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Insilico Toxicity Prediction of Bioactive Compounds of *Eurycoma longifolia* Jack.

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ABSTRACT

Pasak bumi (*Eurycoma longifolia* Jack.) has been used by the community for a long time and has proven its effectiveness in overcoming male sexual arousal disorders. Therefore, a toxicity test is needed to ensure its safety. To minimize the use of test animals, a screening method through computation is needed. In this study, Toxtree software was applied to predict Cramer rule while carcinogenicity (genotox-non genotox) and mutagenicity used ISS rules. In addition, ISS in vitro mutagenicity (Ames test) by ISS, skin irritation-corrosion, and eye irritation-corrosion. ProTox predicts hepatotoxicity, immunotoxicity, reproductive toxicity and LD50 in rodents. pKSCM predicts heRG II inhibitors, Max. tolerated dose (human), chronic toxicity to rodents. Based on Cramer's rules, the chemical contents of *E. longifolia* were High (class III). The next step was to predict the potential toxic compound using comparative Cramer rules, LD50 and scoring system. Based on the results, The conclusion of this study was that the predictions of 9 toxic compounds were Eurycomalactone, n-Pentyl beta-carboline-1-propionate, Melianone, 9-Methoxycanthin-6-one, 5-Methoxycanthin-6-one, 10-Methoxycanthin-6-one, Picrasidine Q, Eurycomalide B, 9-*Methoxycanthin-6-one 3-N-oxide.*

Keywords: *Eurycoma longifolia* Jack, toxicity prediction, Cramer's rule, scoring

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INTRODUCTION

Pasak Bumi (*Eurycoma longifolia* Jack) has been widely known by the public, especially for the people in Sumatra and Kalimantan islands, Indonesia. *E. longifolia* has long been used for generations by the community to increase sexual arousal in men (aphrodisiac). *E. Longifolia* based on many studies can increase male stamina because it contains some minerals and secondary metabolites. Compounds from *E. longifolia* play a role in the synthesis of testosterone, increase androgenic ability and libido. Minerals play a role in spermatozoa maturation (1). Metabolites of the steroid group have a role in increasing testosterone in Leydig cells, especially microsomal enzymes (2). Apart from being an aphrodisiac, another use is to improve the performance of sports athletes without disturbing liver and kidney function under the supervision of the International Olympic Committee Medical Commission (3). This plant has also been evaluated to reduce stress, anger, confusion by decreasing cortisol and increasing testosterone (4).

Beside effectiveness evaluation, a toxicological test also has a povital rule to determine the level of safety. Toxicity testing usually uses test animals to obtain factual values. However, the use of test animals must meet some requirements and go through some processes, starting with the ethical clearance process, preparation of test animals and conducting tests and finally data analysis. In this stage, disturbances sometimes arise, for example, animals experience death or illness caused by other factors, not just the factor of administering the wrong sample test. Therefore, another approach is needed to carry out toxicity tests, namely with certain software or webservers. This study was conducted to predict the toxicity of compounds consisting of *E. longifolia* using computational methods.

MATERIALS AND METHODS

Tools and Materials

The equipment used was hardware, namely a laptop with 2Gb ram, software was Marvin Bean, Toxtree, webserver, namely pKSCM and ProTox. Marvin Beans.

The ingredients used was compounds from *E. longifolia* with the help of the Knapsack database, namely Canthin-6-one, Eurycomalactone, n-Pentyl beta-carboline-1-propionate, Picrasidine L, Picrasidine O, beta-Carboline-1-propionic acid, Eurycomaoside, Melianone, 7-Methoxy-beta-Carboline 1-Propionic acid, 9-Methoxycanthin-6-one, Eurycolactone E, Eurycolactone F, Niloticin, 5-Methoxycanthin-6-one, 10-Methoxycanthin-6-one, Methyl beta -Carboline-1-carboxylate, Picrasidine Q, 14,15beta-Dihydroxyklaineanone, 6alpha-Hydroxyeurycolactone E, 6alpha-Hydroxyeurycomalactone, 7alpha-Hydroxyeurycomalactone, Ailanquassin A, Eurycomalide A, Eurycomalactone A, 9-6-Meoxycolactone 3-N-oxide, Longilene peroxide.

Ways of working

2D format of 28 *E. longifolia* compounds were prepared in Marvin Bean (ChemAxon, 2016) and stored in mol2 or sdf form. After that, converted mol2 into a canonical smile to be included in the program. The first program was Toxtree software to predict cramer rule, carcinogenicity (genotox-non genotox) and mutagenicity with ISS rules, in vitro mutagenicity (Ames test) by ISS, skin irritation-corrosion, and eye irritation-corrosion. ProTox predicted hepatotoxic, immunotoxic, reproductive toxic and LD50 in rodents while pKSCM predicted heRG II inhibitors, Max. tolerated dose (human), chronic toxicity to rodents.

Data analysis

Data analysis was displayed with a positive score of 1 and a negative score of 2, as well as the predictive value of the test animals that used to assess the score of each compound while docking scores, cramer rules were compared with the LD50 value (5)

RESULTS AND DISCUSSION

The contents of *E. Longifolia* from Knapsak, all of them show a high toxicity category according to the cramer rules of Toxtree with a code of High (Class III), so that long-term large use cannot be guaranteed safety due to the compounds are carcinogenic and mutagenic by the presence of heterocyclic rings, ,β unsaturated carbonyls, epoxides and aziridines. Compounds have a lactone or cyclic diester and a lactone

joined in a ring so that it becomes an ,β unsaturated lactone for example Eurycomalactone, Eurycolactone F, 14,15beta-Dihydroxyklaineanone, 6alpha-Hydroxyeurycomalactone, 7alpha-Hydroxyeurycomalactone, Eurycomalide A, Eurycomalactone A, Laurycomalactone (6-8). Presence of heterocyclic polycyclic arom atic hydrocarbons e.g. Canthin-6-one, n-Pentyl beta-carboline-1-propionate, Picrasidine L, Picrasidine O, beta-Carboline-1-propionic acid, 7-Methoxy-beta-Carboline 1-propionic acid, 9-Methoxycanthin-6-one, 5-Methoxycanthin-6-one, 10-Methoxycanthin-6-one ,Methyl beta-Carboline-1-carboxylate, Picrasidine Q, 9-Methoxycanthin-6-one 3-N-oxide. There is also the presence of epoxides and aziridines such as Melianone Niloticin (9, 10). Compounds that are not at risk of causing carcinogenic and mutagenic only Eurycomaoside, Eurycolactone E, 6alpha-Hydroxyeurycolactone E, Ailanquassin A, Longilene peroxide.

While in prediction of skin irritation and corrosion tests, some compounds are at risk for this disorder due to they have O=CO and lactone groups for example Eurycomalactone, Eurycomalide B, Laurycolactone A. Other examples have O=CN groups and aromatic amines such as Picrasidine O and picrasidine L (11-13). For the prediction of toxicity to the eye, it shows its safety, so the content of this plant does not cause eye irritation. Prediction of hepatotoxicity occurs only in one compound, namely Methyl beta-Carboline-1-carboxylate. For immunotoxicity occurs in Eurycomalactone, n-Pentyl beta-carboline-1-propionate, Eurycomaoside Melianone, 7-Methoxy-beta-Carboline 1-propionic acid, 9-Methoxycanthin-6-one, Eurycolactone E, Eurycolactone F, Niloticin, 5- Methoxycanthin-6-one, 10-Methoxycanthin-6-one, Picrasidine Q, 14,15beta-Dihydroxyklaineanone, 6alpha-Hydroxyeurycolactone E, 6alpha-Hydroxyeurycomalactone, 7alpha-Hydroxyeurycomalactone, Ailanquassin A, Eurycomalactone A, 9-Methoxycanthin-6-one 3-N-oxide . Toxicity to reproductive organs occurs in Melianone, Niloticin, Ailanquassin A (14, 15). Prediction of cardiac toxicity with the hERG II inhibitor approach occurs in n-Pentyl beta-carboline-1-propionate, 9-Methoxycanthin-6-one 3-N-oxide, Longilene peroxide (16). Because ProTox and pKSCM use a webserver, the explanations regarding hepatotoxic, immunotoxic, reproductivetoxic and cardiac toxicity are the results of machine learning. The rules for calculating the score are showed in Table 1 while The Toxicity Prediction Using Toxtree, Protox dan pKSCM can be seen in Table 2.

Table 1: The rules for calculating the score

positif	:1
negatif	:2
Carcinogenicity genotox Toxtree	:A
Carcinogenicity non genotox Toxtree	:B
In vitro mutagenicity (Ames test) Toxtree	:C
skin irritation/corrosion Toxtree	:D
eye irritation/corrosion Toxtree	:E
hepatotoxicity ProTox	:F
imunotoxicity ProTox	:G
reproductivetoxicity ProTox	:H
hERG II inhibitor pkSCM	:I
Max. tolerated dose (human) (log mg/Kg day) pKSCM	:J
kronik oral rodent (log mg/kg/day) pKSCM	:K

The LD50 from the Protox webserver was used to evaluate the toxicity category of the compound *E. longifolia* (17) in Figure 1. Category I is fatal if ingested with LD50 requirements < 5 mg/Kg BW in this study not present. Category II is also fatal if swallowed, the requirements are 5 < LD50 < 50 mg/Kg BW, which includes this category are Eurycolactone F, 6alpha-Hydroxyeurycolactone E, Ailanquassin A. Category III is toxic if swallowed 50 < LD50 < 300 mg/Kg BW, included in this category are beta-Carboline-1-propionic acid, Eurycomaoside, 7-Methoxy-beta-Carboline 1-propionic acid, Eurycomalide B. Category IV is dangerous if swallowed 300 < LD50 < 2000, which is included in this category namely Canthin -6-one, Eurycomalactone, n-Pentyl beta-carboline-1-propionate, Picrasidine L, Picrasidine O, Melianone, 9-Methoxycanthin-6-one, 5-Methoxycanthin-6-one, 10-Methoxycanthin-6-one, Methyl beta-Carboline-1-carboxylate, Picrasidine Q, 6alpha-Hydroxyeurycomalactone, 7alpha-Hydroxyeurycomalactone, Eurycomalide A, Longilactone, 9-Methoxycanthin-6-one 3-N-oxide. Category V may be harmful if

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swallowed 2000 < LD50 < 5000, which includes Eurycolactone E, 14,15beta-Dihydroxyklaineanone, Laurycolactone A, Longilene peroxide. Category VI is not harmful if swallowed. LD50 > 5000 i.e. Niloticin.

Table 2: The Toxicity Prediction Using Toxtree, Protox dan pKSCM

Ligand Name	A	B	C	D	E	F	G	H	I	J	K	Scoring
Canthin-6-one	1	2	1	2	2	2	2	2	2	-1,139	1,641	16,502
Eurycomalactone	1	2	1	1	2	2	1	2	2	-0,285	2,371	16,086
n-Pentyl beta-carboline-1-propionate	1	2	1	2	2	2	1	2	1	0,163	1,651	15,814
Picrasidine L	1	2	1	1	2	2	2	2	2	0,047	2,181	17,228
Picrasidine O	1	2	1	1	2	2	2	2	2	0,054	2,018	17,072
beta-Carboline-1-propionic acid	1	2	1	2	2	2	2	2	2	1,086	1,454	18,54
Eurycomaoside	2	2	2	2	2	2	1	2	2	0,015	3,135	20,15
Melianone	1	2	1	2	2	2	1	1	2	-0,874	1,374	14,5
7-Methoxy-beta-Carboline 1-propionic acid	1	2	1	2	2	2	1	2	2	1,109	1,438	17,547
9-Methoxycanthin-6-one	1	2	1	2	2	2	1	2	2	-0,950	1,468	15,518
Eurycolactone E	2	2	2	2	2	2	1	2	2	0,095	2,703	19,798
Eurycolactone F	1	2	1	2	2	2	1	2	2	-0,070	2,374	17,304
Niloticin	1	2	1	2	2	2	1	1	2	-0,727	1,718	14,991
5-Methoxycanthin-6-one	1	2	1	2	2	2	1	2	2	-0,113	1,560	16,447
10-Methoxycanthin-6-one	1	2	1	2	2	2	1	2	2	-0,801	1,634	15,833
Methyl beta-Carboline-1-carboxylate	1	2	1	2	2	1	2	2	2	0,550	2,157	17,707
Picrasidine Q	1	2	1	2	2	2	1	2	2	0,098	0,585	15,683
14,15beta-Dihydroxyklaineanone	1	2	1	2	2	2	1	2	2	0,251	3,660	18,911
6alpha-Hydroxyeurycolactone E	2	2	2	2	2	2	1	2	2	-0,385	2,822	19,437
6alpha-Hydroxyeurycomalactone	1	2	1	2	2	2	1	2	2	-0,600	2,627	17,027
7alpha-Hydroxyeurycomalactone	1	2	1	2	2	2	1	2	2	-0,201	2,265	17,064
Ailanquassin A	2	2	2	2	2	2	1	1	2	0,199	2,192	18,391
Eurycomalide A	1	2	1	2	2	2	1	2	2	-0,056	2,098	17,042
Eurycomalide B	1	2	1	1	2	2	1	2	2	-0,047	2,093	16,046
Laurycolactone A	1	2	1	1	2	2	1	2	2	0,202	2,928	17,13
Longilactone	1	2	1	2	2	2	1	2	2	0,202	2,928	18,13
9-Methoxycanthin-6-one 3-N-oxide	1	2	1	2	2	2	1	2	1	-1,053	0,568	13,515
Longilene peroxide	2	2	2	2	2	2	2	2	1	-0,442	0,470	17,028

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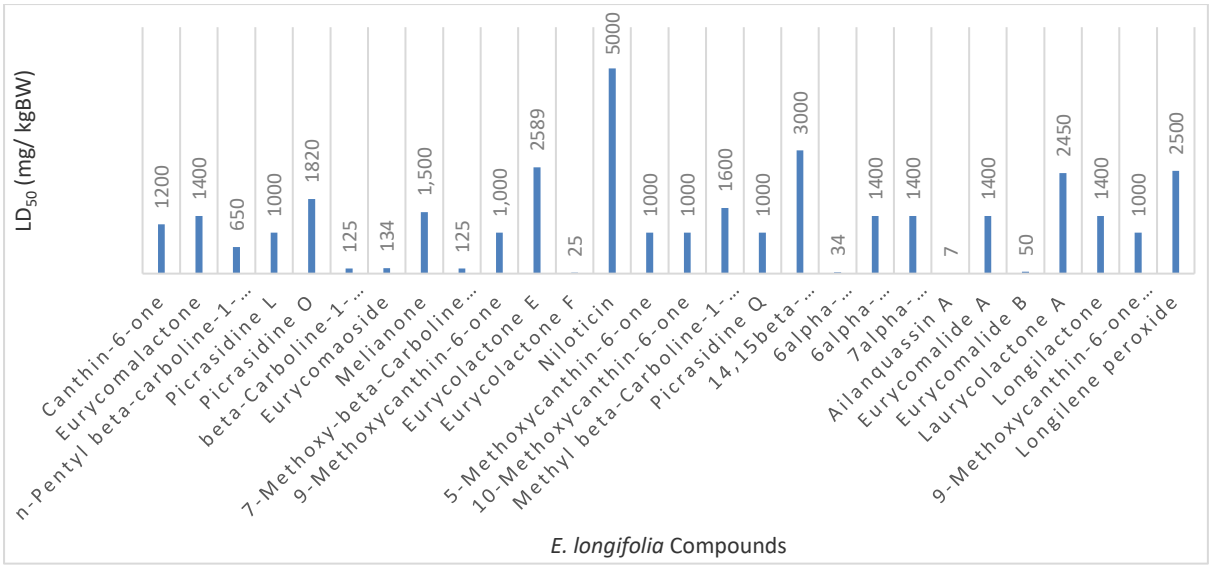

Figure 1: Predicted LD_{50} of *E. longifolia* compounds in rats

For further analysis, Venn diagram is used to see potential toxic compounds as showed in Figure 2 by comparing Cramer's rules, LD50 of Protox and docking scores (18) From the Cramer rules, there are 28 compounds with toxic risk, from Protox 23 compounds and a score of 10 compounds. In the final stage, 9 compounds at risk were obtained, namely Eurycomalactone, n-Pentyl beta-carboline-1-propionate, Melianone, 9-Methoxycanthin-6-one, 5-Methoxycanthin-6-one, 10-Methoxycanthin-6-one, Picrasidine Q , Eurycomalide B, 9-Methoxycanthin-6-one 3-N-oxide.

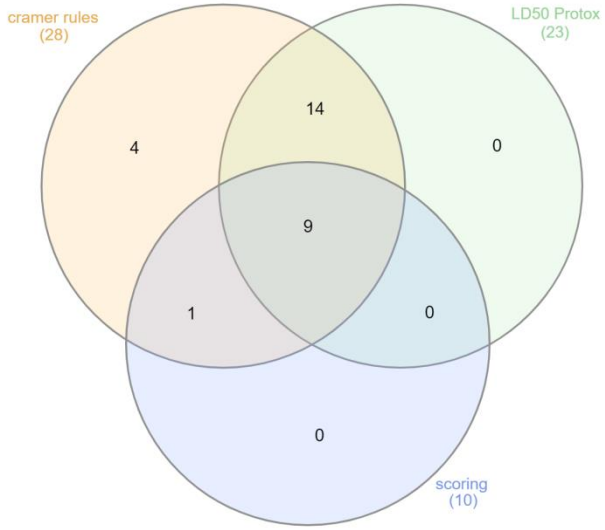

Figure 2: Venn Diagram from Cramer's rules, LD_{50} ProTox and docking scores

CONCLUSION

In conclusion, the potentially toxic compounds of *E. Longifolia* are Eurycomalactone, n-Pentyl beta-carboline-1-propionate, Melianone, 9-Methoxycanthin-6-one, 5-Methoxycanthin-6-one, 10-Methoxycanthin-6-one, Picrasidine Q, Eurycomalide B, 9-Methoxycanthin-6-one 3-N-oxide.

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