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Original Article

IN SILICO STUDY OF CHEMICAL COMPOUNDS FROM ARECA NUT (*ARECA CATECHU* L.) ON $GABA_A$ RECEPTOR AS ANTI-INSOMNIA CANDIDATES

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ABSTRACT

Objective: In silico study of chemical compounds from areca nut (*Areca catechu*) on $GABA_A$ receptor as anti-insomnia candidates.

Methods: Prediction and molecular docking of chemical compounds from areca nut with $GABA_A$ receptors to find out which compounds are most likely to be anti-insomnia therapy candidates.

Results: Molecular docking with AutoDock Vina and ADMET prediction via PreADMET website. Molecular docking and ADME predictions show that there is one potential anti-insomnia compound called syringic acid that has the most amino acid residues in common with the native ligand and standard drug compared to other compounds, as well as producing free energy (ΔG) and inhibition constants (Ki) lower than the native ligand. Syringic acid also has a weak bond with plasma proteins. However, in the parameters of toxicity, syringic acid exhibits carcinogenic and mutagenic properties.

Conclusion: Based on the results of molecular docking and ADME prediction obtained one compound with the best results can be used as a candidate for anti-insomnia drugs, namely syringic acid.

Keywords: Insomnia, Areca nut, Molecular docking, ADMET prediction

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INTRODUCTION

Insomnia is a sleep disorder that causes a person difficulty to start or maintain sleep so it can affect daytime activities [1–3]. Insomnia based on etiology is divided into two types, namely primary and secondary insomnia. Primary insomnia is caused by psychophysiological and hyperarousal disorders or high levels of anxiety [4], while the cause of secondary insomnia is due to manifestations of other diseases or in other words, is a symptom of disease [5].

Insomnia is reported as a frequent sleep disorder in patients with chronic diseases. More than half (56% and 61.8%) hemodialysis patients in end-stage renal disease in Jakarta and Semarang, Indonesia, are recorded as insomniacs [6, 7] A total of 42.8% of COVID-19 inpatients in Wuhan, China, suffer insomnia [8]. Insomnia also occurs in 67% of HIV patients in Miami, Florida [9]. Not only attacking patients with chronic disease but insomnia is also suffered by the community. As many as 79.5% of millennials in Jabodetabek (Jakarta, Bogor, Depok, Tanggerang, and Bekasi), Indonesia, experience sleep duration of fewer than 6.5 h per day or tend to insomnia [10]. In 2014, it was recorded that 35.2% of United State (US) adults had a short sleep duration or less than 7 h. It also occurs in adolescence, 59.7-76.6% of US high school adolescents experience a lack of sleep duration, while a teenager needs a sleep duration of about 8 to 10 h per night [11]. Most of the causes of insomnia in patients with chronic diseases are depression, anxiety disorders, low quality of life, severe fatigue levels, and health status got worsen.

Molecularly, insomnia can be caused by a decrease in the neurotransmitter GABA in the brain [12]. The neurotransmitter GABA can induce sedation or sleep by binding to $GABA_A$ receptors [13]. This mechanism is also implemented in the most commonly used pharmacological therapy of insomnia and became the standard of the Food and Drug Administration (FDA) which is a Benzodiazepine Receptor Agonist (BzRA) drugs that provides an agonist effect on GABAergic transmission and hyperpolarization of nerve membranes resulting in a decrease in sleep-wake time. However, there are warnings from the FDA regarding BzRA drugs because they are known to cause facial angioedema, anaphylaxis, complex sleep behaviors (excessive sleep behavior, falling asleep while driving, eating, etc.), and the phenomenon of rebound insomnia when BzRA therapy is discontinued.

It is known empirically in the Dayak Tulung tribe, East Kalimantan, Indonesia, that areca nut decoction water can produce a sedative effect, warming, and comfort [14, 15]. Also, water and methanol extract of areca nut (300 and 250 mg/kg BW) were shown to produce better anxiolytic effects than diazepam as positive controls in in mice. However, it is not yet known which chemical compounds are responsible for such anxiolytic activity [16]. As it is known that drugs classified as anxiolytic have a tendency to induce sleep through the relaxation effect given so that it can be used as a candidate for anti-insomnia therapy.

In the discovery and development of drugs, to minimize failure in future clinical trials, it is necessary to predict the absorption, distribution, metabolism, excretion, and toxicity (ADMET) of the compound [17]. Alkaloid compounds from areca nut include arecoline, homoarecoline, arecolidine, arecaidine, guvacine, isoguvacine, and guvacoline based on Lipinski's rule of five (RO5) known to show good oral absorption and bioavailability processes so that it has great potential to be developed into oral drugs [18]. However, it is not yet known in other areca nut compounds, so, this study will be conducted molecular docking and ADMET prediction of chemical compounds from areca nut against $GABA_A$ receptors to find out which compounds are potential candidates for anti-insomnia therapy.

MATERIALS AND METHODS

Receptor and ligand preparation

$GABA_A$ receptor and native ligand (benzamidine) were obtained from the online database protein data bank (https://www.rcsb.org/) with PDB ID 4COF. Receptor was separated from the native ligand, water molecules, and other impure compounds using BIOVIA Discovery Visualizer 2016. The structure of the standard drug and all compounds from the areca nut were drawn using ChemDraw Ultra 12.0. and Chem3D Pro 12.0. All compounds of areca nut were then selection based on Lipinski's

RO5. Furthermore, each compound is given charge and torsion using AutoDock Tools 1.5.6 [19-21].

Validation program

The receptors and native ligand that have been prepared are then redocking using AutoDock Vina, the results are visualized with BIOVIA Discovery Studio 2016. RMSD is calculated in PyMOL 2008, if the RMSD value generated<2 Å this method can be declared valid [19].

Molecular docking

Molecular docking is performed between the standard drug compound (diazepam) or compounds from areca nut against $GABA_A$ receptors using AutoDock Vina. The size of the grid box is adjusted to that used in method validation (redocking). The results are visualized in BIOVIA Discovery Visualizer 2016. The score docking the compound from areca nut was then compared with the score docking of standard drug and native ligand redocking results.

ADMET prediction

ADMET prediction compound test of areca nut tested through PreADMET website (https://preadmet.bmdrc.kr/). The results were interpreted based on the reference parameters that have been determined [22].

RESULTS AND DISCUSSION

Validation program

Validation aimed to know the validity of a method so that the results obtained can be accounted for [23]. Validation program is done by redocking the native ligand (benzamidine) on $GABA_A$ receptors with the following grid box coordinates x =-20.554, y =-19.539, and z = 128.123, size box 40 x 40 x 40; number of binding modes 100; and exhaustiveness 16. This grid box will limit the scope of finding for ligand conformation. The RMSD value obtained from the validation must be<2.00 Å because the lower the RMSD value, the closer the native ligand position of the redocking result with the native ligand from crystallography results as shown in fig. 1 [24]. In this study, RMSD was obtained at 1.8 Å so that the method was declared valid.

Table 1: Validation result of native ligand (benzamidine)

Ligand	ΔG (Kcal/mol)	Ki (μM)	Amino acid residues
Benzamidine	-4.6	0.998	Thr202, Tyr205, Tyr157, Glu155, Tyr97, Phe200

Description: the bolded amino acid was an amino acid with hydrogen bonds

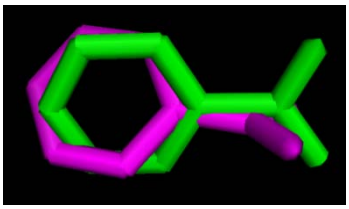

Fig. 1: Visualization overlay of native ligand from crystallography (pink) with the redocking result (green)

The visualization of the native ligand 2D redocking result, benzamidine, against $GABA_A$ receptors as shown in fig. 2. The native ligand interacted with $GABA_A$ receptors through hydrogen bonds with Glu155; van der Waals bonds on Tyr205, Ser156, Tyr157, and Thr202; donor-donor interactions with Tyr97; and phi-phi interactions on Phe200. Some of these amino acid residues have also been mentioned by Miller and Aricescu (2014), that the interaction between benzamidine and $GABA_A$ receptors involves the following amino acid residues, including Phe200, Tyr62, Glu155, Ser156, Tyr157, and Tyr205 [20].

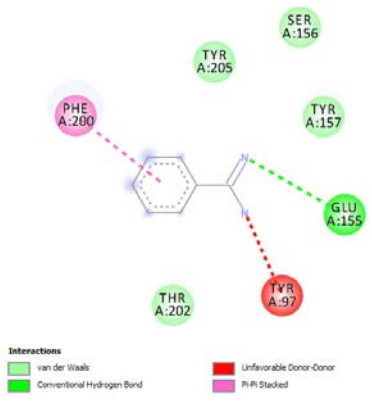

Fig. 2: Visualization of benzamidine interaction with $GABA_A$ receptors

Molecular docking of chemical compounds from areca nut on $GABA_A$ receptors

In the background, it has been mentioned that water decoction, water extract, and methanol extract from areca nut produce a sedative and anxiolytic effect [16]. Thus, molecular docking is carried out on the water and methanol-soluble areca nut compounds; based on literature studies, there were 25 compounds from areca nut. These compounds also have similar structures with the native ligand and standard drug that were there, benzene rings or amines (primary, secondary, or tertiary). In the native ligand and standard drug, the benzene ring will bind to Phe200. Meanwhile, primary and secondary amines in the native ligand will interact with Tyr97 and Glu155. But on the standard drug, Tyr97 interacts with tertiary amines.

The results of molecular docking of chemical compounds from areca nut showed a negative energy interaction or free energy (ΔG) as stated in table 2. ΔG value is a parameter that explains the level of strength and affinity of the interaction between ligand and receptor or protein, if the value of ΔG is negative, then it can be said that the reaction takes place spontaneously; the smaller the value of ΔG obtained then the ligand affinity to receptors is higher because the energy needed to bind is less and otherwise. Also, there is a constant inhibition parameter (Ki); this value is directly proportional to the value of ΔG based on the following formula:

$$\Delta G = R.\ T.\ \ln Ki$$

$$Ki = \exp(\Delta G/(R.\ T)) \text{ [25]}$$

Ki shows the concentration required by a compound to produce an inhibition effect on receptor [23]. Then, the parameters of amino acid residues, the results obtained are not in the form of values, but in the form of visualization of some amino acid residues involved in the interaction of compounds with these receptors. Based on the results of molecular docking obtained two compounds with the best results, namely syringic acid and rhapontigenin. The two compounds have the most amino acid residues in common with the native ligand (benzamidine) and its standard drug (diazepam) as stated in table 2 and table 3, compared to other compounds. Syringic acid and rhapontigenin bind to all the essential amino acids in the binding site of the standard drug, diazepam. The similarity of amino acid residues provides an opportunity for the compound to produce the same biological activity as its standard drug, diazepam. On its

binding site, diazepam produces hydrogen bonds with Tyr97; phi interaction with Leu99 and Phe200; and van der Waals on Glu155, Ala201, Thr202, Tyr157, and Tyr205. Syringic acid forms a hydrogen bond with Glu155 and Tyr97; interaction of phi-phi with Phe200; and van der Waals bonds with Tyr205, Thr202, Ala201, Leu99, Ser156, and Tyr157. Meanwhile, rhapontigenin form hydrogen bonds with Leu99, Tyr205, and Glu155; pi interaction on Phe200, Tyr205, also Leu99; and van der Waals with Tyr157, Tyr97, Arg207, Asn100, Ala201, Thr202, and Ser156.

Table 2: Molecular docking of chemical compounds from areca nut

No	Ligand	ΔG (Kcal/mol)	Ki (μM)	Amino acid residues
1.	Standard drug (Diazepam)	-6.4	0.997	Glu155, Leu99, Ala201, Phe200, Thr202, Tyr157, Tyr205, Tyr97
2.	(+)-isolariciresinol	-6.3	0.997	Tyr97, Lys112, Leu128, Tyr126, Val93, Pro94, Asp95, Thr96, Phe105, Val106, Ser104, Ile130, Phe63, Phe98
3.	8-demethyleucalyptin	-6.6	0.997	Ala135, Glu153, Ser209, Ser211, Arg192, Asn149, Thr151, Arg196, Arg207, Leu99, Asn100
4.	Arecaidine	-4.1	0.998	Phe200, Thr202, Tyr205, Tyr97, Leu99, Glu155
5.	Arecoline	-4.2	0.998	Tyr167, Tyr66, Trp67, Cys37, Val36, Arg68
6.	Arecolidine	-4.0	0.998	Thr123, Tyr167, Tyr66, Trp67, Arg68, Val36, Cys37
7.	Syringic acid	-4.9	0.998	Tyr205, Thr202, Ala201, Phe200, Leu99, Glu155, Tyr97, Ser156, Tyr157
8.	Calquiquelignan M	-6.4	0.997	Phe63, Leu128, Phe98, Val106, Phe105, Ser104, Ile130, Tyr126, Arg114, Ala88, Asp89, Leu91, Ile25, Trp92, Val93, Asp95, Thr96, Pro94, Gln65
9.	Calquiquelignan N	-7.7	0.996	Phe63, Phe98, Phe105, Val106, Ser104, Ile130, Tyr126, Ala88, Arg114, Asp89, Ile25, Tyr159, Arg26, Trp92, Asp95, Leu91, Val93, Thr96, Pro94, Gln65, Leu128
10.	Epicatechin	-6.7	0.997	Tyr97, Phe200, Thr202, Glu155, Tyr205, Arg207, Leu99
11.	Eucalyptin	-6.7	0.997	Thr96, Phe98, Phe63, Tyr97, Ile130, Val106, Lys112, Arg114, Asp89, Val93, Tyr126, Leu128, Pro94, Gln65
12.	Glyceryl-2-vanillic acid methyl ester	-4.9	0.998	Tyr97, Asp101, Val106, Phe105, Ser104, Ile130, Leu128, Gln65, Pro94, Phe63, Tyr126, Thr96, Phe98
13.	Guvacoline	-4.4	0.998	Trp67, Tyr66, Tyr167, Arg68, Thr123, Cys37, Pro35, Asp30, Val36
14.	Guvacine	-4.2	0.998	Trp67, Arg68, Cys37, Pro35, Asp30, Val36
15.	Homoarecoline	-4.5	0.998	Tyr167, Tyr66, Trp67, Arg68, Val36, Cys37, Asp30
16.	Catechin	-7.4	0.997	Val106, Tyr126, Gln65, Pro94, Thr96, Phe63, Leu128, Ile130, Phe98, Asp101, Tyr97, Phe105, Ser104
17.	Quercetin	-6.8	0.997	Pro94, Tyr126, Val93, Ala88, Asp89, Lys112, Arg114, Leu128, Val106, Ile130, Ser104, Phe98, Phe63, Thr96, Gln65
18.	Leucocyanidin	-6.8	0.997	Ser156, Thr202, Ala201, Phe200, Leu99, Tyr157, Glu155, Tyr97
19.	Liquiritigenin	-6.6	0.997	Arg26, Ile25, Trp92, Leu91, Val93, Tyr159, Asp95, Gly158
20.	Nobiletin	-6.0	0.997	Arg207, Leu99, Asn100, Ala135,Thr151, Glu153, Asn149, Arg192, Ser211, Val194, Ser209, Arg196
21.	Procyanidin A1	-9.3	0.996	Asp89, Ile25, Arg26, Tyr159, Trp92, Tyr157, Asp95, Val93, Pro94, Ile130, Thr96, Gln65, Tyr126, Leu128, Lys112, Arg114
22.	Procyanidin B1	-8.9	0.996	Phe63, Ile130, Phe98, Phe105, Ser104, Val106, Tyr157, Asp95, Val93, Ala88, Asp89, Arg114, Lys112, Pro94, Tyr126, Leu128, Thr96, Gln65
23.	Procyanidin B2	-8.8	0.996	Ile130, Phe98, Tyr97, Asp101, Ser104, Lys103, Tyr157, Asp95, Val93, Arg114, Tyr126, Pro94, Lys112, Leu128, Thr96, Gln65, Phe63, Val106
24.	Procyanidin B7	-8.6	0.996	Tyr126, Arg114, Lys112, Phe63, Gln65, Thr96, Leu128, Ile130, Phe105, Ser104, Phe98, Leu99, Asp101, Tyr97, Val106
25.	Rhapontigenin	-6.4	0.997	Tyr157, Tyr97, Leu99, Arg207, Asn100, Ala201, Thr202, Phe200, Tyr205, Glu155, Ser156
26.	Sinensetin	-6.1	0.997	Asp95, Ile25, Leu91, Trp92, Val93, Tyr159, Phe31, Arg26, Gly158

Description: the bolded amino acid was an amino acid with hydrogen bonds

Table 3: Molecular docking of chemical compounds selected from areca nut

No	Ligand	ΔG (Kcal/mol)	Ki (μM)	Amino acid residues
1.	Native ligand (Benzamidine)	-4.6	0.998	Thr202, Tyr205, Tyr157, Glu155, Tyr97, Phe200
2.	Standard drug (Diazepam)	-6.4	0.997	Glu155, Leu99, Ala201, Phe200, Thr202, Tyr157, Tyr205, Tyr97
3.	Syringic acid	-4.9	0.998	Tyr205, Thr202, Ala201, Phe200, Leu99, Glu155, Tyr97, Ser156, Tyr157
4.	Rhapontigenin	-6.4	0.997	Tyr157, Tyr97, Leu99, Arg207, Asn100, Ala201, Thr202, Phe200, Tyr205, Glu155, Ser156

Description: the bolded amino acid was an amino acid with hydrogen bonds

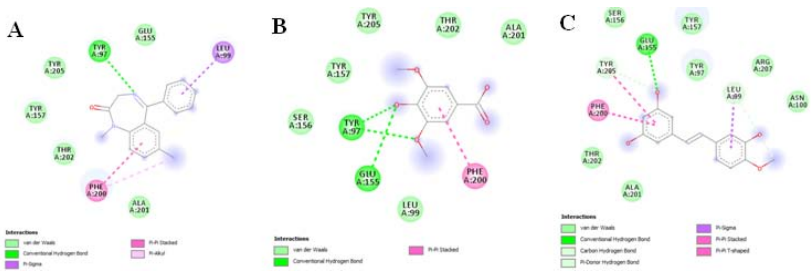

Fig. 3: Visualization of interactions of diazepam (a), syringic acid (b), and rhapontigenin (c) with $GABA_A$ receptor

Syringic acid and rhapontigenin have lower ΔG than the native ligand. Also, rhapontigenin has a value of ΔG which is equivalent to the standard drug, diazepam, but not with syringic acid, so it can be said that the level of the spontaneity of interactions that occur in rhapontigenin against receptors is same as diazepam and better than the native ligand and syringic acid.

Screening compounds based on lipinski rule of five (RO5)

In oral drug discovery, of course, it is necessary to profile solubility and good permeability. To speed up the process of drug discovery based on the solubility and permeability of drug candidates can be used Lipinski's RO5. The rule explains that the molecular weight of the drug should not be>500 because it is known to decrease the permeability of the drug in the gut and central nervous system. Furthermore, the log P or solubility value of a compound in an unmixed solvent i.e. octanol and water describing the lipophilicity of the compound or the ability of the compound to penetrate the bilayer lipid cell membrane. Then, aside from molecular weight and lipophilicity, compounds should also be seen from hydrogen bond donors and acceptors. When there are many hydrogen bond donors in a compound it will be difficult to penetrate the bilayer lipid membrane because it will tend to be partitioned in solvents with strong hydrogen bonds such as water. Similarly, if the number of hydrogen bond acceptors will affect the permeability of the compound through interaction with solvents that have strong hydrogen bonds, such as water [26]. The selected compounds, namely syringic acid and rhapontigenin, are qualified for Lipinski's RO5, so they can be used as candidates for the oral drugs.

Table 4: Screening compound from areca nut based on lipinski's rule of five

No	Compounds	MW (g/mol)	Log P	Hydrogen bond donor	Hydrogen bond acceptor	Description
1.	(+)-isolariciresinol	360.4	2	4	6	Qualify
2.	8-demethyleucalyptin	312.3	2.8	1	5	Qualify
3.	Arecaidine	141.17	-2.3	1	3	Qualify
4.	Arecoline	155.19	0.3	0	3	Qualify
5.	Arecolidine	155.19	0.7	0	3	Qualify
6.	Syringic acid	198.17	1	2	5	Qualify
7.	Calquiquelignan M	420.4	0.5	6	9	Not Qualify
8.	Calquiquelignan N	542.5	1.1	3	11	Not Qualify
9.	Epicatechin	290.27	0.4	5	6	Qualify
10.	Eucalyptin	326.3	4	1	5	Qualify
11.	Glyceryl-2-vanillic acid methyl ester	256.25	-0.9	4	6	Qualify
12.	Guvacoline	141.17	0.1	1	3	Qualify
13.	Guvacine	127.14	-2.5	2	3	Qualify
14.	Homoarecoline	169.22	0.7	0	3	Qualify
15.	Catechin	290.27	0.4	5	6	Qualify
16.	Quercetin	302.23	1.5	5	7	Qualify
17.	Leucocyanidin	306.27	-0.8	6	7	Not Qualify
18.	Liquiritigenin	256.25	2.2	2	4	Qualify
19.	Nobiletin	402.4	3	0	8	Qualify
20.	Procyanidin A1	576.5	2.4	9	12	Not Qualify
21.	Procyanidin B1	578.5	2.4	10	12	Not Qualify
22.	Procyanidin B2	578.5	2.4	10	12	Not Qualify
23.	Procyanidin B7	578.5	2.4	10	12	Not Qualify
24.	Rhapontigenin	258.27	3.1	3	4	Qualify
25.	Sinensetin	372.4	3	0	7	Qualify

Table 5: Screening of the selected compounds from areca nut based on lipinski's rule of five

No	Compounds	MW (g/mol)	Log P	Hydrogen bond donor	Hydrogen bond acceptor	Description
1.	Syringic acid	198.17	1	2	5	Qualify
2.	Rhapontigenin	258.27	3.1	3	4	Qualify

Description: Lipinski's RO5 are molecular weight (MW) ≤ 500 g/mol, log P ≤ 5, hydrogen bond donor ≤ 5, and hydrogen bond acceptor ≤ 10 [25]

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction

Characteristics of a drug such as absorption, distribution, metabolism, excretion, and toxicity are critical points that need to be known to determine the route of drug administration, dosage form, efficacy, pharmacological profile, and drug safety. However, optimization of the overall parameters takes a long time, the biggest and fastest possibility is to test them *in silico*. ADMET prediction *in silico* can be done through PreADMET website (https://preadmet.bmdrc.kr/) based on parameters such as permeability in Caco2 cells, Human Intestinal Absorption (HIA), Blood Brain Barrier (BBB), Plasma Protein Binding (PPB), carcinogenic in rats and mice, and mutagenic.

These parameters are tested for a variety of purposes. Human Intestinal Absorption (HIA) parameter is a parameter that determines the bioavailability of an oral drug with its manual formula is HIA = $D_{blood}/D_{oral,}$ with D_{blood} was a number of drugs on the portal vein and D_{oral} = a number of oral medications administered. This formula can also determine how many doses of oral drugs should be administered [27]. Similar to the HIA parameter, the permeability parameter of Caco2 cells were tested to know the permeability of the drug into the intestine through Caco2 cells which is a human colon adenocarcinoma cell that will certainly produce a different permeability to other normal cells [28]. Then, there is the Blood Brain Barrier (BBB) parameter, the resulting value provides information about the ability of the drug to penetrate the blood vessels of the brain which is a membrane separating the central nervous system with blood circulation [29]. BBB parameters are certainly very expected for this study because the target of the drug, $GABA_A$ receptors, is located in the brain. Meanwhile, Plasma Protein Binding (PPB) describes distribution parameters based on the percentage of drugs bound to plasma proteins. Generally, only drugs in a free or unbound state can enter the cell and interact with pharmacological targets [22].

ADMET predictions of the two selected compounds were listed in table 6 and table 7. This prediction shows both syringic acid and rhapontigenin were well-perceived in the gut so it can be said that the bioavailability of both is also good. Both compounds also have intermediate permeability values in Caco2 cells and intermediate absorption in the central nervous system (CNS) so there is an opportunity for both to interact with $GABA_A$ receptors located in the brain. In the PPB parameters, Syringic acid produces a percentage of

69.77% which indicates that the compound is weakly bound to plasma proteins, allowing the presence of unbound compounds that can enter the cell and then interact with the target receptor. While the PPB on rhapontigenin shows a strong bond with plasma proteins. Based on the hypothesis of free drug hypothesis, in a steady state, the concentration of free drugs (unbound) in plasma will be equal to the concentration in the tissues. Then, the concentration of free drugs in these tissues will provide pharmacological action on the target site. Although, there is another opinion that PPB does not greatly affect the efficacy *in vivo*, therefore, more research is needed on this matter [30]. Plasma protein binding can be reduced by modifying the structure to be more polar through the addition of polar groups such as carbamate, sulfone, and amide at the binding site to plasma proteins [31].

Table 6: ADME prediction of chemical compounds from areca nut

No	Compounds	HIA (%)	CACO-2 cell (nm/s)	PPB (%)	BBB	Water solubility (mg/l)
1.	(+)-isolariciresinol	84.23	21.09	85.05	0.11	753.81
2.	8-demethyleucalyptin	95.98	39.61	89.29	0.11	10.46
3.	Arecaidine	92.82	21	0	0.89	466510
4.	Arecoline	100	26.32	8.13	1.05	232569
5.	Arecolidine	100	56.41	74.49	0.80	47875.3
6.	Syringic acid	82.02	18.83	69.77	0.53	17371.2
7.	Calquiquelignan M	86.30	2.65	76.01	0.02	44.40
8.	Calquiquelignan N	87.41	6.80	85.56	0.04	0.64
9.	Dihidrotrisin	87.31	10.52	100	0.5	251.402
10.	Epicatechin	66.70	0.65	100	0.39	1240.55
11.	Eucalyptin	95.97	42.65	89.51	0.19	7.33
12.	Glyceryl-2-vanillic acid methyl ester	83.77	0.37	53.79	0.05	9491.66
13.	Guvacoline	91.64	21.30	17.36	0.40	189187
14.	Guvacine	83.89	20.71	0	0.41	375743
15.	Homoarecoline	100	46.99	16.77	1.04	151467
16.	Catechin	66.70	0.65	100	0.39	1240.55
17.	Quercetin	63.48	3.41	93.23	0.17	96.43
18.	Leucocyanidin	46.51	3.37	92.51	0.14	1086.99
19.	Liquiritigenin	92.35	17.64	97.99	0.64	164.18
20.	Naringenin	86.47	2.99	94.56	0.06	190.69
21.	Nobiletin	99.07	54.02	84.85	0.02	5.33
22.	Procyanidin A1	35.29	9.23	100	0.07	0.78
23.	Procyanidin B1	19.51	13.67	100	0.06	3.97
24.	Procyanidin B2	19.51	13.67	100	0.06	3.97
25.	Procyanidin B7	19.50	12.87	100	0.05	8.71
26.	Rhapontigenin	88.44	3.26	100	0.82	124.95
27.	Sinesetin	98.88	51.22	86.24	0.02	3.52

Table 7: ADME prediction of two selected compounds from areca nut

No	Compounds	HIA (%)	Caco2 cell (nm/s)	PPB (%)	BBB	Water solubility (mg/l)
1.	Syringic acid	82.02	18.83	69.77	0.53	17371.2
2.	Rhapontigenin	88.44	3.26	100	0.82	124.95

Description: % Human Intestinal Absorption (% HIA): (a) 70-100% Well absorbed compounds, (b)20-70% Moderately absorbed compounds, (c) 0-20% Poorly absorbed compounds, Caco-2 cell permeability (nm/s): (a)>70 High permeability, (b) 4-70 Middle permeability, (c)<4 Low permeability, Plasma Protein Binding (%): (a)>90% strongly bound, (b)<90% weakly bound. Blood Brain Barrier: (a)>2.0 High absorption to CNS, (b) 2.0-0.1 Middle absorption to CNS, (c)<0.1 Low absorptions to CNS

Table 8: Toxicity prediction of chemical compounds from areca nut

No	Compounds	Carcinogenic (Mouse	Carcinogenic (Rat)	Ames test/Mutagenic
1.	(+)-isolariciresinol	Negative	Negative	Mutagen
2.	8-demethyleucalyptin	Negative	Positive	Mutagen
3.	Arecaidine	Positive	Negative	Mutagen
4.	Arecoline	Negative	Negative	Mutagen
5.	Arecolidine	Positive	Negative	Mutagen
6.	Syringic acid	Negative	Positive	Mutagen
7.	Calquiquelignan M	Negative	Negative	Non-mutagen
8.	Calquiquelignan N	Negative	Negative	Non-mutagen
9.	Epicatechin	Negative	Negative	Mutagen
10.	Eucalyptin	Negative	Positive	Mutagen
11.	Glyceryl-2-vanillic acid methyl ester	Negative	Negative	Mutagen
12.	Guvacoline	Negative	Negative	Mutagen
13.	Guvacine	Positive	Negative	Mutagen
14.	Homoarecoline	Negative	Negative	Mutagen
15.	Catechin	Negative	Negative	Mutagen
16.	Quercetin	Negative	Positive	Mutagen
17.	Leucocyanidin	Negative	Negative	Mutagen
18.	Liquiritigenin	Negative	Positive	Mutagen
19.	Nobiletin	Negative	Positive	Mutagen
20.	Procyanidin A1	Negative	Positive	Non-mutagen
21.	Procyanidin B1	Negative	Positive	Non-mutagen
22.	Procyanidin B2	Negative	Positive	Non-mutagen
23.	Procyanidin B7	Negative	Positive	Non-mutagen
24.	Rhapontigenin	Positive	Negative	Mutagen
25.	Sinesetin	Negative	Positive	Mutagen

Table 9: Toxicity prediction of two selected compounds from areca nut

No	Compounds	Carcinogenic (Mouse)	Carcinogenic (Rat)	*Ames Test*/Mutagenic
1.	Syringic acid	Negative	Positive	Mutagen
2.	Rhapontigenin	Positive	Negative	Mutagen

Description: positive, mean no evidence of carcinogenic activity; negative mean clear evidence of carcinogenic activity.

Predictions of mutagenic on the PreADMET website are based on the Ames test. The test is a simple method to determine the mutagenic properties of a compound by using several strains of *Salmonella typhimurium* as carriers of mutation genes whose growth depends on the synthesis of the amino acid histidine. The results of the test will show the mutagen ability of a compound to trigger growth in a medium free of histidine.

In contrast to mutagenic predictions, carcinogenic predictions in PreADMET were obtained from the National Toxicology Program (NTP) database and the US FDA regarding *in vivo* carcinogenic testing in mouse and rat during a 2 y. If the test results are positive, NTP defines that there is no evidence of carcinogenic activity in the compound, while negative results indicate carcinogenic activity. If you look at the results listed in table 9 states that the two selected compounds of areca nut have mutagenic and carcinogenic effects in either mouse or rat. This toxicity can be caused by certain functional groups such as ortho-dihydrobomatic and carboxylic acid found in compounds of areca nut, although of course, it must be through further research. Ortho-dihydroxic is known to be susceptible to forming oxidative compounds i.e. ortho-quainons that can produce reactive metabolites and irreversible alkylation in proteins or DNA. Meanwhile, carboxylic acid can cause toxicity through its main metabolic route by forming acyl glucuronides which are reactive metabolites and can cause irreversible modification of proteins [32]. This can be addressed by replacing part or all of the structure of the compound [33] or lower the dose to reduce or eliminate the formation of reactive metabolites and oxidative stress [34].

CONCLUSION

Based on the results of molecular docking and ADME prediction obtained one compound with the best results can be used as a candidate for anti-insomnia drugs, namely syringic acid. The compound has the most amino acid residues in common with the native ligand (benzamidine) and standard drug (diazepam) that interact with Tyr157, Tyr97, Leu99, Ala201, Thr202, Phe200, Tyr205, and Glu155, then have lower ΔG and Ki values than the native ligands. Another advantage is that syringic acid has a weak bond with plasma proteins. However, based on toxicity parameters, syringic acid is potentially carcinogenic and mutagenic.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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