Nusantara Hasana Journal
Volume 5 No. 4 (September 2025), Page: 173-181
E-ISSN: 2798-1428

IN SILICO STUDY INVESTIGATING TO KEAP1-KELCH INHIBITORY POTENTIAL OF *PLUCHEA INDICA* LEAVES BIOACTIVE COMPOUNDS

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Abstract
Pluchea indica (beluntas) is a herbal drug candidate that has a high antioxidant capacity. This research reports the analysis of ligand-protein interactions, drug-like properties, biological activity, and prediction of ADMET profile of bioactive compounds contains in P. indica leaves as antioxidants. The interaction of the ligand-KEAP1 resulted in the lowest binding affinity value for the stigmasterol compound with a weight of -12.1 Kcal/mol with several chemical bonds formed and produced amino acid residues. Analysis of drug-like properties using SwissADME showed that P. indica complied with all parameters of Lipinski's rule. Biological activity with online PASS test showed high biological activity as an antioxidant. Finally, prediction of ADMET profile using admetSAR showed that P. indica had good pharmacological criteria.
Keywords: ADMET, Antioxidant, In silico, Pluchea indica

INTRODUCTION

Pluchea indica (beluntas) is one of the medicinal plants that has been known for a long time by the public, but its use as a medicine has not been carried out optimally. The content of phytochemical compounds in *P. indica* leaves has antioxidant activity because of large amounts of phenolic compounds. The phenolic compounds in *P. indica* leaves, such as caffeoylquinic acid derivatives, may be responsible for the antioxidant and anti-alpha glucosidase activity (Vongsak et al., 2018). Flavonoids content in *P. indica* are included in a large group of polyphenol antioxidants consisting of anthocyanidins, flavones, catechins, flavanones, flavones, and flavonols (Hudha, 2014). Andarwulan et al. (2010) stated that the activity to radical scavenging in *P. indica* extract was higher than in other vegetables. Triggering antioxidant enzymes such as peroxidase, catalase, and SOD (superoxidase dismutase) in human body plays an essential role in the response to oxidative stress causing inflammation and regulatory mechanism (Dayalan et al. al., 2020; Panieri et al., 2020).

Oxidative stress can activate various transcription factors, including nuclear factor kappa-B (NF-κB), p53, HIF-hypoxia inducible factor 1α (HIF-1α), peroxisome proliferator-activated receptor (PPAR-γ), erythroid 2-related nuclear factor protein (Nrf2), and catenin/Wnt (Dayalan et al., 2020). Nrf2 is critical in the antioxidant response through the Nrf2/KEAP1 pathway, whereas KEAP1 is the inhibitor of Nrf2. The complex of Nrf2-KEAP1 is present in cytoplasm. Under oxidative stress, Nrf2 dissociated from the complex and translocate to nucleus with electrophiles stabilize in it (Canning et al., 2015; Shan et al., 2015). The free form of Nrf2 will activate the antioxidant genes such as SOD, catalase, and peroxidase (David et al, 2017). The inhibitory interaction between Nrf2/KEAP1 is one of the appropriate antioxidant targets to prevent that are responsible to exogenous or endogenous oxidative strees causing inflammation and metabolic disease (Abed et al., 2015; Xing et al., 2015).

Recently, the development of drug candidates based on medicinal plant products

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as antioxidants has led to a lot of research directed at drug design and discovery through computational studies. Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) is a term that describes the pharmacokinetic characteristics and toxicity of drugs or compounds while in the human body. ADMET information provides a profile of drug candidates and is used to design drugs effectively and safely. In addition, modeling the interaction between compounds and proteins can also be used for initial screening in predicting the biological activity in cells (Bare et al., 2020). In this in silico study, the bioactive compound of *P. indica* leaves were investigated as KEAP1 inhibitor and determined the ADMET profile.

METHODE

Secondary metabolites of *P. indica* leaves were obtained from KNApSAcK-3D (http://www.knapsackfamily.com/knapsack_core/top.php), downloaded from the PubChem database and then modified (minimize energy and conversion in PDB format) using PyMOL software (Dallakyan & Olson, 2015). In addition, KEAP1 protein as a target antioxidant protein was downloaded from the PDB database with an access code of 5cgj and interacted with four bioactive compounds using PyRx version 0.8 using vina wizard (Dallakyan & Olson, 2014). Docking keap1-nrf2 using Cluspro 2.0 to see the binding side of Nrf2 on Keap1.The Discovery Studio 2016 observing include bond affinity (kcal/mol), the 3D structure of the ligand-protein bond complex, amino acid residues bound to ligands and the 2D structure of the ligand-protein complex. The PASS test was carried out online at http://way2drug.com/PassOnline/predict.php to determine the potential antioxidant activity and free radical scavengers of the secondary metabolite of *P. indica* leaves. Pharmacokinetic and toxicity were carried out using SwissADME (http://swissadme.ch/) and admetSAR (http://lmmd.ecust.edu.cn/admetsar1/predict/). The prediction of bioactivity of secondary metabolites was calculated by molinspiration (http://www.molinspiration.com/cgibin/properties).

RESULT AND DISCUSSION

The study of the mechanisms underlying KEAP1 plays an essential role in developing antioxidant agents. KEAP1 consists of two canonical domains: an N-terminal BTB and a C-terminal DC (or Kelch) domain, linked by an intervening region (IVR) (Itoh et al., 1999). Keap1 contains highly conserved reactive cysteine residues that act as electrophilic sensors responding to endogenous and exogenous ROS. Several studies have shown that KEAP1 is a basic unit of the Keap1-Nrf2 system that protects cells from oxidative damage by sensing oxidative stress to regulating Nrf2 activity (Itoh et al., 1999). Nrf2 plays an essential role in maintaining the redox homeostasis of cells by regulating the expression of cytoprotective proteins. The activity of Nrf2 is strongly influenced by its interaction with Kelch-like ECH-associated protein 1 (Keap1) (Taguchi, 2011).

In this study, four active compounds of *P. indica*, including stigmasterol, lirioresinol B, pinoresinol, and plucheoside A, were obtained from the results of KNApSACk-3D. The analysis of drug-like properties (drug-likeness) was carried out based on Lipinski's rule of five, which stated that a compound has properties similar to drugs if the molecular weight (MW) of the compound is less than 500 Dalton (g/mol), the partition coefficient value log P less than 5, the number of hydrogen bond donors (hydrogen bond donors) is less than 5, and the number of hydrogen bond acceptors is less than 10 (Lipinski, 2000). The four active compounds *P. indica* leaves met all of Lipinski's criteria, namely BM 250.33-418.44 g/mol, HBA <10, and HBD had values of 1 and 2.

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MlogP in lirioresinol B, pinoresinol, and plucheoside A met the criteria, namely MLogP value < 5, but MLog P stigmasterol did not meet the criteria of 6.62 (Table 1).

Much of the bioavailability of a drug is dependent on its solubility and ability to cross the intestinal membranes, and this in turn relates to the physicochemical properties of a compound such as water solubility, LogP, number of rotatable bonds, nonpolar surface area, etc. The compounds that fail to comply with the famous Lipinski's rule of five and the Verber's rules generally have poor pharmacokinetic properties. Such drugs may show poor absorption, faster rate of metabolism and excretion, unfavorable distribution, and might be toxic in nature. The drug likeness of a compound can easily be predicted by filtering the compounds based on the Lipinski's rule of five (Mandlik et al., 2016).

Table 1. The chemical properties of the active compound of *P. indica*

Active compound	MW (g/mol)	TPSA (Å²)	HBA	HBD	Rotatable Bond	MLogP	Water solubility
Stigmasterol	412,69	20.23	1	1	5	6,62	Moderately soluble
Lirioresinol B	418.44	95.84	8	2	6	1,93	Soluble
Pinoresinol	358,39	77,38	6	2	4	2,04	Soluble
Plucheosida A	250,33	46,53	3	1	0	2,47	Soluble

Based on the results of pharmacokinetic predictions, the four compounds had positive values for HIA and HOB (Table 2). These results indicated an excellent absorption in the human body and a reasonable absorption rate in the human intestine. This also showed that all these compounds could spread throughout the body as antioxidants when they enter the human body. Furthermore, the distribution prediction results for the four active *P. indica* had sound blood-brain barrier (BBB+) ability. Good distribution required good BBB distribution capability. The BBB parameter is the ability that allowed blood vessels to vascularize the central nervous system, which regulated the movement of ions, molecules, and cells between the blood and the brain (Daneman and Prat, 2015). Prediction of distribution with inhibitory parameters and substrates of P-glycoprotein (Pgp) is essential because P-glycoprotein is one of the drug transporters that determined the absorption and release of various drugs. The all compounds content of *P. indica* were non-substrate (except pinoresinol) and non-inhibitor to P-glycoprotein. Furthermore, the carcinogenicity profile showed non-carcinogenic properties with acute oral toxicity, safe with acute oral toxicity category III, but stigmasterol belongs to group I.

Table 2. Prediction of the toxicity of the active compound of *P. indica* leaves

Active compound	HIA	BBB	inhibition/substrate of CYP	Carcinogenicity	Acute oral toxicity
Stigmasterol	+	+	Non-substrate Non-inhibitor	Non-carcinogen	I
Lirioresinol B	+	+	Non-substrate Non-inhibitor	Non-carcinogen	III
Pinoresinol	+	+	Substrate Non-inhibitor	Non-carcinogen	III
Plucheosida A	+	+	Non-substrate Non-inhibitor	Non-carcinogen	III

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Predictions of bioactive were shown in Table 3. In this term, a score of more than 0.0 indicated high activity, 0.0 to -0.5 indicated moderate activity, and less than -0.5 indicated inactivity (Paramashivam et al., 2015). Based on the predictions of bioactive results, stigmasterol, pinoresinol, and plucheoside A had high activity, whereas lirioresinol B had low activity as G protein-coupled receptor ligands. In terms of ion channel modulators, plucheoside A showed high activity, while lirioresinol B, stigmasterol, and pinoresinol showed moderate activity. Lirioresinol B, stigmasterol, and pinoresinol had the moderate ability as kinase inhibitors, while plucheoside A had low activity. Stigmasterol, pinoresinol, and plucheoside A also had high nuclear receptor ligands, while lirioresinol B had a moderate activity value. All active compounds in *P. indica* were found as protease inhibitors with moderate activity, and all active compounds content of *P. indica* also had high biological activity as enzyme inhibitors. It can be concluded that all active compounds content of *P. indica* had good potency in all of these parameters.

Table 3. Prediction bioactivity of the active compound of *P. indica*

Active compound	GCPR	ICM	KI	NRL	PI	EI
Stigmasterol	0,12	-0,08	-0,48	0,74	-0,02	0,53
Lirioresinol B	-0,01	-0,23	-0,17	-0,01	-0,14	0,08
Pinoresinol	0,01	-0,26	-0,21	0,02	-0,17	0,07
Plucheosida A	0,26	0,08	-0,61	0,68	-0,23	0,82

GPCR: G protein-coupled receptor ligands; ICM: ion channel modulators; KI: kinase inhibitor; NRL: nuclear receptor ligands; PI: protease inhibitor; EI: enzyme inhibitor

Based on the prediction results of the PASS test, all active compounds in *P. indica* leaves had the potential as antioxidants (Table 2). The stigmasterol and plucheoside A have Pa value >0.8. The compound has high activity if the Pa value is >0.7 (Chelliah, 2008). PASS determined various biological activities based on the Structure-Activity Relationship (SAR) or the relationship between the structure of a compound and its biological activity (Filimonov et al., 2014).

Table 4. The biological activity and Pa value of the bioactive compound of *P. indica* leaves based on the PASS test

Active compound	Pa	Biological activity
Stigmasterol	0.970	Antihypercholesterolemic
	0.965	Cholesterol antagonist
	0.933	Oxidoreductase inhibitor
	0.915	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
	0.913	Prostaglandin-E2 9-reductase inhibitor
	0.910	Alkenylglycerophosphocholine hydrolase inhibitor
	0.902	CYP3A4 substrate
	0.900	Hypolipemic
	0.900	DELTA14-sterol reductase inhibitor
	0.898	Alkylacetylglycerophosphatase inhibitor
	0.866	CYP3A substrate
	0.872	Alcohol O-acetyltransferase inhibitor
	0.869	CYP7 inhibitor

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	0.868	Acylcarnitine hydrolase inhibitor
Lirioresinol B	0.865	Feruloyl esterase inhibitor
	0.871	Aspulvinone dimethylallyltransferase inhibitor
	0.824	Antineoplastic
	0.810	JAK2 expression inhibitor
Pinoresinol	0.891	Feruloyl esterase inhibitor
	0.881	Aspulvinone dimethylallyltransferase inhibitor
	0.867	JAK2 expression inhibitor
	0.832	Membrane integrity agonist
	0.804	Cardiovascular analeptic
	0.814	Chlordecone reductase inhibitor
Plucheosida A	0.916	Cytostatic
	0.914	CDP-glycerol glycerophosphotransferase inhibitor
	0.892	Antineoplastic
	0.883	Antiprotozoal (Leishmania)
	0.886	Alkenylglycerophosphocholine hydrolase inhibitor
	0.879	CYP2H substrate
	0.873	Analeptic
	0.855	Antihelmintic (Nematodes)
	0.846	Antifungal

Molecular docking is used to predict the molecular mechanism between the ligand as an antioxidant agent and KEAP1. The binding energy obtained from the docking results indicated that the bond strength between the ligand and KEAP1. Binding energy is the strength of the interaction between two or more molecules. Binding affinity is the value of the strength of the interaction between the protein and the ligand which increases the lower the value. These interactions consist of hydrogen bonds, electrostatic interactions, van der Waals interactions, and hydrophobic interactions (Hanif et al., 2021). The greater the binding energy value, the lower the affinity between the receptor and the ligand. On the other hand, the lower the binding energy value, the higher the affinity between the receptor and the ligand (Kastritis and Bonvin, 2012). The docking results showed that the stigmasterol has the lowest binding affinity value of -12.1 Kcal/mol, whereas the liriosterol has the highest binding affinity value of -8 Kcal/mol (Table 1).

The interaction formed between the four *P. indica* compounds and KEAP1 protein contributes to the strength of the bonds indicated by the binding affinity, in addition to the high and low hydrophobicity, the role of donor/acceptor in ligand-protein (Chen et al., 2016). A low-affinity value will strengthen the interaction between the ligand and protein. Strong ligand and protein interactions stabilize the ligand-protein complex.

Table 5. The value of binding affinity and amino acid residue of the ligand-KEAP1

Complex Ligand-Protein	Binding Affinity (Kcal/mol)	Residue
Stigmasterol-KEAP1	-12.1	VAL (A:467), ARG (A:470), VAL (A:514), VAL (A:608), THR (A:560), VAL (A:420), GLY (A:325)
Lirioresinol B-KEAP1	-8	VAL (A:604), CYS (A:513), ILE (A:559), VAL (A:463)

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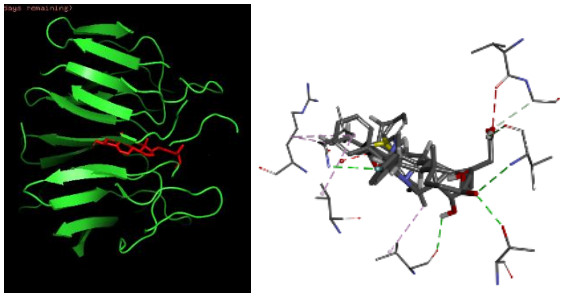
Pinoresinol-KEAP1	-9	CYS (A:368), VAL (A:418), THR (A:560), VAL (A:512), ILE (A:559), VAL (A:604), LEU (A:365)
Plucheosida A-KEAP1	-9.3	PHE (A:577), TYR (A:572), SER (A:602), SER (A:363), SER (A:508), ARG (A:483)

The molecular docking result include binding affinity values, chemical bonds formed, and amino acid residues. Based on the visualization results in 2D, several bonds that formed from each compound are unfavorable bump, alkyl, unfavorable donor-donors, conventional hydrogen bonds, carbon-hydrogen bonds, unfavorable acceptor-acceptors, unfavorable donors, pi-cation, pi-donor hydrogen bond, pi-pi stacked, pi-sigma, and pi-alkyl (Figure 1). The results of KEAP1-Nrf2 protein docking showed different binding sites with the four compounds in *P. indica*. The binding of four *P.indica* compounds indicated that these compounds acted as antioxidants by interacting with KEAP1. Stigmasterol, lirioresinol B, pinoresinol and plucheoside A compounds contained in *P. indica* are predicted to have potential as Keap1 protein inhibitors. The four compounds that bind to the KEAP1 protein may prevent the Nrf2 protein from binding to its active site. The release of Nrf2 from the Nrf2-KEAP1 complex stimulates the reduction of ROS in cells to achieve cell homeostasis (Canning et al., 2015).

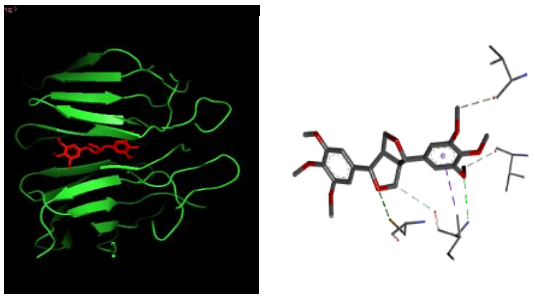
Stigmasterol

A B



Lirioresinol B

A B



Pinoresinol

A B

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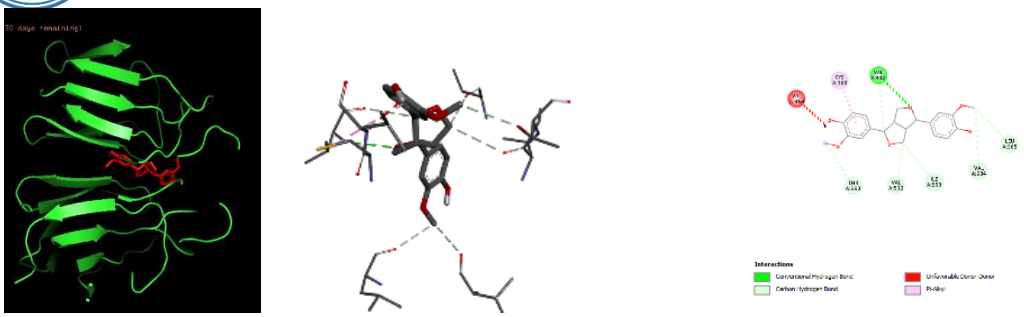
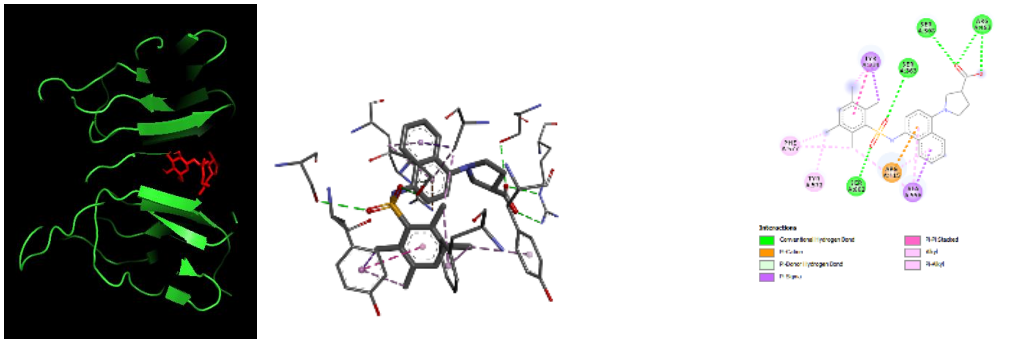


Plucheosida A

A B



KEAP1-Nrf2

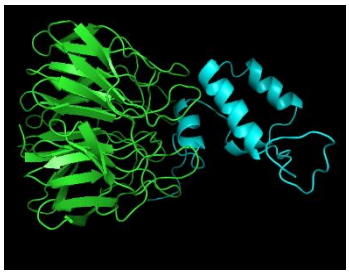


Figure 1. The molecular docking between the Keap1 and the stigmasterol, lirioresinol B, pinoresinol, plucheosida A and Nrf2. (A) 3D structure; (B) 2D structure.

CONCLUSION

The computational study show that the active compounds of *P. indica* are predicted have high antioxidant capacity with high affinity value interaction to KEAP1. In the ADMET profile, drug similarity analysis showed that all compounds of *P. indica* leaves complied with Lipinski rules and had good absorption, distribution, metabolism, and excretion profiles, non-carcinogenic with acute oral toxicity, which is relatively safe.

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