Jurnal Farmasi Indonesia
Vol. xx, No. xx, Month 20xx, page. xx~xx
ISSN: 1693-8615 , e-ISSN:2302-4291 , DOI: 10.31001/jfi.v20i1.paperID

In Silico Pharmacokinetic and Toxicity Analysis on Clitoria Ternatea Flower

In Silico Pharmacokinetic and Toxicity Analysis on Clitoria Ternatea Flower

Erna Yovi Kurniawati[1*], Noor Pramono[2], Syarief Thaufik Hidayat[2], Endang Mahati[3]
[1]Doctoral Program Medical and Health Sciences, Faculty of Medicine, DIponegoro University, Indonesia
[2]Departement Fertility and Endocrinology, Faculty of Medicine, Diponegoro University, Indonesia
[3]Departement Pharmacology and Therapy, Faculty of Medicine, Diponegoro University, Indonesia

Article Info

Article history:

Received 14 09, 2023
Revised 14 11, 2023
Accepted 17 11, 2023

Kata kunci

Clitoria Ternatea
In Silico
Farmakokinetik
Toksisitas

Keywords:

Clitoria Ternatea
In Silico
Pharmacokinetics
Toxicity

ABSTRAK

Penelitian ini mengeksplorasi aspek farmakokinetik dan toksisitas dari Clitoria ternatea, yang terkenal dalam pengobatan tradisional karena potensi farmakologisnya yang menjanjikan. Dengan menggunakan metode komputasi, penelitian ini berupaya mengungkap interaksi senyawa aktif dalam tubuh manusia, melibatkan prediksi tidak hanya sifat farmakokinetik tetapi juga mengeksplorasi korelasi antara struktur kimia dan parameter seperti lipofilisitas dan status substrat P-glikoprotein. Urgensi penelitian ini berasal dari kebutuhan akan sumber terapi yang aman. Melalui penilaian toksisitas in silico yang mendalam, potensi efek samping dari senyawa diidentifikasi dengan cermat, untuk memastikan evaluasi keamanan yang komprehensif.. Penelitian ini memperkenalkan perspektif baru dalam mengeksplorasi tanaman obat tradisional, dengan menekankan pentingnya alternatif terapi yang aman. Tujuan utamanya adalah untuk membangun fondasi yang kuat untuk mengembangkan C. ternatea lebih lanjut sebagai sumber daya yang berharga untuk aplikasi farmakologis dan memajukan pengobatan alami. Hasil penelitian mengungkapkan banyak senyawa dengan potensi farmakologis yang signifikan, yang menunjukkan harapan untuk aplikasi pengobatan alami di masa depan. Namun, keharusan untuk penelitian tambahan dan validasi eksperimental menggarisbawahi komitmen kami untuk memahami aspek farmakologis dan toksikologis dari senyawa ini.

ABSTRACT

This study explores the pharmacokinetic and toxicity aspects of Clitoria ternatea, which is well-known in traditional medicine for its promising pharmacological potential. Using computational methods, research seeks to unravel the interaction of active compounds in the human body, predicting not only pharmacokinetic properties but also exploring the correlation between chemical structure and parameters such as lipophilicity and P-glycoprotein substrate status, which improves our understanding of the compound's behaviour. The urgency of this research stems from the need for a safe source of therapeutics. Through in-depth in silico toxicity assessments, potential side effects of compounds are carefully identified, ensuring a comprehensive safety evaluation. This important step lays the foundation for responsible pharmacological development. This research introduces a new perspective on exploring traditional medicinal plants, emphasising the importance of safe therapeutic alternatives. The ultimate goal is to establish a solid foundation to further develop C. ternatea as a valuable resource for pharmacological applications and advance natural medicine. The results revealed many compounds with significant pharmacological potential, which show promise for future natural medicine applications. However, the imperative for additional research and experimental validation underscores our commitment to understanding the pharmacological and toxicological aspects of these compounds.

Corresponding Author:
Erna Yovi Kurniawati
Doctoral Program Medical and Health Sciences, Diponegoro University
Jl. Prof. Dr. Soedarto, Tembalang Semarang Jawa Tengah
email: yovi.raharjanto@gmail.com

1. INTRODUCTION

C. ternatea is a plant that has a long history of being used as a natural source of ingredients for a variety of health purposes. *C. ternatea* extract has potential pharmacological benefits, which have attracted the interest of academics and practitioners of traditional medicine. Clitoria ternatea extract has shown potential in modulating gut microbiota and improving overall hormonal balance [1]. Clitoria ternatea, also known as butterfly pea flower, has been studied for its potential therapeutic effects in PCOS. It contains compounds such as flavonoids, alkaloids, saponins, and tannins [2], which have antioxidant and anti-inflammatory properties [3]. Studies have shown that Clitoria ternatea extract can inhibit the production of pro-inflammatory cytokines, reduce oxidative stress, and protect against liver and kidney damage. It may also help regulate lipid metabolism, lower cholesterol levels, and improve insulin sensitivity [4]. This content makes *C. ternatea* a promising natural ingredient in the field of medicines and health supplements. However, the study of the use of *C. ternatea* as a potential drug requires understanding related pharmacokinetic profiles and toxicity using in silico methods such as Swiss ADME and Protox II, as well as further validation through laboratory tests.

Pharmacokinetic analysis is correlate to bioavailability which can affect the pharmacology effect and the toxic effects of C. ternatea extract[5]. Conventional laboratory trials, however, can be expensive and time-consuming for pharmacokinetic and toxicity studies. The in silico method, involving Swiss ADME and Protox II, provided valuable insights into the pharmacokinetic and toxicity profiles of C. ternatea. The first advantage is the higher speed of analysis, which allows this study to provide results quickly. In addition, the in silico approach can identify detailed trends in the interaction of C. ternatea's active compounds with the human body, providing in-depth understanding without the need for a long time. As a result, we will use the in silico method with the Swiss ADME and Protox II programs in this study. The pharmacokinetics of a substance, including the processes of absorption, distribution, metabolism, and excretion, can be estimated with the help of software called Swiss ADME. Protox II is a toxicity predictor to assess the potential of chemical poisoning. This in silico method also has limitations. The resulting predictions still require further experimental validation in the laboratory to ensure their accuracy. Nonetheless, recognising these limitations, the use of in silico methods remains a smart choice, providing a faster and more cost-effective perspective on the pharmacological properties and toxicity of natural compounds.

The objective of this study is to predict the potential pharmacological effects of *C. ternatea* extract so that it can study important details about how *C. ternatea* extract interacts with the human body, how the active ingredients are distributed within the body, and the potential toxic effects that may be caused by using the in silico method. The results of this research will further explain the potential applications of *C. ternatea* extracts as medications. The silico approach used in this study can also offer a faster and more cost-efficient alternative to understanding the pharmacokinetic properties and toxicity of natural compounds. The findings of this analysis could serve as a starting point for further research and potential advances in the pharmaceutical industry and the use of local wisdom as a herbal medicine. Through this in silico approach, this study aims to optimise the understanding of the pharmacological potential of C. ternatea while considering the advantages and limitations of this methodology.

2. METHOD

3D and Canonical SMILES structures were identified through the PubChem Database (National Library of Medicine). Analyze of the physiochemical descriptor as well

as predict ADME parameters and drug-like properties (druglikeness) using SwissADME (Swiss Institute of Bioinformatics). Website Access http://www.swissadme.ch, upload SMILES compounds, SwissADME will provide results and ADME parameters. Toxicity of compounds tested using ProTox II (Prediction of Toxicity of Chemicals). Website Access https://tox-new.charite.de/protox_II/, upload SMILES compounds, select any additional models to predict and start Tox-Prediction. The dose of toxicity is based on LD50 in mg/kg of body weight. LD50 is a median lethal dose, which means a dose at which 50% of the test subjects die after exposure to a compound.

The toxicity class is determined by the globally harmonised chemical labeling classification system (GHS). The LD50 value is given in mg/kg. Class I: fatal if swallowed (LD50 ≤ 5). Class II: lethal if swelled (5 < LD50 ≥ 50). Class III: toxic if swallened (50 < LD 50 ≤ 300). Class IV: hazardous if swelted (300 < LD50) ≤ 2000). Class V: may be hazardous if swollen (2000 < LD50, ≤ 5000). Class VI: non-toxic (LD50 > 5000). Toxicity models analyze target hepatotoxicities, carcinogenicity, mutagenicity, and cytotoxics[5], [6]

3. RESULT

The solubility and bioavailability potential of a chemical compound in the human body are based on Lipinski's Rule. Lipinski's rule consists of four simple criteria that must be met by the chemical to be considered to have the potential to be a drug with good bioavailability, including: molecular weight less than 500 g/mol, Log P (octanol-water partition coefficient) less than 5, the number of hydrogen receptor atom donors from a bound hydrogen bond is less than or equal to 5, and the number of receptors of a hydrogen atom donor from a bonded hydrogen is less than or equivalent to 10. A compound meets at least four of the above criteria, so it is assumed that it has the potential to have good solubility in water and can easily pass through cell membranes. Compounds that meet Lipinski's Rule tend to have higher bioavailability, which means they are more likely to be absorbed by the body and produce the desired pharmacological effects[6], [7].

Table 1. Druglikeness *Clitoria Ternatea* Compounds

Compound	*Lipinski's Rule Parameter*				*Druglikeness* (Lipinski)
	Molecular Weight ≤500	MLOGP ≤4.15	Num H-bond acceptors ≤10	Num H-bond donors ≤5	
Kaempferol	286.24 g/mol	-0.03	6	4	Yes
Quercetin	302.24 g/mol	-0.56	7	5	Yes
Myricetin	318.24 g/mol	-1.08	8	6	Yes
Ternatin	374.34 g/mol	-0.12	8	2	Yes
Petunidin	317.27 g/mol	0.03	7	5	Yes
Peonidin	301.27 g/mol	0.57	6	4	Yes
Delphinidin	338.70 g/mol	0.03	7	6	Yes
Malvidin	331.30 g/mol	0.28	7	4	Yes
Cyanidin	287.24 g/mol	0.32	6	5	Yes
Epicatechin	290.27 g/mol	0.24	6	5	Yes
Scutellarin	462.36 g/mol	-2.12	12	7	No
Baicalein	270.24 g/mol	0.52	5	3	Yes
Luteolin	286.24 g/mol	-0.03	6	4	Yes
Apigenin	270.24 g/mol	0.52	5	3	Yes
Chlorogenic	354.31 g/mol	-1.05	9	6	Yes
Protocatechuic	154.12 g/mol	0.40	4	3	Yes
Gallic	170.12 g/mol	-0.16	5	4	Yes
Anthraquinone	208.21 g/mol	1.86	2	0	Yes
Taraxerol	426.72 g/mol	6.92	1	1	Yes
Alpha Tocopherol	430.71 g/mol	6.14	2	1	Yes
Gamma-Tocopherol	416.68 g/mol	5.94	2	1	Yes
Campesterol	400.68 g/mol	6.54	1	1	Yes
Stigmasterol	412.69 g/mol	6.62	1	1	Yes

Compound	*Lipinski's Rule Parameter*				*Druglikeness* (Lipinski)
	Molecular Weight ≤500	MLOGP ≤4.15	Num H-bond acceptors ≤10	Num H-bond donors ≤5	
Beta-Sitosterol	414.71 g/mol	6.73	1	1	Yes
Sitostanol	416.72 g/mol	6.88	1	1	Yes
Palmitic Acid	256.42 g/mol	4.19	2	1	Yes
Stearic Acid	284.48 g/mol	4.67	2	1	Yes
Petroselinic Acid	282.46 g/mol	4.57	2	1	Yes
Linoleic Acid	280.45 g/mol	4.47	2	1	Yes
Arachidic Acid	312.53 g/mol	5.13	2	1	Yes
Behenic Acid	340.58 g/mol	5.58	2	1	Yes
Phytanic Acid	312.53 g/mol	5.13	2	1	Yes
3-deoxy-3, 11-epoxy cephalotaxine	313.35 g/mol	1.56	5	0	Yes

Forecasting absorption, distribution, metabolism, and excretion is then done by entering the SMILES code of the compound through the ADME Tools (Swiss ADME).

Table 2. ADME Prediction Analysis (Absorption, Distribution, Metabolism, Excretion)

Compounds	ADME Prediction				
	Absorption		**Distribution** (Lipophilicity-Log $P_{o/w}$)	**Metabolism** (Cytochrome metabolism)	**Excretion** (P-gp substrate)
	GI Absorption	Skin permeation (Log Kp)			
Kaempferol	High	-6.7	1.58	CYP1A2 CYP2D6 CYP3A4	No
Quercetin	High	-7.05	1.23	CYP1A2 CYP2D6 CYP3A4	No
Myricetin	Low	-7.4	0.79	CYP1A2 CYP3A4	No
Ternatin	High	-6.37	2.50	CYP1A2 CYP2C9 CYP3A4	No
Petunidin	High	-6.88	0.63	CYP1A2	Yes
Peonidin	High	-6.53	0.97	CYP1A2	Yes
Delphinidin	High	-7.5	-0.98	No	Yes
Malvidin	High	-6.73	0.92	CYP1A2	Yes
Cyanidin	High	-7.51	0.32	CYP1A2	Yes
Epicatechin	High	-7.82	0.85	No	Yes
Scutellarin	Low	-8.59	-0.20	No	Yes
Baicalein	High	-5.7	2.24	CYP1A2 CYP2D6 CYP3A4	No
Luteolin	High	-6.25	1.73	CYP1A2 CYP2D6 CYP3A4	No
Apigenin	High	-5.8	2.11	CYP1A2 CYP2D6 CYP3A4	No
Chlorogenic	Low	-8.76	-0.38	No	No
Protocatechuic	High	-6.42	0.65	CYP3A4	No
Gallic	High	-6.84	0.21	CYP3A4	No
Anthraquinone	High	-5.16	2.64	CYP1A2 CYP2C19	No
Taraxerol	Low	-2.3	7.22	No	No
Alpha Tocopherol	Low	-1.33	8.27	No	Yes
Gamma-Tocopherol	Low	-1.51	7.95	No	Yes

Compounds	ADME Prediction				
	Absorption		Distribution (Lipophilicity-Log $P_{o/w}$)	Metabolism (Cytochrome metabolism)	Excretion (P-gp substrate)
	GI Absorption	Skin permeation (Log Kp)			
Campesterol	Low	-2.5	6.90	No	No
Stigmasterol	Low	-2.74	6.97	CYP2C9	No
Beta-Sitosterol	Low	-2.2	7.19	No	No
Sitostanol	Low	-2.93	7.07	No	No
Palmitic Acid	High	-2.77	5.20	CYP1A2 CYP2C9	No
Stearic Acid	High	-2.19	5.93	CYP1A2	No
Petroselinic Acid	High	-2.6	5.70	CYP1A2 CYP2C9	No
Linoleic Acid	High	-3.05	5.45	CYP1A2 CYP2C9	No
Arachidic Acid	Low	-1.61	6.62	CYP1A2	No
Behenic Acid	Low	-1.01	7.40	CYP1A2	No
Phytanic Acid	High	-2.31	6.15	CYP2C9	Yes
3-deoxy-3, 11-epoxy cephalotaxine	High	-7.25	1.95	CYP2D6	No

The ability of a compound or substance to harm living creatures is known as toxicity. The level of toxicity of a substance is determined by general toxicities, which include natural substances found in plants, animals, and other natural resources. The toxicity of natural compounds, including hepatotoxicity, carcinogenity, mutagenity, and cytotoxics, is critical for many pharmaceutical, medical, and environmental applications. Toxicity testing of compounds from natural ingredients is crucial because it can help in identifying potentially hazardous or toxic substances and provide guidance for safe and effective drug design [10], [11]

Table 3. Toxicity Test Results of *Clitoria Ternatea* Compound

Compounds	Toxicity						
	LD50 (mg/kg)	*Toxicity Class*	*Hepatotoxicity*	*Carcinogenicity*	*Immunotoxicity*	*Mutagenicity*	*Cytotoxicity*
Kaempferol	3919	5	Inactive	Active	Inactive	Active	Inactive
Quercetin	159	3	Inactive	Active	Inactive	Active	Inactive
Myricetin	159	3	Inactive	Active	Inactive	Active	Inactive
Ternatin	5000	5	Inactive	Inactive	Active	Inactive	Inactive
Petunidin	5000	5	Inactive	Active	Active	Inactive	Inactive
Peonidin	5000	5	Inactive	Inactive	Inactive	Inactive	Inactive
Delphinidin	5000	5	Inactive	Inactive	Inactive	Inactive	Inactive
Malvidin	5000	5	Inactive	Inactive	Active	Inactive	Inactive
Cyanidin	5000	5	Inactive	Active	Inactive	Inactive	Inactive
Epicatechin	10000	6	Inactive	Inactive	Inactive	Inactive	Inactive
Scutellarin	5000	5	Inactive	Active	Inactive	Inactive	Inactive
Baicalein	3919	5	Inactive	Active	Inactive	Active	Inactive
Luteolin	3919	5	Inactive	Inactive	Inactive	Inactive	Inactive
Apigenin	2500	5	Inactive	Inactive	Inactive	Inactive	Inactive
Chlorogenic	5000	5	Inactive	Inactive	Active	Inactive	Inactive
Protocatechuic	2000	4	Inactive	Active	Inactive	Inactive	Inactive
Gallic	2000	4	Inactive	Active	Inactive	Inactive	Inactive
Anthraquinone	5000	5	Inactive	Inactive	Inactive	Active	Inactive
Taraxerol	70000	6	Inactive	Inactive	Active	Inactive	Inactive
Alpha Tocopherol	5000	5	Inactive	Inactive	Inactive	Inactive	Inactive

Compounds	Toxicity						
	LD50 (mg/kg)	*Toxicity Class*	*Hepatotoxicity*	*Carcinogenicity*	*Immunotoxicity*	*Mutagenicity*	*Cytotoxicity*
Gamma-Tocopherol	5000	5	Inactive	Inactive	Inactive	Inactive	Inactive
Campesterol	890	4	Inactive	Inactive	Active	Inactive	Inactive
Stigmasterol	890	4	Inactive	Inactive	Active	Inactive	Inactive
Beta-Sitosterol	890	4	Inactive	Inactive	Active	Inactive	Inactive
Sitostanol	500	4	Inactive	Inactive	Active	Inactive	Inactive
Palmitic Acid	900	4	Inactive	Inactive	Inactive	Inactive	Inactive
Stearic Acid	900	4	Inactive	Inactive	Inactive	Inactive	Inactive
Petroselinic Acid	48	2	Inactive	Inactive	Inactive	Inactive	Inactive
Linoleic Acid	100000	6	Inactive	Inactive	Inactive	Inactive	Inactive
Arachidic Acid	900	4	Inactive	Inactive	Inactive	Inactive	Inactive
Behenic Acid	900	4	Inactive	Inactive	Inactive	Inactive	Inactive
Phytanic Acid	900	4	Inactive	Inactive	Inactive	Inactive	Inactive
3-deoxy-3, 11-epoxy cephalotaxine	28	2	Inactive	Active	Active	Inactive	Active

4. DISCUSSION

Druglikeness *Clitoria Ternatea* Compounds

Kaempferol, Quercetin, Myricetin, Ternatin, Petunidin, Peonidin, Delphinidin, Malvidin, Cyanidin, Epicatechin, Baicalein, Luteolin, Apigenin, Chlorogenic, Protocatechuic, Galic, Anthraquinone, Taraxerol, Alpha Tocopherol, Gamma Tocopherol, Campesterol, Stigmasterol, Beta-Sitosterol, Sitostanol, Palmitic Acid, Stearic Acid, Petroselinic Acid, Linoleic Acid, Arachidic Acid, Behenic Acid, Phitanic Acid, and 3-deoxy-3,11-epoxy cephalothinone met the criteria for drug similarity according to Lipinski's Rule. Scutellarin has a number of H-bond acceptors and donors that exceeds the limit set by Lipinski's Rule, so it does not fulfil the druglikeness criteria. This could be an indication that this compound may have potential limitations in absorption and distribution in the body.

There was one Clitoria ternatea compound that did not meet the drug similarity criteria based on Lipinski's Rule due to the number of hydrogen bond acceptors and donors exceeding the set limit. This does not directly exclude the possibility of developing Clitoria ternatea as a natural medicine. Although Lipinski's Rule provides initial guidelines for drug evaluation, many successful drugs on the market do not adhere to all of these criteria. The decision to proceed with Clitoria ternatea natural substance drug development research depends on several additional factors, such as the results of preclinical trials and additional information on the pharmacological and toxicological properties of the compound. Some compounds that do not fulfil Lipinski's Rule but have significant pharmacological activity can be the object of further development, especially if they show high therapeutic potential and can be addressed by formulation improvements or structural modifications.

Further natural substance drug development is possible through further validation steps required to better understand the properties of the compound; this may include toxicity testing, determination of the mechanism of action, and additional research to understand how the compound interacts with biological systems. In the drug development of natural substances such as Clitoria ternatea, it is important to take a holistic approach and consider the diversity of compounds present as well as the potential synergies between them. Therefore, while one compound may not fulfil certain criteria,

further studies at the cellular and animal level may provide a more complete insight into the medicinal potential of the plant extract as a whole.

Gastrointestinal Absorbtion

Based on the ADME prediction table, the GI absorption prediction results compounds in the "high" category (kaempferol, quercetin, ternatin, petunidin, peonidin, delphinidin, malvidin, cyanidin, epicatechin, isorhamnetin, bainicale, luteolin, apigenin, protocatechuic, gallic, anthraquinone, palmitic acid, stearic acid, petroselinic acids, linoleic acides, phytanic acidos, 3-deoxy-3, 11-epoxy cephalotaxine). There are compounds in the category "low" (myricetin, scutellarin, chlorogenic, taraxerol, alpha-tocopherol, gamma-tocopherol, campesterol, stigesterol, beta-sitosterol, sitstigostanol/mastanol, arachidic acid, and behenic acid).

Low GI absorption of a compound can have a significant impact on the effectiveness and availability of the compound in the human body. If a compound has low GI absorption, it means that the amount of the compound absorbed by the gastrointestinal tract may be limited, which may hinder the expected therapeutic effect as the compound may not reach the required concentration levels in the blood or target organs. Low absorption can reduce the efficacy of a compound, as sufficient amounts of the compound may not reach its biological target. This can reduce the therapeutic potential and make the compound less effective as a drug.

Low Clitoria ternatea contains compounds with low GI absorption; this can be an important consideration in its development as a natural medicine. However, many factors affect absorption, including formulation, dosage, and the properties of the compound. Certain formulation modifications or processing techniques may be required to improve the bioavailability and absorption of these compounds. Compounds with high GI absorbtion tend to have better bioavailability, which means they are more efficient in reaching the target tissue and producing the desired therapeutic effect[6], [7].

Skin Permeation

The level of skin permeability that is considered good for transdermal patches is usually in the range of about -3 to -1 cm/s. A higher level of permeability in this range indicates that the compound is more easily penetrating the skin and has the potential to provide an effective therapeutic dose. Compounds with a low log Kp value tend to remain on the skin surface without penetrating deeper. Composites with a positive log kp value (values above zero) tend to have the potential to penetrate or absorb through the skin better than compounds with a negative log kp value (values below zero). A positive Log K p value indicates that the compound is more prone to transitioning from the water (skin) phase to the non-polar phase (such as the skin layer)[6], [8]. There are 13 compounds that are predicted to be developed into transdermal patches of ternate clitoral, including taraxerol, alpha-tocopherol, gamma-tocopherol, campesterol, stigmasterol, beta-sitosterol, sitostanol/stigmastanol, palmitic acid, stearic acid, petroselinic acids, arachidic acids, behenic acid, and phytanic acid.

If certain compounds in Clitoria ternatea have high skin permeation scores, it can be considered to optimise the patch formulation by focusing on these compounds. The selection of compounds with good skin permeability characteristics can improve the efficiency of drug delivery through transdermal patches. It is necessary to adjust the patch formulation, including the selection of a carrier material (a vehicle) that supports skin penetration. The selection of suitable carrier materials can increase the availability and penetration of compounds from Clitoria ternatea through the skin layer. It is important to conduct preclinical and clinical trials to evaluate the effectiveness and safety of transdermal patch use of Clitoria ternatea. Data from these studies will provide a better

understanding of the potential use of these patches in clinical applications. The drug development of transdermal patches should also consider the potential toxicity and safety of the compounds in Clitoria ternatea. The use of patches allows continuous absorption of compounds through the skin, so careful evaluation of side effects and toxicity is important[8], [9].

Lipophilicity

All compounds are lipophobic, except the three hydrophilic. (delphinidin, scutellarin, chlorogenic). One of the important elements in pharmacokinetics and drug effects on the body is lipophilia. Better membrane and biological tissue penetration is characteristic of compounds with high lipophilicity, which allows for more effective distribution to various organs and tissues of the body. However, excessive lipophilization can also lead to an accumulation of substances in certain fat tissues and organs, which can affect the way the body distributes and removes those substances. Therefore, determining lipophilicity is an important component of pharmaceutical research and drug design[9].

Although three compounds in Clitoria ternatea are hydrophilic (delphinidin, scutellarin, and chlorogenic), the development of Clitoria ternatea as an oral natural medicine is still possible. The development of appropriate formulations and innovative delivery strategies can help improve the availability and efficacy of these compounds in the body. Research is needed to design optimal oral formulations or transdermal patches. The choice of binders, absorption aids, and replacements can affect the solubility, stability, and absorption of these hydrophilic compounds. The application of specialised delivery technologies, such as nanoparticles or liposomes, can improve the solubility and absorption of hydrophilic compounds. These technologies can help to include these compounds in formulations and improve absorption efficiency in the gastrointestinal tract. The combination of hydrophilic compounds with lipophilic compounds in formulations can expand development options. Lipophilic compounds can facilitate absorption and distribution in the body, while hydrophilic compounds still provide the desired health benefits. Adjustment of dosage and frequency of administration can be an important strategy to ensure optimal availability and efficacy in oral use[6]–[8].

Cytochrome metabolism

Some compounds, such as kaempferol, quercetin, ternatin, petunidin, peonidin, malvidin, and cyanidin, have predictions of metabolism by the CYP1A2 enzyme, which is one of the most active cytochrome P450 enzymes in the human body, suggesting that these compounds may undergo significant breakdown and modification in the body. The compounds baicalein, luteolin, and apigenin have predictions of metabolism by CYP2D6 and cYP3A4, which are other P450 cytochrome enzymes that are important in the breakdown of different compounds.

Compounds involved in CYP enzyme metabolism generally undergo chemical transformations, such as oxidation, which can produce active or inactive metabolites. Such metabolites can have different pharmacological activities or toxic effects from the original compound. The involvement of CYP enzymes may lead to potential drug interactions. Compounds that use the same metabolic pathway as other compounds metabolised by the same CYP enzymes may compete for those enzymes, affecting the rate of elimination and availability of both. Genetic polymorphisms in CYP enzymes may lead to variations in individual responses to compounds metabolised by those enzymes. Some individuals may have higher or lower CYP enzyme activity, affecting the rate of metabolism of drug compounds. In clinical development, dosage adjustments may be required to accommodate variability in metabolism by CYP enzymes. This is important to achieve the desired therapeutic concentration and avoid toxic effects. It is important to

consider the impact of CYP enzyme involvement in drug development, especially if the compound has the potential to be a natural medicine. Some compounds metabolised by CYP enzymes can still be used as natural medicines if, through formulation or dosage adjustments, individualised responses can be predicted or monitored well, and drug interactions can be addressed by dosage adjustments or the selection of appropriate compounds[8].

Based on the table provided, if the "Cytochrome metabolism" for a compound is listed as "No", it means that the compound is not significantly metabolised by the listed CYP enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4). However, some compounds, such as delphinidine, epicatechin, scutellarin, chlorogenic, alpha-tocopherol, gamma-tocopherol, taraxerol, and a number of other compounds, have no prediction of metabolism by a particular cytochrome P450 enzyme. Compounds that do not undergo significant metabolism by CYP enzymes may be more stable in the body, as they will not undergo chemical changes that can accelerate elimination. Involvement of CYP enzymes in drug metabolism can lead to drug interactions if the compounds compete for the same enzymes. If a compound is not altered by CYP enzymes commonly involved in drug metabolism, the potential for drug interactions may be reduced. Compounds that do not undergo significant metabolism may have higher bioavailability as they do not undergo chemical changes that may affect absorption and distribution in the body [8].

P-gp (P-glycoprotein)

There are 10 compounds (peonidin, delphinidin, malvidin, petunidin, cyanidin, epicatechin, scutellarin, alpha-tocopherol, gamma-tocopherol, and phytanic acid) with Pgp substrate status "yes", as well as 24 compounds with "no" status. Compounds that are P-gp substrates tend to experience reduced bioavailability because they can be pumped out (efflux) from cells involving P-gp. This can affect the level of absorption and availability of the compound in the body. For compounds with a P-gp "yes" status, formulation optimisation and dose adjustment are necessary. Special formulation techniques or combinations with P-gp modulators may help improve bioavailability. Compounds that are P-gp substrates may interact with other drugs that also use the same transport pathway. This requires special consideration in the development of combination formulations or dose adjustments. Compounds with "no" P-gp status may have higher bioavailability as they do not experience the effect of reduced absorption by P-gp. The presence of compounds with P-gp "no" status may reduce the risk of drug interactions involving the P-gp pathway, facilitating the development of formulations involving these compounds. Compounds with P-gp "no" status may be more suitable for oral formulations, as they are less affected by the influence of P-gp on absorption [8]–[10].

For both compounds with P-gp status "yes" and "no", clinical trials and safety evaluation are important stages in the drug development of herbal ingredients. This will provide further understanding of patient responses and potential side effects. Formulation optimisation and the use of innovative formulation methods can help overcome challenges that may be associated with P-GP status. The choice of compound combinations in formulations can help harness the synergistic potential and minimise the negative impact of a particular P-gp status.

Toxicity

A huge amount of research and toxicity testing should be done on any natural ingredient intended to be used in pharmaceutical products, food supplements, or medicines. Toxicity studies help determine safe doses and help in the development of products that are safe for human health. Compounds found in natural products can vary in toxicity depending on their chemical and biological properties. Plants that are

traditionally used as medicines contain compounds with low toxicity or even beneficial effects on health, but certain ingredients of natural products can also be highly toxic and harmful to humans[10].

The results of the analysis showed large variations in the LD50 of various compounds, with the highest value being 100,000 mg/kg (linoleic acid) and the lowest being 28 mg/kg. (3-deoxy-3, 11-epoxy cephalotaxine). Test results of LD50 compounds petroselinic acid and 3-deoxy-3, 11-epoxy cephalotaxine in class 2 compounds with LD50 class 2 considered to be toxic with high potential to cause negative effects on test organisms in moderate doses These compounds have the ability to cause damage or disruption to the body's functions when consumed or exposed in sufficiently high doses. The LD50 test results of compounds in class 3 are quercetin and myricetin. Compounds with class 3 LD50 are considered to be toxic, with a moderate level of toxicity. Although its toxicity is lower than that of compounds with grade 1 and 2 LD50s, the compound still has the potential to cause negative effects on test organisms at higher doses. Even higher doses are needed to cause death in 50% of the test animals, but they still have the potential to cause toxic effects if swallowed in significant doses[11].

The LD50 test results of the compounds in class 4 are: protocatechuic, campesterol, stigmasterol, beta-sitosterol, sitostanol/stigmastanol, palmitic acid, stearic acid, arachidic acids, behenic acids, phytanic acid, and gallic acid. This classification suggests that much higher doses are needed to cause death or toxic effects, but we still need to be cautious as it can be dangerous if swallowed in significant doses. The LD50 test results of compounds in class 5 are: kaempferol, ternatin, sinensetin, peonidin, petunidin, delphinidin, malvidin, cyanidin, isorhamnetin, scutellarin, baicalein, luteolin, apigenin, chlorogenic, anthraquinone, alpha-tocopherol, and gamma-tocopherol. This category indicates that the compound has a low to moderate toxicity potential if swallowed. The doses required to cause toxicity effects in 50% of the test animals are within a sufficiently high range, so the compounds tend to be less harmful at doses commonly used in human applications or exposures. The LD50 test results of the compounds epicatechin, taraxerol, and linoleic acid fall into the class 6 category. Very high doses are required to cause toxicity effects, and this classification suggests that such a compound is considered non-toxic at commonly used doses. The majority of the compounds in *C. ternatea* are classified in classes 4 and 5, which show moderate to low levels of toxicity.[10], [11].

Analysis suggests that the compound *C. ternatea* is predicted to have no hepatotoxic activity, which can damage the liver. Some compounds are predicted to have carcinogenic potential, which means they can cause cancer. Compounds such as kaempferol, quercetin, myricetin, petunidin, cyanidin, scutellarin, baicalein, protocatechuic, gallic, and 3-deoxy-3, 11-epoxy cephalotaxine are classified as active in terms of carcinogenicity, so use or exposure to these compounds should be cautious. Some compounds are predicted to have immunotoxic potential, which can affect the immune system. These compounds, such as ternatin, petunidin, chlorogenic, taraxerol, campesterol, stigmasterol, beta-sitosterol, cytostanol, and 3-deoxy-3, 11-epoxy cephalotaxine, may affect the body's immune response to infection or disease. Mutagenicity refers to the ability of a compound to cause mutations in genetic material. Some compounds in this study are classified as active in terms of mutagenicity, such as kaempferol and quercetin, myricetin, baicalein, and anthraquinone, suggesting that these compounds can cause potentially harmful genetic changes. The compounds 3-deoxy-3, 11-epoxy cephalotaxine are predicted to have cytotoxic potential, which can affect the health of cells in the body. Further studies may be needed to confirm these predictions and identify more detailed toxic mechanism[10], [12].

5. CONCLUSSION

The compounds in *C. ternatea* have a molecular weight that conforms to Lipinski's Rule, indicating good absorption potential. The hydrophobic properties of some compounds affect their distribution and permeability. Predicting metabolism and P-gp status provides insight into the pharmacokinetic effects. Experimental validation is required for confirmation.

6. ACKNOWLEDGEMENT

This journal article is written as preliminary research funded by the Ministry of Education, Culture, Research, and Technology through the Indonesian Educational Scholarship Program of the Doctoral Program. The content is entirely the responsibility of the author. The author wishes to state that there is no conflict of interest that may affect the outcome or interpretation in this article.

7. REFERENCE

[1] Y. Liang, Q. Ming, J. Liang, Y. Zhang, H. Zhang, and T. Shen, "Gut microbiota dysbiosis in polycystic ovary syndrome: Association with obesity — A preliminary report," *Can J Physiol Pharmacol*, vol. 98, no. 11, pp. 803–809, 2020, doi: 10.1139/cjpp-2019-0413.

[2] Gejalakshmi.S and N. Harikrishnan, "Exploring the Pharmacological Potential of Clitoria Ternatea: In vivo Assessment of its CNS Activity as a Medicinal Herb," *Journal of Population Therapeutics and Clinical Pharmacology*, vol. 30, no. 4, pp. 523–532, Apr. 2023, doi: 10.47750/JPTCP.2023.30.04.052.

[3] Y. Wang *et al.*, "Clitoria ternatea blue petal extract protects against obesity, oxidative stress, and inflammation induced by a high-fat, high-fructose diet in C57BL/ 6 mice," *Food Research International*, vol. 162, pp. 963–9969, 2022, doi: 10.1016/j.foodres.2022.112008.

[4] W. Hakam Maulidy, A. Mustika, and I. Safitri Mukono, "The Effect of Butterfly Pea (Clitoria Ternatea) Extract on Reducing Total Cholesterol Levels in Rattus norvegicus with the Hypercholesterolemia Model," *International Journal of Research Publications*, vol. 115, no. 1, Dec. 2022, doi: 10.47119/IJRP10011511220224349.

[5] K. Linggam, S. Ramanathan, S. Sasidharan, and S. M. Mansor, "Toxicity evaluation of methanol extract of clitoria ternatea L. Leaf," *Malaysian Journal of Medicine and Health Sciences*, vol. 8, no. 2, pp. 33–39, 2012, Accessed: Jun. 25, 2023. [Online]. Available: https://www.researchgate.net/publication/288117872

[6] P. Rani, B. K. Rajak, and D. V. Singh, "Physicochemical parameters for design and development of lead herbicide molecules: Is 'Lipinski's rule of 5′ appropriate for herbicide discovery?," *Pest Manag Sci*, vol. 79, no. 5, pp. 1931–1943, May 2023, doi: 10.1002/PS.7367.

[7] R. Roskoski, "Rule of five violations among the FDA-approved small molecule protein kinase inhibitors," *Pharmacol Res*, vol. 191, p. 106774, May 2023, doi: 10.1016/J.PHRS.2023.106774.

[8] B. Bakchi *et al.*, "An overview on applications of SwissADME web tool in the design and development of anticancer, antitubercular and antimicrobial agents: A medicinal chemist's perspective," *J Mol Struct*, vol. 1259, p. 132712, Jul. 2022, doi: 10.1016/J.MOLSTRUC.2022.132712.

[9] K. M. Al Azzam, "SwissADME and pkCSM Webservers Predictors: an integrated Online Platform for Accurate and Comprehensive Predictions for In Silico ADME/T Properties of Artemisinin and its Derivatives," *Kompleksnoe Ispolzovanie*

Mineralnogo Syra = Complex use of mineral resources, vol. 325, no. 2, pp. 14–21, Jun. 2023, doi: 10.31643/2023/6445.13.

[10] H. K. Shin, R. Huang, and M. Chen, "In silico modeling-based new alternative methods to predict drug and herb-induced liver injury: A review," *Food and Chemical Toxicology*, vol. 179, p. 113948, Sep. 2023, doi: 10.1016/J.FCT.2023.113948.

[11] E. Benfenati, Q. Chaudhry, G. Gini, and J. Lou Dorne, "Integrating in silico models and read-across methods for predicting toxicity of chemicals: A step-wise strategy," *Environ Int*, vol. 131, p. 105060, Oct. 2019, doi: 10.1016/J.ENVINT.2019.105060.

[12] P. K. P. Krishnamoorthy *et al.*, "In-silico study reveals immunological signaling pathways, their genes, and potential herbal drug targets in ovarian cancer," *Inform Med Unlocked*, vol. 20, p. 100422, Jan. 2020, doi: 10.1016/J.IMU.2020.100422.