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Original Research Article

In Silico Toxicity Prediction of Bioactive Compounds of *Dioscorea alata* L.

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ABSTRACT

Yam (*Dioscorea alata L.*) contains various compounds that are widely known to have various health benefits. This study aims to analyze the toxicity of the active compounds in *D. alata* L. *in silico*. This involves the screening of the bioactive compounds, drug-likeness screening based on the Lipinski rule of five, and toxicity prediction using ADMETSAR (ADMET) and Protox II (hepatotoxicity, immunotoxicity, mutagenicity, and cytotoxicity). The bioactive compounds in *D. alata* L were 64 obtained from the Kanava Knapsack and the USDA database. 23 of these compounds with potential as drugs include 6 flavonoids compounds (26.09%), 5 stilbenoid compounds (21.74%), 4 glycosides compounds (17.39%), 3 carboxylic acid compounds (13.04%), vitamins (8.69%), phenol, propiophenone and steroid (4.35%). Based on the results of LD-50, there were 2 non-toxic compounds: diosgenin and catechin. While other compounds were classified as having a low toxicity. There are 2 compounds predicted to have Human Hepatotoxicity activity, 14 compounds that have drug-induced liver injury activity, 2 hepatotoxicity compounds, 5 carcinogenicity compounds, 6 immunotoxicity compounds, 2 mutagenicity compounds, and 1 cytotoxicity compound. The toxicity is predicted to appear if used as a single compound. This study proved that most of the active compounds in *D. alata* L. possess very low toxicity with only 2 non-toxic compounds (catechin and diosgenin). However, the use of the compounds as a single compound is predicted to have some toxic activities. It is important to carry out further research to validate the toxicity of the active compounds *D. alata* L. *in vivo*.

***Keywords*:** Toxicity, Yam, Diosgenin, Catechin, ADMETSAR, Protox.

Introduction

Yam (*Dioscorea alata L.*) comes from the Dioscoreaceae family. This type of yam is native to Africa and Asia. It is a staple in tropical countries. In addition, *D. alata* L. is also widely cultivated to be used as a basic substance for traditional medicine. *D. alata* L. has long been known to contain diosgenin.[1] Diosgenin is the most important steroidal saponin compound from *D. alata* L. because it has several biological functions, including immunomodulatory activity. *D. alata* L. has been shown to regulate metabolism, improve heart function, and have restorative effects. It has been shown to reduce fat in the liver and repair the cecum and colon after a high-fat diet. In addition, research on *D. alata* L. is concerned with the activity of scavenging reactive oxygen species, antioxidants, antidiabetics, anticlastogenic, antiosteoporotic, hypoallergenic, and immunomodulatory activities.[1–7] However, *D. alata* L. is used as food and traditional medicine empirically from generation to generation. Based on the development of knowledge and research, it is necessary to know the safety of traditional medicine to avoid unwanted harmful or toxic effects, such as toxicity effect. The toxicity effects include human hepatotoxicity (H-HT), drug-induced liver injury (DILI), carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity. Hepatotoxicity is defined as an injury to the liver whereas DILI is an acute or chronic response to a natural or manufactured compound. Both of them cause impairment of liver function.[8,9] Carcinogenicity is the ability of some compounds to cause cancer.[10] Immunotoxicity is defined as adverse effects on the immune system.[11] Mutagenicity is a term used to describe the properties of a chemical or natural drug substance that can induce genetic mutations.[12] Cytotoxicity is toxicity caused by the action of chemotherapeutic agents on living cells.[13] They are over 600 species of Dioscorea, but only 15 to 20 are edible. Common *Dioscorea sp.* contains low amounts of a toxic bitter alkaloid which disappears in cooking. Bitter components were identified as furanoid norditerpenes (diosbulbins A and B).[14] But naturally, plant proteins found in Dioscorea sp. can be toxic and cause illness if consumed raw.[15] Currently, no data support information on the safety of *D. alata* L. consumption. Therefore, it is necessary to carry out further research to find out whether there is a toxic effect or not. This study aims to analyze the profile of the active compound contained in *D. alata* L. and its toxicity *in silico*.

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Materials and Methods

Screening of Bioactive Compounds in D. alata L.

The screening for the bioactive compound contained in *D. alata* L. was conducted using the Kanaya Knapsack database (http://www.knapsackfamily.com/KNApSAcK/) and the USDA Dr. Duke Phytochemicals (https://phytochem.nal.usda.gov/). The canonical or isomeric simplified molecular-input line-entry system (SMILE) of the bioactive compounds was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

Drug-likeness Analysis and Toxicity Prediction of Bioactive Compound in D. alata L.

Drug-likeness analysis was used to determine the bioactive compounds of *D. alata* L. which have molecular properties such as drugs and non-drugs based on the Lipinksi rule of five. The ADMETlab v.2.0 database

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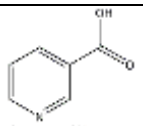
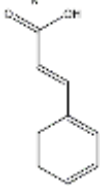
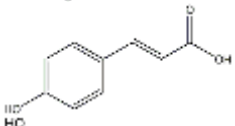
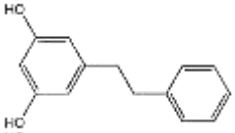
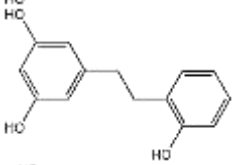
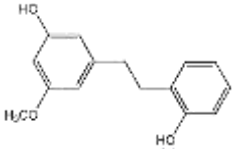
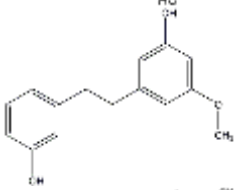
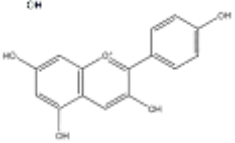
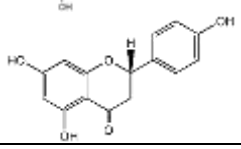
(https://admetmesh.scbdd.com/service/evaluation/index) was used to obtain the Lipinski rule and ADME/T predictions of a compound.[16]
The Protox II database (https://tox-new.charite.de/protox_II/index.php?site=compound_input) was used to obtain predictions of the Lipinski rule and toxicity.[17,18] The Canonical SMILE structure of each bioactive compound of *D. alata* L. was selected and entered into the ADMETSAR and Protox II search keywords. The criteria for compounds that meet Lipinski's rules are those with at least 2 of 5 main properties.[19] The criteria used as the basis for grouping and selection are: Molecular mass of fewer than 500 Daltons, High lipophilicity (expressed as LogP less than 5), Having less than 10 hydrogen bond acceptors, Having less than 5 hydrogen bond donors, and Molar refractivity of between 40 -130.
Toxicity was carried out with ADMETSAR it is used to predict the pharmacokinetic activities including Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET). The Protox II software was used to predict the toxicity of the compounds using additional models including Hepatotoxicity, Carcinogenicity, Immunotoxicity, Mutagenicity, and Cytotoxicity. Additionally, based on the Lethal Dose (LD)-50 value, the toxicity of compounds is grouped into 6 classes,[17] 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) and class VI: non-toxic (LD50 > 5000).

Results and Discussions

Bioactive Compounds Contained in D. alata L.
As shown in Table 1, the bioactive compounds of *D. alata* L. obtained from the KnapSack Kanaya database were 32 bioactive compounds. The bioactive compounds of *D. alata* L. obtained by Dr. Duke's Phytochemical and Ethnobotanical also found 32 bioactive compounds (Table 2). Furthermore, these two databases were used as the basis for further analysis.

Table 1: Screening Results of Bioactive Compounds *D. alata* L. from the Knapsack Database

Metabolite	Molecular formula	MW	Chemical structure	PubChem ID
Vitamin B3	$C_6H_5NO_2$	123.0320284		938
Cinnamate	$C_9H_8O_2$	148.0524295		444539
p-Coumaric acid	$C_9H_8O_3$	164.0473441		637540
Dihydropinosylvin	$C_{14}H_{14}O_2$	214.0993797		442700
Demethylbatatasin IV	$C_{14}H_{14}O_3$	230.0942943		442699
Batatasin IV	$C_{15}H_{16}O_3$	244.1099444		181271
Batatasin III	$C_{15}H_{16}O_3$	244.1099444		10466989
Pelargonidin	$C_{15}H_{11}O_5$	271.0606485		440832
Naringenin	$C_{15}H_{12}O_5$	272.0684735		932

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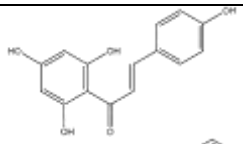
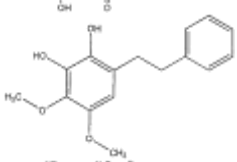
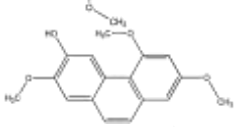
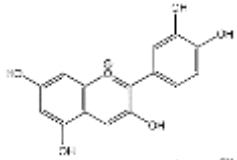
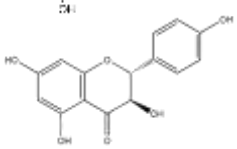
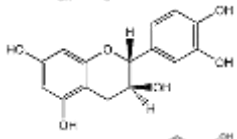
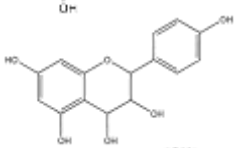
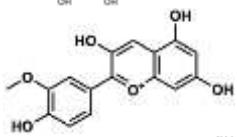
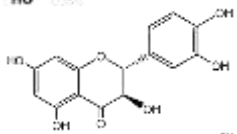
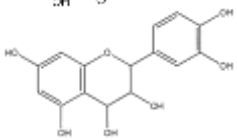
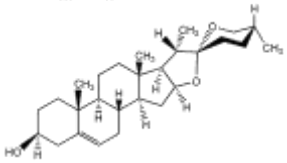
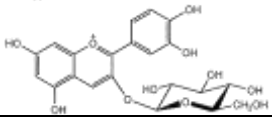
Metabolite	Molecular formula	MW	Chemical structure	PubChem ID
Naringenin chalcone	$C_{15}H_{12}O_5$	272.0684735		5280960
Batatasin II	$C_{16}H_{18}O_4$	274.1205091		85806015
Batatasin I	$C_{17}H_{16}O_4$	284.104859		442694
Cyanidin	$C_{15}H_{11}O_{6+}$	287.0555631		128861
Dihydrokaempferol	$C_{15}H_{12}O_6$	288.0633881		122850
Catechin	$C_{15}H_{14}O_6$	290.0790382		9064
Leucopelargonidin	$C_{15}H_{14}O_6$	290.0790382		3286789
Peonidin	$C_{16}H_{13}O_6$	301.0712132		441773
Taxifolin	$C_{15}H_{12}O_7$	304.0583027		39533
Leucocyanidin	$C_{15}H_{14}O_7$	306.0739528		71629
Diosgenin	$C_{27}H_{42}O_3$	414.3133952		99474
Cyanidin 3-O-glucoside	$C_{21}H_{21}O_{11+}$	449.1083865		44256715

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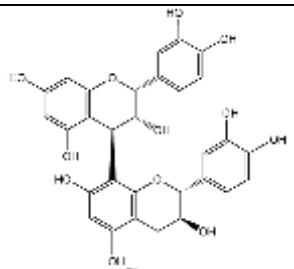
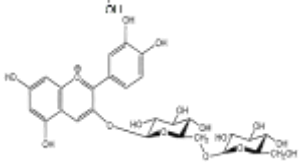
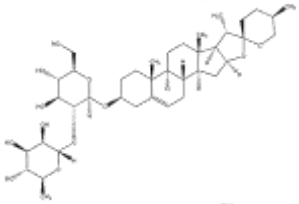
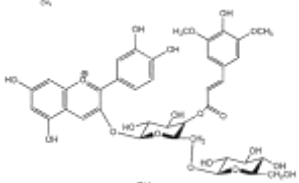
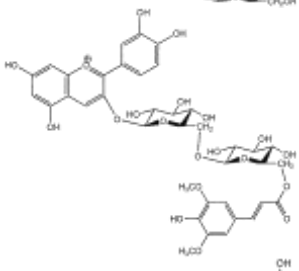
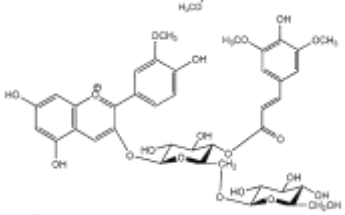
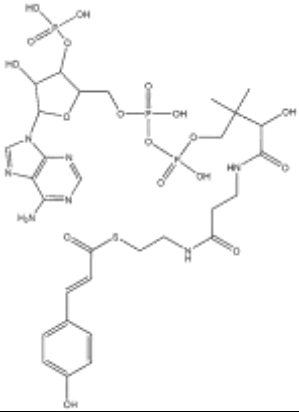
Metabolite	Molecular formula	MW	Chemical structure	PubChem ID
Procyanidin B1	$C_{30}H_{26}O_{12}$	578.1424263		11250133
Cyanidin 3-gentiobioside	$C_{27}H_{31}O_{16}$	611.16121		11968393
Diosgenin 3-O-alpha-L-rhamnopyranosyl(1->2)-beta-D-glucopyranoside	$C_{39}H_{62}O_{12}$	722.4241275		11061578
Alatanin 1	$C_{38}H_{41}O_{20}$	817.2191188		154496127
Alatanin C	$C_{38}H_{41}O_{20}$	817.2191188		44256751
Alatanin 2	$C_{39}H_{43}O_{20}$	831.2347688		14757905
p-Coumaroyl CoA	$C_{30}H_{42}N_7O_{18}P_3S$	913.1519878		6440013

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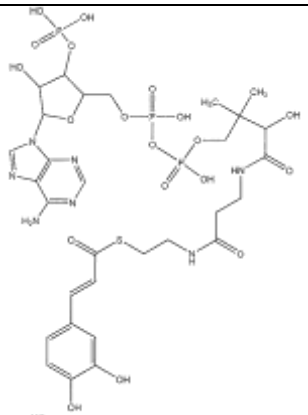
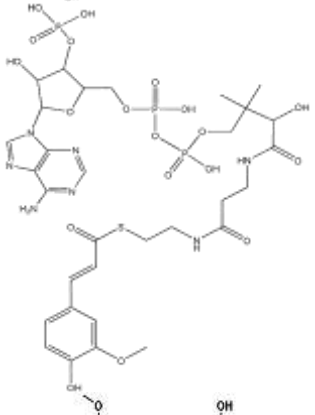
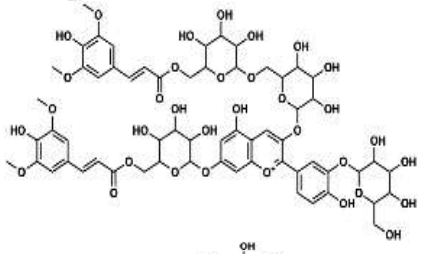
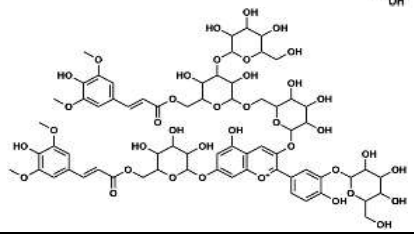
Metabolite	Molecular formula	MW	Chemical structure	PubChem ID
Caffeoyl-CoA	$C_{30}H_{42}N_7O_{19}P_3S$	929.1469024		11966126
Feruloyl-CoA	$C_{31}H_{44}N_7O_{19}P_3S$	943.1625525		11966129
Alatanin B	$C_{61}H_{71}O_{34}$	1347.382674		131753052
Alatanin A	$C_{67}H_{81}O_{39}$	1509.435498		131753053

Drug-likeness of the bioactive compounds D. alata L.

To advance the discovery and development of new drugs, great efforts are being made to evaluate the similar 'drug-like' properties of molecules in the early stages of the discovery-research process. There are different approaches to solving this problem, but the simplest and most used approach is developed by Chris Lipinski and his colleagues at Pfizer, which is generally referred to either as the Lipinski Rules or the Rule of Five.[20] Rule of five is a rule of thumb to evaluate drug-likeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans.[19] Based on drug-likeness screening in this study, there were 23 bioactive compounds in *D. alata* L. that met the Lipinski rule criteria (Table 3). A total of 23 bioactive compounds of *D. alata* L. are included in the flavonoid group (26.09%), stilbenoids (21.74%), glycoside (17.39%), the carboxylic acid (13.04%), vitamins (8.69%), phenol, propiophenone and steroids (4.35%).

Toxicity Prediction of Bioactive Compounds D. alata L.

This study showed that the toxicity of the bioactive compounds contained in *D. alata* L. was low because the average LD-50 value (blue bar; Figure 1) and the toxicity classes of the 23 bioactive compounds of *D. alata* L. ranged around class 4 and class 6 (orange line; Figure 1). However, several compounds need to be aware that can cause Human Hepatotoxicity (H-HT), Drug-Induced Liver Injury (DILI), Hepatotoxicity, Carcinogenicity, Immunotoxicity, Mutagenicity, and Cytotoxicity when used as a single compound (Tables 4 and 5). In Table 4, the results of the ADMETlab analysis show that several bioactive compounds in *D. alata* L. (indicated in yellow) are predicted to be toxic in a single compound form. This yellow mark was found in the H-HT activity of 2 compounds: cinnamic acid and *p*-coumaric acid. Other compounds predicted to potentially induce DILI, are cyanidin, nicotinamide/vitamin B3, cyanidin-3-O-glucoside, ascorbic acid, ferulic acid, cinnamic acid, p-Coumaric acid, pelargonidin, naringenin, naringenin chalcone, batatasin I, dihydrokaempferol, peonidin, and taxifolin.

Furthermore, the analysis of Pgp-inhibitor, Pgp-substrate, Human Intestinal Absorption, F (20% Bioavailability), and F (30% Bioavailability) was carried out based on the ADMETlab results. Table 4 showed some of the bioactive compounds in *D. alata* L. (marked in green) which indicates that the compounds possess poor absorption and bioavailability values are not good enough.

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Table 2: Screening Results of Bioactive Compounds *D. alata* L. from the Dr. Duke Database

Activities Count	Chemical	Plant Part	Low PPM	High PPM	StdDev
0	Cyanidin-Glycoside	Bulb			
0	Cyanidin-3-Gentiobioside	Plant			
61	Ferulic-Acid	Plant			
14	Manganese	Plant			
5	Aluminum	Root	1	12	-0.30604
112	Ascorbic-Acid	Root		100	-0.58881
0	Ash	Root	7000	35045	-0.92845
53	Beta-Carotene	Root	0	0.2	-0.43514
4	Boron	Root	0.8	7	-0.63039
28	Calcium	Root	44	290	-0.77898
0	Citrates	Root	1270	5935	-0.67733
12	Copper	Root	0.5	10.7	-0.11297
0	Fat	Root	300	5700	-0.57292
15	Fiber	Root	6000	55600	-0.72204
6	Iron	Root	1.4	25	-0.40533
0	Kilocalories	Root	619	3005	-0.79611
65	Magnesium	Root	66	827	-0.92296
0	Malates	Root	870	5750	-0.83164
39	Niacin	Root	0.7	17	-0.96334
0	Oxalate	Root	150	700	-0.73033
4	Phosphorus	Root	48	2190	-0.2333
14	Potassium	Root	2240	15040	-0.0541
0	Protein	Root	11000	63085	-0.62131
15	Riboflavin	Root	0.2	1.1	-0.65096
1	Sodium	Root	22	335	-0.35679
5	Starch	Root	159000	743000	1.787331
0	Succinate	Root			
0	Sugars	Root	5000	35980	-0.65724
14	Sulfur	Root	98	990	-0.51173
31	Thiamin	Root	0.3	1.4	-0.4692
0	Water	Root	650000	786000	0.109666
77	Zinc	Root	2	17	-0.31439

This green mark is indicated by the Human Intestinal Absorption activity of the Cyanidin-3-O-glucoside compound. Bioavailability (denoted as F and generally expressed as a percentage, F%) quantifies the proportion of compounds absorbed and available to produce systemic effects. The absorption and bioavailability values are not good enough on the F20% and F30% for the cyanidin, cyanidin-3-O-glucoside, ascorbic acid, dihydropinosylvin, dimethylbatatacin IV, batatasin IV, batatasin III, pelargonidin, naringenin, dihydrokaemferol, catechins, leucopelargonidine, dan leucocyanidins.

The results of the Protox II analysis showed that some of the active compounds in *D. alata* L. are marked with a yellow color. This indicates that the predicted compound is toxic in single compound form. This yellow sign was found in the hepatotoxicity activity for nicotinamide/vitamin B3 and cinnamic acid compounds. The carcinogenicity activity for 5 compounds: cyanidin, p-coumaric acid, leucopelargonidin, taxifolin, and leucocyanidin. Immunotoxicity activity in 6 compounds: diosgenin, cyanidin-3-*O*-glucoside, ferulic acid, naringenin chalcone, batatasin I, and leucocyanidin. Mutagenicity activity in 2 compounds: batatasin I and taxifolin. Cytotoxicity activity was only found in 1 compound: naringenin. Recently, the utilization of *D. alata* L. has become more popular as a staple food and for traditional medicine. *D. alata* L. is believed to have many health benefits and is expected to be a good source of protein, vitamin C, and beta-carotene. Based on the database from KnapSack and Dr. Duke, we found 64 bioactive compounds contained in *D. alata* L. While, the bioactive compounds that have potential as drugs are only 23 compounds (Table 3). From those 23 compounds, 6 compounds belong to flavonoid (26.09%), 5 stilbenoids compounds (21.74%), 4 glycoside compounds (17.39%), 3 carboxylic acids compounds (13.04%), 2 vitamins (8.69%), 1 phenol (4.35%), 1 propiophenone (4.35%) and 1 steroid (4.35%). Flavonoids are considered an indispensable component in a variety of nutraceuticals, pharmaceuticals, medicinal and cosmetic applications. This is attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties and their capacity to modulate key cellular enzyme functions.[21] Stilbenoids exert various biological effects such as cardioprotection, neuroprotection, anti-diabetic properties, anti-inflammatory, also cancer prevention and treatment.[22] Carboxylic acids exist as effective drug functional groups because they are involved in specific and critical interactions for binding to their targets.[23] Moreover, plant-derived vitamins have strong antioxidant potential, including water-soluble and lipid-soluble compounds.[24] Propiophenone, a Naringenin chalcone, has a role as a metabolite, an anti-allergic agent, and an anti-inflammatory agent.[25] Naringenin chalcone also suppresses asthmatic symptoms by inhibiting Th2 cytokine production from CD4 T cells.[26] The last is a steroid (Diosgenin) known as the major compound of *D. alata* L. As an antioxidant, Diosgenin is known to have neuroprotective effects and to improve some aging-related deficits such as memory improvement.[27] Diosgenin, which is found in several plant species, is reported to be a promising bioactive biomolecule with diverse important medicinal properties, including hypolipidemic, hypoglycaemic, antioxidant, anti-inflammatory, and anticancer, antiproliferative activities, and used in allergic diseases.[28] Indeed, *D. alata* L. is widely used in traditional medicine because of its bioactive compounds and potential for the treatment of various diseases.

On the other hand, based on the LD-50 value (Figure 1), there are only 2 non-toxic compounds (Catechin and Diosgenin). As many as 10 compounds were classified as class 4 toxic compounds (harmful if swallowed), namely Dihydrokaempferol, Dihydropinosylvin, Demethylbatatasin IV, Batatasin I, Batatasin II, Batatasin III, Batatasin IV, Ferulic Acid, Naringenin, Taxifolin. While, 11 compounds belonging to class 5 toxic compounds (may be harmful if swallowed) namely Cyanidin, Nicotinamide/Vitamin B3, Cyanidin-3-O-glucoside, Ascorbic Acid, Cinnamic acid, p-Coumaric acid, Pelargonidin, Naringenin chalcone, Leucopelargonidin, Peonidin, Leucocyanidin.

Moreover, we try to predict the toxicity of each compound contained in *D. alata* L. through smaller parameters. Based on ADMETSar, Cinnamic acid was predicted to have Human Hepatotoxicity (H-HT) activity (Table 4). Hepatotoxicity is defined as an injury to the liver or impairment of the liver function caused by exposure to xenobiotics such as drugs, food additives, alcohol, chlorinated solvents, peroxidized fatty acids, fungal toxins, radioactive isotopes, environmental toxicants, and even some medicinal plants.[8] Other compounds that are predicted to potentially induce Drug-induced liver injury (DILI) (Table 4), are cyanidin, nicotinamide/vitamin B3, cyanidin-3-*O*-glucoside, ascorbic acid, ferulic acid, cinnamic acid, p-Coumaric acid, pelargonidin, naringenin, naringenin chalcone, batatasin I, dihydrokaemferol, peonidin, and taxifolin. DILI can be classified based on clinical presentation (hepatocellular, cholestatic, or mixed), mechanism of hepatotoxicity, or histological appearance from a liver biopsy that occur due to a natural or manufactured compound.[9] The toxicity prediction result based on Protox II (Table 5) nicotinamide/vitamin B3 and cinnamic acid compounds were predicted to have hepatotoxicity activity. Cyanidin, p-coumaric acid, leucopelargonidin, taxifolin, and leucocyanidin have carcinogenicity activity.

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Table 3: Lipinski's Rule of Five Results of *D. alata* L. Bioactive Compounds

Compounds	Class		Mol weight	Num. H-bond acceptors	Num. H-bond donors	Molecular refractivity	Log P	Violation	Qualify	Lipinski
Diosgenin	Steroid	Spirostan	414.31	3	1	121.6	5.71	0	5	Qualify
Cyanidin	Glycoside	Anthocyanin	287.06	6	5	76.17	2.91	1	4	Qualify
Nicotinamide/ Vitamin B3	Vitamin		122.05	3	2	32.33	0.88	0	5	Qualify
Cyanidin-3-O-glucoside	Glycoside	Anthocyanin	449.11	11	8	103.92	-0.01	2	3	Qualify
Ascorbic Acid	Vitamin		176.03	6	5	35.12	-1.41	1	4	Qualify
Ferulic Acid	Carboxylic Acids	Cinnamate	194.06	4	2	51.63	1.5	0	5	Qualify
Cinnamic acid	Carboxylic Acids	Cinnamate	148.05	2	1	43.11	1.78	0	5	Qualify
p-Coumaric acid	Carboxylic Acids	Cinnamate	164.05	3	2	45.13	1.49	0	5	Qualify
Dihydropinosylvin	Phenol	Benzenediol	214.1	2	2	64.75	2.88	0	5	Qualify
Demethylbatatasin IV	Organic aromatic	Stilbenoid	230.09	3	3	66.77	2.59	0	5	Qualify
Batatasin IV	Organic aromatic	Stilbenoid	244.11	3	2	71.24	2.89	0	5	Qualify
Batatasin III	Organic aromatic	Stilbenoid	244.11	3	2	71.24	2.89	0	5	Qualify
Pelargonidin	Glycoside	Anthocyanin	271.06	5	4	74.15	3.2	0	5	Qualify
Naringenin	Flavonoids	Flavonone	272.07	5	3	71.57	2.51	0	5	Qualify
Naringenin chalcone	Propiophenone	Chalcone	272.07	5	4	74.34	2.41	0	5	Qualify
Batatasin II	Organic aromatic	Stilbenoid	274.12	4	2	77.73	2.9	0	5	Qualify
Batatasin I	Organic aromatic	Stilbenoid	284.1	4	1	82.95	3.72	0	5	Qualify
dihydrokaempferol	Flavonoids	Dihyroflavonols	288.06	6	4	72.73	1.48	0	5	Qualify
Catechin	Flavonoids	Flavanol	290.08	6	5	74.33	1.55	1	4	Qualify
Leucopelargonidin	Flavonoids	Flavan 3,4-diols	290.08	6	5	73.47	1.33	1	4	Qualify
Peonidin	Glycoside	Anthocyanin	301.07	6	4	80.64	3.21	0	5	Qualify
Taxifolin	Flavonoids	Flavanol	304.06	7	5	74.76	1.19	1	4	Qualify
Leucocyanidin	Flavonoids	Hydroxyflavan	306.07	7	6	75.5	1.04	1	4	Qualify

Note: The red font color indicates the rule of 5 criteria that do not match

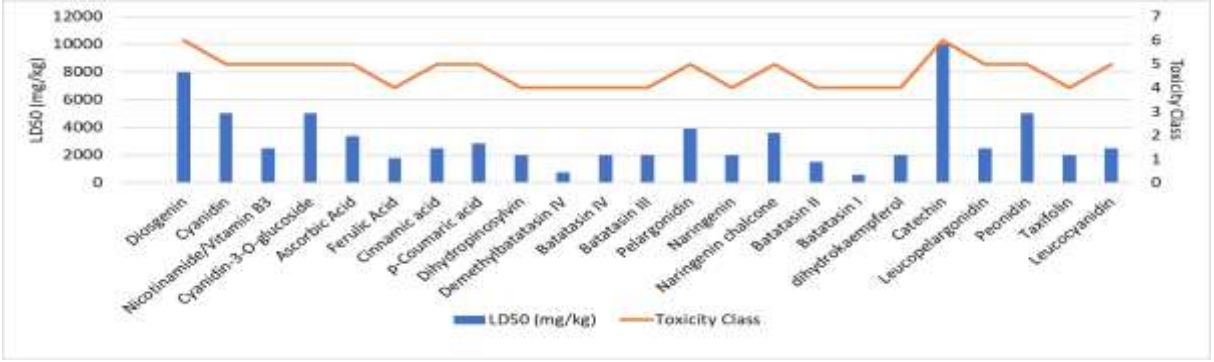

Figure 1: The Toxicity Prediction of *D. alata* L. Bioactive Compounds Based on LD50 and

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Table 4: ADMETlab Analysis to Determine Metabolism Potential and Toxicity of *D. alata* L. Bioactive Compounds

No	Compounds	Mol weight	AlogP	Num. H-bond acceptors	Num. H-bond donors	TPSA	LogS (log mol/L)	Pgp-inhibitor		Pgp-substrate		Human Intestinal Absorption		F (20% Bioavailability)		F (30% Bioavailability)		Blood-Brain Barrier		H-HT (Human Hepatotoxicity)		DILI (Drug Induced Liver Injury)		FDAMDD (Maximum Recommended Daily Dose)	
								Value	Prob	Value	Prob	Value	Prob	Value	Pro	Value	Prob	Value	Prob	Value	Prob	Value	Prob	Value	Prob
1	Diosgenin	414,31	5,556	3	1	38,69	-5,869	--	0,281	---	0,001	---	0,005	---	0,005	---	0,05	++	0,701	--	0,204	---	0,057	+	0,646
2	Cyanidin	287,06	2,895	6	5	112,45	-3,432	---	0,012	---	0,022	---	0,028	+++	0,997	+++	0,998	---	0,011	--	0,165	+++	0,969	++	0,811
3	Nicotinamide/Vitamin B3	122,05	-0,246	3	2	55,98	0,451	---	0	--	0,15	---	0,009	---	0,002	---	0,012	++	0,876	---	0,059	+++	0,95	---	0,022
4	Cyanidin-3-O-glucoside	449,11	0,007	11	8	191,6	-3,544	---	0,003	++	0,746	++	0,823	+++	0,93	+++	0,998	---	0,099	--	0,119	+++	0,968	---	0,042
5	Ascorbic Acid	176,03	-1,42	6	5	114,29	-0,613	---	0,001	---	0,089	---	0,069	+++	0,918	+++	0,978	---	0,073	--	0,168	+++	0,936	---	0,009
6	Ferulic Acid	194,06	1,803	4	2	66,76	-1,761	---	0	---	0,086	---	0,03	---	0,047	+	0,584	-	0,329	-	0,345	+	0,511	---	0,076
7	Cinnamic acid	148,05	2,363	2	1	37,3	-2,051	---	0	---	0,028	---	0,013	---	0,005	---	0,059	+	0,614	+	0,685	-	0,433	---	0,033
8	p-Coumaric acid	164,05	1,923	3	2	57,53	-1,363	---	0	---	0,017	---	0,009	---	0,004	--	0,194	--	0,29	+	0,673	--	0,2	---	0,031
9	Dihydropinosylvin	214,1	3,138	2	2	40,46	-2,758	--	0,234	---	0,011	---	0,039	+++	0,995	+++	0,992	--	0,116	--	0,155	---	0,016	++	0,845
10	Demethylbatatasin IV	230,09	2,804	3	3	60,69	-2,087	--	0,281	---	0,005	---	0,077	+++	0,999	+++	0,996	---	0,06	--	0,132	---	0,018	+	0,516
11	Batatasin IV	244,11	3,313	3	2	49,69	-2,802	--	0,176	---	0,004	---	0,006	+++	0,98	+++	0,983	---	0,086	---	0,083	---	0,023	++	0,738
12	Batatasin III	244,11	3,186	3	2	49,69	-2,973	-	0,358	---	0,006	---	0,008	+++	0,989	+++	0,986	---	0,072	--	0,167	---	0,021	+++	0,909
13	Pelargonidin	271,06	3,306	5	4	92,22	-3,367	---	0,014	---	0,047	---	0,014	+++	0,991	+++	0,996	---	0,011	--	0,126	+++	0,964	++	0,719
14	Naringenin	272,07	2,562	5	3	86,99	-3,876	---	0,007	---	0,001	---	0,018	+++	0,972	+++	0,997	---	0,042	---	0,098	++	0,853	--	0,177
15	Naringenin chalcone	272,07	2,957	5	4	97,99	-3,048	---	0,018	---	0,01	---	0,022	-	0,488	+	0,609	---	0,028	--	0,213	++	0,866	+	0,629
16	Batatasin II	274,12	2,926	4	2	58,92	-3,221	--	0,166	---	0,003	---	0,004	---	0,003	---	0,004	---	0,1	---	0,083	---	0,019	---	0,065
17	Batatasin I	284,1	3,7	4	1	47,92	-5,422	---	0,024	++	0,728	---	0,005	---	0,041	-	0,367	---	0,046	--	0,156	+++	0,931	+++	0,903
18	Dihydrokaempferol	288,06	0,853	6	4	107,22	-2,725	---	0,005	---	0,021	---	0,012	++	0,715	---	0,099	---	0,029	--	0,253	+++	0,94	--	0,201
19	Catechin	290,08	1,142	6	5	110,38	-2,99	---	0,007	---	0,002	---	0,096	+++	0,99	+++	0,999	---	0,025	---	0,071	--	0,109	---	0,082
20	Leucopelargonidin	290,08	0,551	6	5	110,38	-3,19	---	0,003	---	0,002	--	0,101	-	0,399	+++	0,978	---	0,078	---	0,043	--	0,15	---	0,023
21	Peonidin	301,07	3,193	6	4	101,45	-3,498	---	0,009	-	0,305	---	0,012	+++	0,951	+++	0,984	---	0,008	--	0,114	+++	0,963	++	0,859
22	Taxifolin	304,06	0,449	7	5	127,45	-2,662	---	0,005	---	0,016	---	0,014	---	0,892	+	0,678	---	0,031	--	0,176	+++	0,943	-	0,423
23	Leucocyanidin	306,07	0,021	7	6	130,61	-3,174	---	0,003	---	0,002	--	0,213	++	0,828	+++	0,995	---	0,049	---	0,053	--	0,265	---	0,032

Note: The red color indicates the rule of 5 Lipinski criteria that do not match

The yellow color indicates the compounds that must be aware of their toxicity activity in a single compound

The green color indicates the absorption and bioavailability values are not good

Prob is a probability value (If the value is closer to 1 it means a higher confidence score)

+ Value shows significant activity

- Value indicates activity that is not significant

Carcinogenicity is the tendency of some compounds to cause cancer (carcinogen). These compounds could reduce the time for tumor occurrence.[10] Carcinogenicity is closely related to mutagenicity. Mutagenicity is a genetic mutation induced by a chemical or drug substance. Detection of mutagenicity at the preclinical drug discovery stage is important to stop the development of potentially dangerous drugs and assist the development of safe therapeutic agents.[12] In this study, batatasin I and taxifolin were predicted to have mutagenicity activity. Other compounds including diosgenin, cyanidin-3-O-glucoside, ferulic acid, naringenin chalcone, batatasin I, and leucocyanidin were predicted to have immunotoxicity activity. Immunotoxicity occurs due to exposure to toxic substances that cause a decrease in the function of the immune system.[13] The last, naringenin was predicted to have cytotoxicity activity. Cytotoxicity is toxicity to living cells caused by drug substances. Cytotoxicity tests are very important in nanoparticles as they help in the determination of proposed biomedical uses.[13] Hoskins found that Cinnamic acid has low toxicity.[29] Its molecular structure and the known toxicity of similar molecules, such as styrene, have brought it to the toxicologist's attention. Furthermore, based on previous research, p-Coumaric acid is considered a safe compound because of its very low toxicity.[30] Leung et al. explained that vitamin B3 (niacin) induces direct hepatotoxicity, but little is known about its mechanism.[31] Previous studies report that Ferulic acid, Naringenin, Leucopelargonidin, Leucocyanidin, and Diosgenin have low toxicity and many physiological functions.[32–35] There have been no reports about the toxicity of cyanidin, cyanidin-3-O-glucoside, ascorbic acid, pelargonidin, naringenin chalcone, batatasin I, dihydrokaempferol, peonidin, taxifolin. A recent study only found their medicinal functions. Therefore, although the compounds contained in *D. alata* L. were suspected to have H-HT, DILI, hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity activity, their toxicity was low.

Conclusion

This study proved that most of the active compounds in *D. alata* L. showed very low toxicity, with only 2 non-toxic compounds (Diosgenin and Catechin) based on LD-50 value. The use of some compounds in the single compound form was predicted to have some H-HT, DILI, hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity activity, even though their toxicity was low. Therefore, it is important to carry out further research to prove the prediction of toxicity of each bioactive compound contained in *D. alata* L. *in vivo*.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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