ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 10| Issue: 2| February 2024|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2024: 8.402 || ISI Value: 1.188

INSILICO TOXICITY AND PHARMACOKINETICS TESTS -AN ANTITUMOR DRUG

Shweta[1], Hedgapure Mahesh[2], Kvln Murthy[3]
[1]*Asst. Professor, S V E T[s] College of Pharmacy. Humnabad, Karnataka- 585330*
[2]*Professor and HOD of Pharmaceutics, Sri Shahu Maharaj College of Pharmacy, Naubad, Bidar- 585402*
[3]*Lecturer, SVR Degree College, Macherla, Andhra Pradesh- 522426*

Article DOI: https://doi.org/10.36713/epra15893
DOI No: 10.36713/epra15893

ABSTRACT
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. Cytochrome P450 (CYP) enzymes, predominantly found in hepatocyte endoplasmic reticulum, play a central role in metabolizing numerous small molecule drugs through diverse oxidative and reductive biotransformation's. The study of absorption, distribution, metabolism, and excretion (ADME) is integral to drug design, exploring the fate of drug molecules post-administration. Rosmarinus 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. In pharmacokinetic research, the six phenolic acid compounds in Rosemary exhibited superior properties compared to the reference ligand Sorafenib.

KEYWORDS: *Rosemary, Rosmarinus Acid, Insilico, Pharmacokinetics, 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.

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 (1).

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,

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 10| Issue: 2| February 2024|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2024: 8.402 || ISI Value: 1.188

contributing to the body's ability to maintain homeostasis and prevent the accumulation of potentially toxic substances (2).

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 (3).

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 (4). 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 (3).

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 (5).

RESULTS

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%.

Table 1 Absorption results

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

Table 2 Distribution Results

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

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.

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 10| Issue: 2| February 2024|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2024: 8.402 || ISI Value: 1.188

Table 3 Metabolic Results

S.No	Compound	Metabolism	
		CYP2D6	CYP3A4
1.	Carnosic Acid	No	No
2.	Carnosol	No	Yes
3.	Rosmanol	No	No
4.	Ursolat Acid	No	Yes
5.	Betulinic Acid	No	Yes
6.	Rosmarinic Acid	No	No

Table 4 Excretion Results

S.No	Compound	Excretion	
		Total Clearance (Log ml/min/kg)	Renal OTC2 Substrate
1.	Carnosic Acid	0,379	No
2.	Carnosol	0,28	No
3.	Rosmanol	0,289	No
4.	Ursolat Acid	0,083	No
5.	Betulinic Acid	0,116	No
6.	Rosmarinic Acid	0,25	No

Table 5. Toxicity results

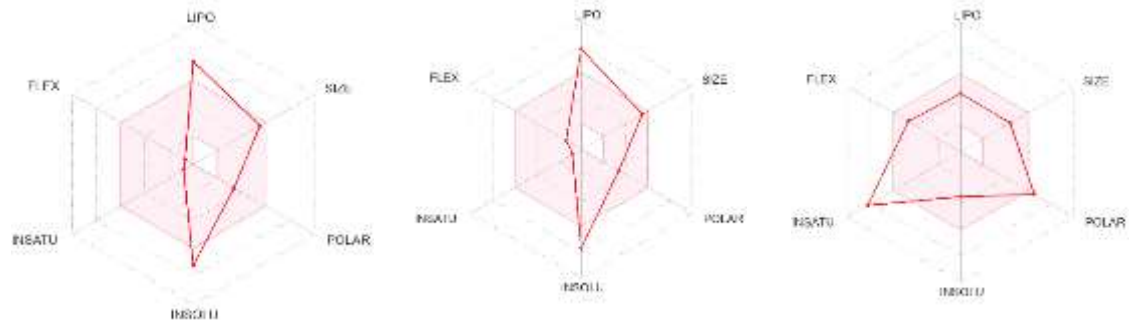
S.No	Compound	Toxicity				
		AMES toxicity	Oral Rat Toxicity (LOAEL)	Skin Sensitisation	T.Pyroformis toxicity	Minnow Toxicity
1.	Carnosic Acid	No	1,972	No	0,285	-0,627
2.	Carnosol	No	1,909	No	0,405	-0,636
3.	Rosmanol	No	2,547	No	0,329	0,285
4.	Ursolat Acid	No	2,054	No	0,285	-0,787
5.	Betulinic Acid	No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

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.

2. Drug Similarity Prediction

Carnosic Acid **Carnosol** **Rosmanol**

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 10| Issue: 2| February 2024|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2024: 8.402 || ISI Value: 1.188



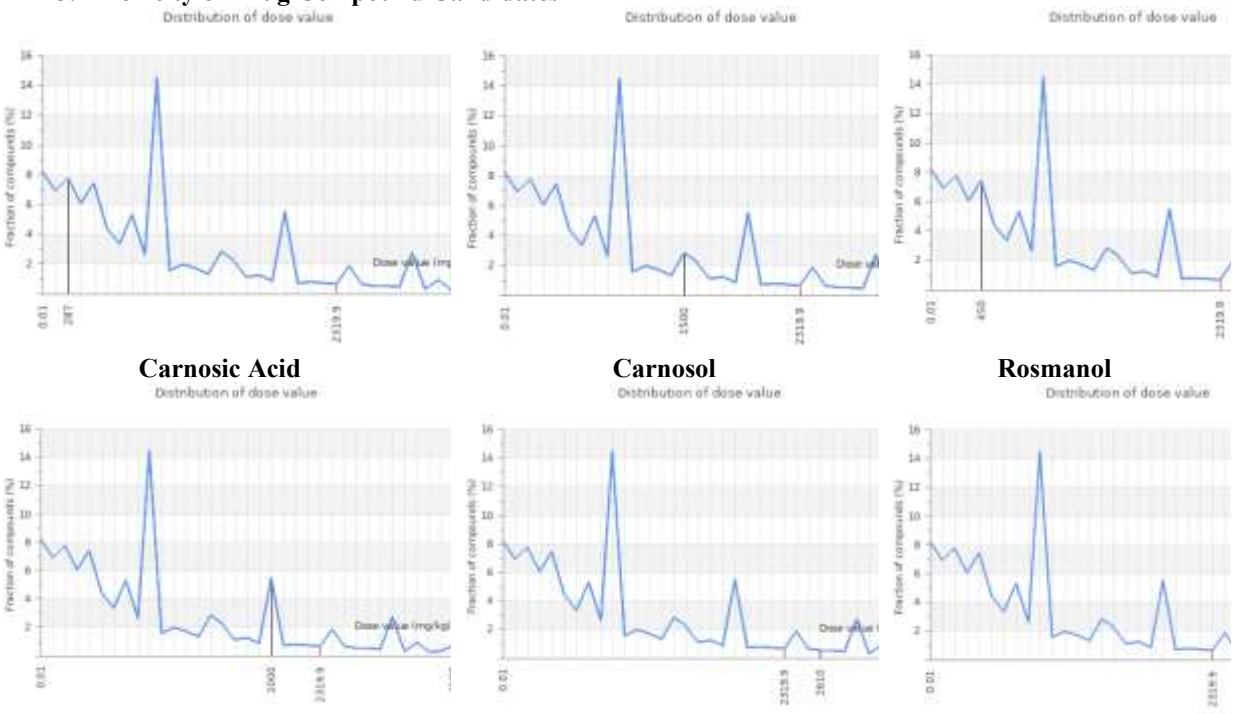
Ursolat Acid **Betulinic Acid** **Rosmarinic Acid**

Figure 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

S.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

3. Toxicity of Drug Compound Candidates



Carnosic Acid **Carnosol** **Rosmanol**

Ursolat Acid **Betulinic Acid** **Rosmarinic Acid**

Figure 2. Comparison of input compounds with dataset compounds.

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] (6) :

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 10| Issue: 2| February 2024|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2024: 8.402 || ISI Value: 1.188

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)

Table 7. Predictions of toxicity class and LD50

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

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 8 Results of Average Similarity and Prediction Accuracy

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

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.

Table 9 Target Organ Toxicity Results

No	Compound	Hepatotoxicity	Carcinogenicity	Immunotoxocoty	Mutagenicity	Cytotoxic
1.	Carnosic Acid	No	No	Yes	No	No
2.	Carnosol	No	No	Yes	No	No
3.	Rosmanol	No	No	Yes	No	No
4.	Ursolic Acid	Yes	Yes	Yes	No	No
5.	Betulinic Acid	No	Yes	Yes	No	No
6.	Rosmarinic Acid	No	No	Yes	No	No

DISCUSSION

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(7).

Based on the results of predicting $CaCO_2$ 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 (8). 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

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 10| Issue: 2| February 2024|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2024: 8.402 || ISI Value: 1.188

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 (7). 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) (8). 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 (8).

A compound is identified as a cytochrome P450 inhibitor if the concentration needed to achieve a 50% inhibitory effect is below 10uM (4). 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 (7). 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 (7). 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 (8). 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 (7). 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 (7). 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

ISSN (Online): 2455-3662
EPRA International Journal of Multidisciplinary Research (IJMR) - Peer Reviewed Journal
Volume: 10| Issue: 2| February 2024|| Journal DOI: 10.36713/epra2013 || SJIF Impact Factor 2024: 8.402 || ISI Value: 1.188

Sorafenib turned out to have effects on hepatotoxicity, immunity and cytotoxicity.

CONCLUSION

From the results of the study, it was concluded that: 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. From the pharmacokinetic research results, the six phenolic acid compounds in Rosemary proved to be better than the comparison ligand Sorafenib.

REFERENCES

1. *Susa ST, Hussain A, Preuss C V. Drug Metabolism. In Treasure Island (FL); 2023.*
2. *Colizzi M, Weltens N, McGuire P, Van Oudenhove L, Bhattacharyya S. Descriptive psychopathology of the acute effects of intravenous delta-9-tetrahydrocannabinol administration in humans. Brain Sci. 2019;9(4).*
3. *Almazroo OA, Miah MK, Venkataramanan R. Drug Metabolism in the Liver. Clin Liver Dis. 2017;21(1):1–20.*
4. *Noor S, Mohammad T, Rub MA, Raza A, Azum N, Yadav DK, et al. Biomedical features and therapeutic potential of rosmarinic acid. Arch Pharm Res [Internet]. 2022;45(4):205–28. Available from: https://doi.org/10.1007/s12272-022-01378-2*
5. *Nursanti O, Aziz A, Hadisoebroto G. Prediksi Toksisitas dan Farmakokinetika untuk mendapatkan Kandidat Obat Analgesik. J Noncommunicable Dis. 2023;3(1):34.*
6. *Banerjee P, Eckert AO, Schrey AK, Preissner R. ProTox-II: A webserver for the prediction of toxicity of chemicals. Nucleic Acids Res. 2018;46(W1):W257–63.*
7. *Pires DEV, Blundell TL, Ascher DB. PKCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. J Med Chem. 2015;58(9):4066–72.*
8. *Ajani TA, Obikeze K, Magwebu ZE, Egieyeh S, Chauke CG. In - silico and in - vitro screening of Asiatic acid and Asiaticoside A against Cathepsin S enzyme. BMC Pharmacol Toxicol [Internet]. 2023;1–9. Available from: https://doi.org/10.1186/s40360-023-00701-x*