid	No	No
	Carnosol	No	Yes
	Rosmanol	No	No
	Ursolat Acid	No	Yes
	Betulinic Acid	No	Yes
	Rosmarinic Acid	No	No

Table 4. Excretion results

No	Compound	Excretion	
		Total Clearance (Log ml/min/kg)	Renal OTC2 Substrate
	Carnosic Acid	0,379	No
	Carnosol	0,28	No
	Rosmanol	0,289	No
	Ursolat Acid	0,083	No
	Betulinic Acid	0,116	No
	Rosmarinic Acid	0,25	No

Table 5. Toxicity results

NO	Compound	Toxicity				
		AMES toxicity	Oral Rat Toxicity (LOAEL)	Skin Sensitisation	T. Pyroformis toxicity	Minnow Toxicity
	Carnosic Acid	No	1,972	No	0,285	-0,627
	Carnosol	No	1,909	No	0,405	-0,636
	Rosmanol	No	2,547	No	0,329	0,285
	Ursolat Acid	No	2,054	No	0,285	-0,787
	Betulinic Acid	No	2,206	No	0,285	-1,174
	Rosmarinic Acid	No	2,907	No	0,302	2,698

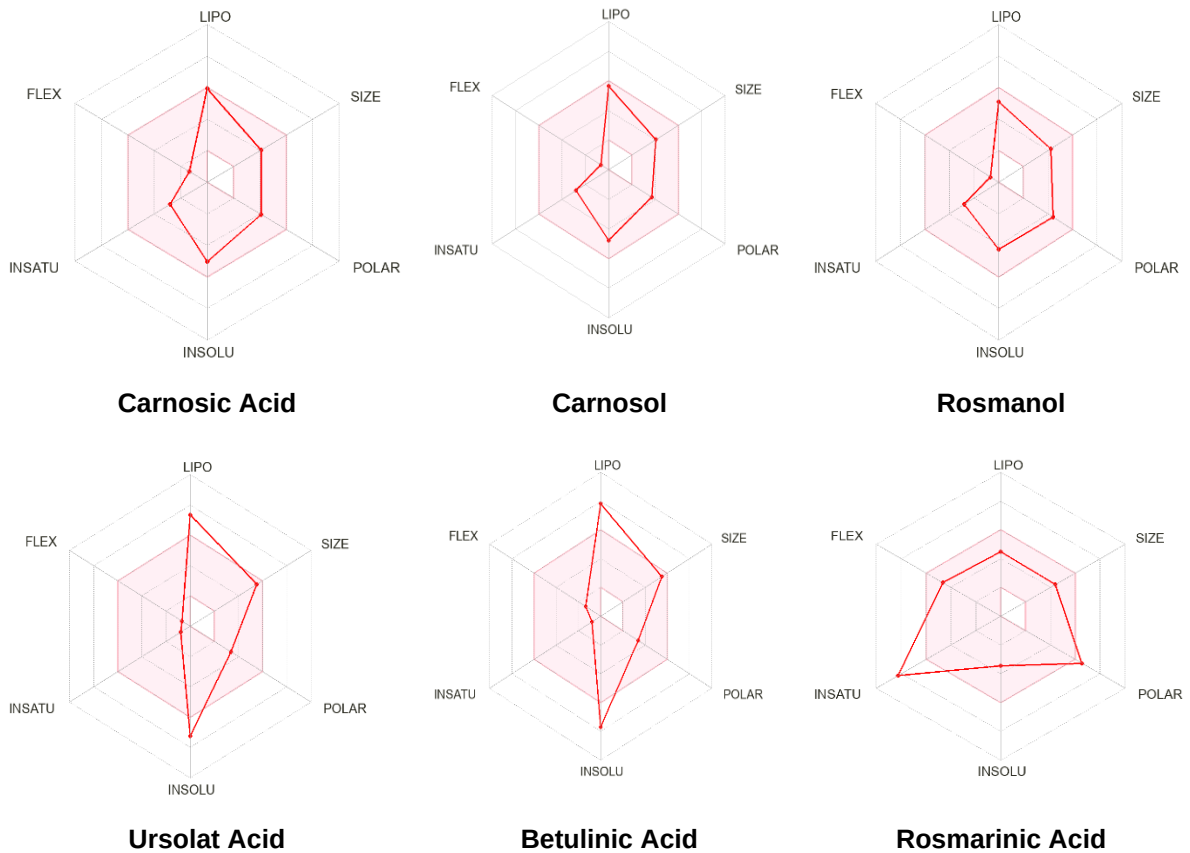
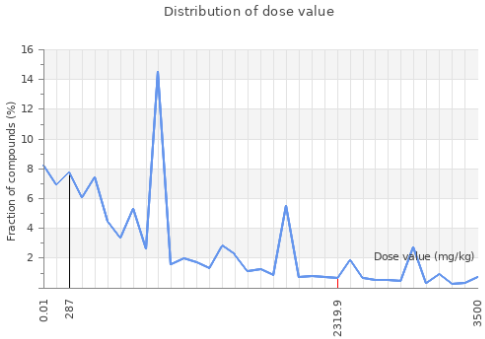


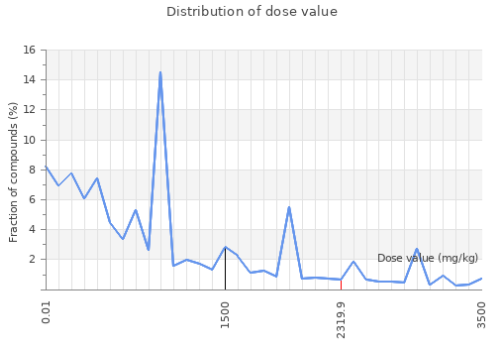
Fig. 1. The toxicity radar chart is intended to quickly illustrate the confidence of a positive toxicity result compared to the class average

Table 6. Test the molecular properties of ligands

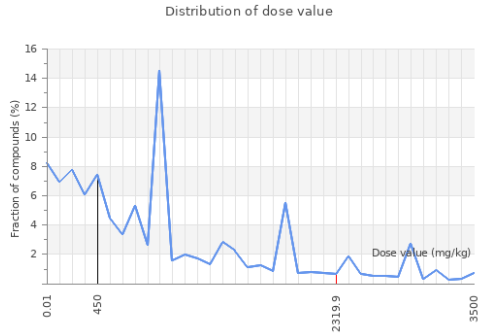
No	Compound	Molecular weight (g/mol)	H-Donor	H-Akseptor	LogP	Qualified/ No
1.	Carnosic Acid	332,43	3	4	4,89	Qualified
2.	Carnosol	330,42	2	4	4,38	Qualified
3.	Rosmanol	346,42	3	5	3,41	Qualified
4.	Ursolat Acid	456,70	2	3	7,34	No
5.	Betulinic Acid	456,70	2	3	8,21	No
6.	Rosmarinic Acid	360,31	5	8	2,36	Qualified



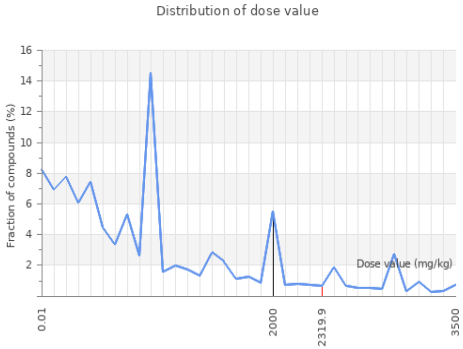
Carnosic Acid



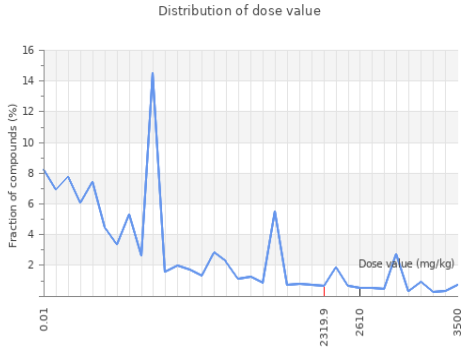
Carnosol



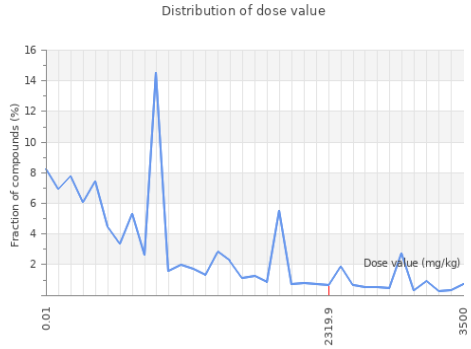
Rosmanol



Ursolat Acid



Betulinic Acid



Rosmarinic Acid

Fig. 2. Comparison of input compounds with dataset compounds

Table 7. Predictions of toxicity class and LD50

No	Compound	LD_{50} mg/kg	Toxicity class prediction	Qualified/ No
	Carnosic Acid	287	3	No
	Carnosol	1500	4	No
	Rosmanol	450	4	No
	Ursolat Acid	2000	4	No
	Betulinic Acid	2610	5	Qualified
	Rosmarinic Acid	5000	5	Qualified

Table 8. Results of average similarity and prediction accuracy

No	Compound	Average similarity (%)	Prediction Accuracy (%)
	Carnosic Acid	72,69%	69,26%
	Carnosol	57,99%	67,38%
	Rosmanol	59,45%	67,38%
	Ursolat Acid	100%	100%
	Betulinic Acid	77,12%	69,26%
	Rosmarinic Acid	63,44%	68,07%

Table 9. Target organ toxicity results

No	Compound	Hepatotoxicity	Carcinogenicity	Immunotoxocoty	Mutagenicity	Cytotoxic
	Carnosic Acid	No	No	Yes	No	No
	Carnosol	No	No	Yes	No	No
	Rosmanol	No	No	Yes	No	No
	Ursolic Acid	Yes	Yes	Yes	No	No
	Betulinic Acid	No	Yes	Yes	No	No
	Rosmarinic Acid	No	No	Yes	No	No

Based on the results of Average Similarity and Prediction Accuracy using Protox Web Server, results were obtained as in the table above, the comparison ligand Sorafenib had lower results with an Average Similarity value of 53.45% and Prediction Accuracy of 67.38%. Meanwhile, the Ursolic Acid ligand showed average similarity and prediction accuracy results with a value of 100% and other ligand compounds with values above the comparison ligand.

4. DISCUSSION

Sorafenib has been recommended as the front-line therapeutic drug by the FDA based on its survival advantages identified in the clinical therapies of liver cancer and kidney cancer. However, the poor pharmacokinetic properties significantly limited the further clinical application in cancer therapy. In order to overcome these limitations, there were various multifunctional nanosized sorafenib delivery systems have been designed and synthesized with the help of nanotechnology [6].

Sorafenib, a molecular targeted multi-kinase inhibitor, has received considerable interests in recent years due to its significant profiles of efficacy in cancer therapy. However, poor pharmacokinetic properties such as limited water solubility, rapid elimination and metabolism lead to low bioavailability, restricting its further clinical application [6].

Recent years have observed the emergence of novel therapeutic opportunities for advanced hepatocellular carcinoma (HCC), such as combination therapies including immune checkpoint inhibitors. The CR rate observed in HCC patients receiving immune-based combinations appears more than twelve times higher compared with sorafenib monotherapy, supporting the long-term benefit of these combinatorial strategies, with even the possibility to cure advanced disease [10].

The utilization of in silico research in drug discovery has augmented the identification of lead compounds, achieving results more swiftly than conventional medicinal chemistry. However, a common setback involves the failure of many promising compounds due to unfavorable ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties. In an effort to

mitigate this risk, in silico screening approaches are employed. Our proposed novel method for predicting pharmacokinetic traits, named pkCSM, introduces a graph-based signature approach. This technique utilizes encoded distance patterns between atoms to represent small molecules and facilitate the training of predictive models [11].

The pkCSM fingerprint has proven effective in constructing predictive regression and classification models across five distinct major pharmacokinetic trait classes. Our findings indicate that pkCSM exhibits comparable or superior performance in assessing a diverse set of pharmacokinetic properties compared to other freely accessible methods. In this context, we introduce a web server, offering an openly accessible and integrated platform for swiftly screening multiple pharmacokinetic properties [11].

Caco2 cell monolayers serve as a commonly utilized in vitro representation of human intestinal mucosa for anticipating the absorption of orally administered drugs. The model incorporates 674 drug-like molecules, associating them with Caco2 permeability values and projecting the logarithm of the apparent permeability coefficient. A compound is deemed to exhibit high Caco2 permeability when its papp exceeds 8x10-6 cm/s. According to the pkCSM predictive model, a substance is predicted to possess high Caco2 permeability if its projected value surpasses 0.90 [11].

Based on the results of predicting Caco2 permeability values using pkCSM, the results shown in the table above show that ligands such as Rosmanol, Ursolic Acid, Betulinic Acid, have higher Caco2 cell values than the Sorafenib ligand as a comparison, namely 0.762. The highest Caco2 value is owned by Betulinic Acid and the value The lowest Caco2 is owned by Rosmarinic Acid. For a given compound, it predicts the percentage that will be absorbed through the human intestine. Molecules with an absorbance of less than 30% are considered poorly absorbed [12]. Based on the results of predicting the HIA value of Caco2 using pkCSM, results were obtained as in the table above, all sample ligands and comparison ligands had HIA values above 30%. In this way, all samples and comparisons are well absorbed in digestion. The highest sample HIA value was owned by Ursolic Acid and the lowest was owned by Rosmarinic Acid. Meanwhile, Sorafenib's HIA value is 85,494.

The volume of distribution (VDss) represents the hypothetical volume necessary for an even distribution of the total drug dose to achieve the same concentration as in blood plasma. A higher VD implies a greater distribution of the drug in tissues rather than plasma, and factors like kidney failure and dehydration can influence this. This predictive model was constructed by calculating the steady-state volume of distribution (VDS) in humans for 670 drugs. The anticipated logarithm of VDss for a compound is expressed as log L/kg. A VDss is considered low if it falls below 0.71 L/Kg (Log VDss <-0.15) and high if it exceeds 2.81 L/Kg (Log VDS > 0.45) [12]. Based on the results of predicting the distribution volume value using pkCSm, the results shown in the table above are obtained, ligands such as Carnosol and rosmanol have a high distribution volume, while the Sama carnosic league, ursolic acid and betulinic acid have a low distribution volume. The Rosmarinic acid ligand has a good distribution volume of 0.393, while the distribution volume value of Sorafenib is -0.009.

Based on the results of predicting BBB Permeability values using pkCSM, the results shown in the table above show that all ligand samples have values less than 0.3 and are considered unable to cross the blood-brain barrier. The highest BBB sample value was owned by Carnosol and the lowest was owned by Rosmarinic acid. Meanwhile, the comparison ligand has a value of -1.473 and is considered less distributed in the brain.

Compounds with a logPS value greater than -2 are chosen based on their potential to penetrate the Central Nervous System (CNS). Conversely, those with a logPS value lower than -3 are deemed incapable of penetrating the CNS [12].

A compound is identified as a cytochrome P450 inhibitor if the concentration needed to achieve a 50% inhibitory effect is below 10uM [7]. Ligand samples that are not metabolized in the liver are Carnosic Acid, Rosmanol and Rosmarinic acid. Meanwhile, Carnosol ligands, ursolic acid and betulinic acid are metabolized by the CYP3A4 enzyme. The comparator ligand Sorafenib is also metabolized in CYP3A4.

The measurement of drug clearance is determined by the proportionality constant CLtot, which primarily involves hepatic clearance. This is interconnected with bioavailability, emphasizing the significance of establishing the dose rate required to attain steady-state

concentrations [11]. All ligands had greater clearance compared to the comparison ligand of -0.213. The Organic Cation Transporter (Renal OTC 2 Substrate) Is an uptake transporter in the kidneys, playing a vital role in the distribution and renal elimination of drugs and endogenous substances. Not all ligands are considered substrates of OTC2, and the reference ligands also do not fall under this category.

The Ames test is a widely used method for assessing the potency of compounds using bacteria. A positive test indicates that the compound is a mutagen and therefore may be a carcinogenic compound [11]. All ligand samples showed negative results on the Ames test, which means that all ligands are not mutagen compounds.

For specific compounds, the prediction of pIGC50, representing the negative logarithm of the concentration needed to inhibit growth by 50% in log ug/L, is conducted. Values exceeding -0.5 log ug/L are regarded as indicative of toxicity [12]. The table above shows that T. Pyroformis toxicity results greater than -0.5 are considered non-toxic.
For a given compound, the prediction will be made for the log LC50. LC50 values falling below 0.5 mM (Log LC50 <-0.3) are categorized as indicating high acute toxicity (Pires, Blundell and Ascher, 2015). The table above shows that almost all ligands above -0.3 are considered low acute toxicity except Rosmanol. The reference ligand compound of -0.515 is considered low acute toxicity.

Skin sensitization is a potential adverse reaction for products applied dermally. Assessing whether a compound in contact with the skin can induce allergic contact dermatitis is a crucial safety consideration [11]. The table above shows that all ligands have no potential to cause allergic contact dermatitis.

Lipinski's Rule of Five has requirements for a molecule, namely: the maximum number of hydrogen bond donors is 5, the number of hydrogen bond acceptors is less than 10, the molecular weight is less than 500g/mol and the logP value is less than [11]. Based on the prediction results of the ligand molecular properties test using pkCSM, the results as shown in the table above were obtained, all sample ligands and comparison ligands met the requirements in accordance with the Lipinski Rule of Five.

Based on the LD50 prediction results using Protox Web Server, results were obtained as in the table above, where the highest result was shown by Rosmarinic acid with an LD50 result of 5000 mg/kg and the lowest was shown by Carnosic acid with an LD50 result of 287 mg/kg. From these results it can be concluded that the one that is categorized as most likely to be non-toxic if ingested is Rosmarinic acid.

Based on the results of Average Similarity and Prediction Accuracy using Protox Web Server, results were obtained as in the table above, the comparison ligand Sorafenib had lower results with an Average Similarity value of 53.45% and Prediction Accuracy 67.38%. Meanwhile, the Ursolic Acid ligand showed average similarity and prediction accuracy results with a value of 100% and other ligand compounds with values above the comparison ligand. Thus Carnosic Acid, Carnosol, Rosmanol, Ursolic Acid, Betulinic Acid, Rosmarinic acid and Sorafenib do not have the same structure.

Based on the prediction results of Target Organ Toxicity using Protox Web Server. The results obtained are as shown in the table above, all ligands such as Carnosic Acid, Carnosol, Rosmanol, Ursolic Acid, Betulinic Acid, Rosmarinic acid do not have mutagenicity and Cytotoxic effects, but have an effect on immunity. Target Organ Toxicity Prediction also shows that only the Ursolic Acid ligand has hepatotoxicity effects. The positive carcinogenicity effect was shown by the ligands Ursolic acid and Betulinic acid. The comparison ligand Sorafenib turned out to have effects on hepatotoxicity, immunity and cytotoxicity.

5. CONCLUSION

From the results of the study, it was concluded that:

1. Based on the compound toxicity test using the protox web server, it is concluded that the six phenolic acid compounds in Rosemary proved to be safer than the comparison ligand Sorafenib.
2. From the pharmacokinetic research results, the six phenolic acid compounds in Rosemary proved to be better than the comparison ligand Sorafenib.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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