Journal of
Food and Pharmaceutical Sciences

Original Article

In silico Pharmacokinetic and Toxicity Prediction of Compounds from *Andrographis Paniculata* (Burm.F.) Nees.

Izatunnafis[1], Yosi Bayu Murti[2], Bambang Sulistyo Ari Sudarmanto[3]*

[1]Magister Program of the Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia; izatunnafis@mail.ugm.ac.id

[2]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia; yosibayu.murti@ugm.ac.id

[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia; arie_sudarmanto@ugm.ac.id

*Corresponding author: Bambang Sulistyo Ari Sudarmanto | Email: arie_sudarmanto@ugm.ac.id

Received: 22 March 2023; Revised: 12 May 2023; Accepted: 8 June 2023; Published: 23 June 2023

Abstract: Many compounds have been isolated from *Andrographis paniculata* (Burm. f.) Nees (AP). In drug discovery and development, plant secondary metabolites are popular as resources for drug candidates. A high-quality drug candidate should not only be effective against the therapeutic target, but it should also be safe and have good pharmacokinetic features. This study aimed to predict the pharmacokinetic features and toxicity potencies of 46 compounds from AP using the pKCSM online tool. According to pKCSM prediction, among the forty-six compounds from AP, compound **1** (14-Deoxy-11,12-didehydroandrographolide), compound **2** (14-Deoxyandrographolide), and compound **39** ((-)-beta-Sitosterol) have good pharmacokinetic features and do not have potencies to be mutagenic and hepatotoxic agents. The lethal dosage values (LD50) of compounds **1, 2,** and **39,** are 1935, 2053, and 2424 (mol/kg), respectively. However, further research is still needed to confirm these predictions.

Keywords: pkcsm; sambiloto; Knapsack; ADMET.

1. INTRODUCTION

Andrographis paniculata (Burm.f.) Nees (AP), an Acanthaceae family member, is widely used in traditional medicine systems and exhibits a wide spectrum of bioactivity, such as anti-inflammation, anti-viral infection, anti-diabetes, and anti-cancer [1,2]. Clinical studies have reported that AP extracts alone or in combination with other medicinal plants were effective to treat the common cold and sinusitis [3], diabetes type II [4,5], malaria [6], and COVID-19 [7]. In the AP extract, compounds from diterpene lactone [8], flavonoid [9], and phenolic group [10] are thought to be responsible for the bioactivities. Several secondary metabolites of AP, including andrographolide as a major compound, were isolated and were reported in many studies, such as neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, andrograpanin, andropanolide, andrographidine A, and 3-O-caffeoylquinic acid [11].

For decades, plant secondary metabolites and their structural analogs have remained popular drug candidates in drug discovery and development [12,13]. However, despite the fact that

some compounds have been shown to have certain bioactivities, they cannot be developed and have failed in clinical trials due to pharmacokinetic issues. Thus, pharmacokinetic screening is needed [14,15]. The evaluation of the pharmacokinetic properties, including absorption, distribution, metabolism, and excretion (ADME) and the toxicity potencies (T) of compounds can be done using in vitro and in vivo methods, however, these experiments are expensive, especially when testing a large number of compounds. Many in silico models are being developed to predict the ADMET features of compounds. This approach has decreased experimental drug trials and increased success rates, making it a helpful method [16].

In this study, AP compounds are collected from the Knapsack, a complete database of plants and their metabolites [17]. The pharmacokinetic properties and the toxicity potencies of AP compounds were evaluated using pKCSM online tool. pKCSM is a free web server that uses graph-based signatures to create predictive models of ADMET features [18]. The computational prediction limitations of this study require future investigation to verify the results.

2. MATERIALS AND METHODS

The compounds of AP were collected from the Knapsack (http://www.knapsackfamily.com/) [17]. The SMILES (simplified molecular input line entry system) strings of each compound were collected from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and were then introduced into pKCSM (http://biosig.unimelb.edu.au) to evaluate the physicochemical properties, pharmacokinetic characteristics, and toxicological potencies of the AP compound. Both Knapsack and pKCSM were accessed in March 2022.

3. RESULTS AND DISCUSSION

3.1 Collection of AP compounds

Forty-six compounds of AP were downloaded from the Knapsack database (see Table 1), including twenty-two compounds from the diterpene lactone group, sixteen flavonoids, a compound from the sterol group, four phenolic acids, and three compounds from the sesquiterpene class. The pKCSM online tool [18] was then used to predict the physicochemical properties, pharmacokinetic characteristics, and toxicity potencies of compounds.

Table 1. List of *Andrographis paniculata* compounds by Knapsack

Compound	C_ID	Metabolite	Formula
		Diterpene lactone	
1	C00022240	14-Deoxy-11,12-didehydroandrographolide	C20H28O4
2	C00022232	14-Deoxyandrographolide	C20H30O4
3	C00022255	14-Deoxy-11-oxoandrographolide	C20H28O5
4	C00038325	7R-Hydroxy-14-deoxyandrographolide	C20H30O5
5	C00038326	7S-Hydroxy-14-deoxyandrographolide	C20H30O5
6	C00022415	Andrograpanin	C20H30O3
7	C00023362	Andrographolide	C20H30O5
8	C00041336	Andropanolide	C20H30O5
9	C00041603	Isoandrographolide	C20H30O5
10	C00041244	14-Deoxy-17-hydroxyandrographolide	C20H32O5

Continued Table 1…

11	C00029683	Andrographic acid	C20H28O6
12	C00038155	12R,13R-Hydroxyandrographolide	C20H32O6
13	C00038156	12S,13S-Hydroxyandrographolide	C20H32O6
14	C00041241	12S-Hydroxyandrographolide	C20H32O6
15	C00034370	14-Acetylandrographolide	C22H32O6
16	C00034369	14-Acetyl-3,19-isopropylideneandrographolide	C25H36O6
17	C00022416	Neoandrographolide	C26H40O8
18	C00022233	Ninandrographolide	C26H40O9
19	C00041274	3-O-beta-D-Glucopyranosylandrographolide	C26H40O10
20	C00041335	Andrographiside	C26H40O10
21	C00041377	Bisandrographolide B	C40H56O8
22	C00041378	Bisandrographolide C	C40H56O8
		Flavonoid	
23	C00001016	5-Hydroxy-7,4'-dimethoxyflavone	C17H14O5
24	C00003810	7-O-Methylwogonin	C17H14O5
25	C00008154	5-Hydroxy-7,8-dimethoxyflavanone	C17H16O5
26	C00014122	Dihydroskullcap flavone I	C17H16O6
27	C00013310	5-Hydroxy-7,2',6'-trimethoxyflavone	C18H16O6
28	C00035795	5,7,2',3'-Tetramethoxyflavanone	C19H20O6
29	C00004610	5-Hydroxy-3,7,8,2'-tetramethoxyflavone	C19H18O7
30	C00003952	5,4'-Dihidroxy-7,8,2',3'-tetramethoxyflavone	C19H18O8
31	C00004129	Wogonin 5-glucoside	C22H22O10
32	C00004132	5-Hydroxy-7,8-dimethoxyflavone 5-glucoside	C23H24O10
33	C00008449	Andrographidin A	C23H26O10
34	C00013653	Skullcapflavone 1,2'-O-beta-D-glucopyranoside	C23H24O11
35	C00004260	5-Hydroxy-7,8,2'-trimethoxyflavone 5-glucoside	C24H26O11
36	C00004449	5,2',3'-Trihydroxy-7,8-dimethoxyflavone 3'-glucoside	C23H24O12
37	C00004450	5-Hydroxy-7,8,2',3'-tetramethoxyflavone 5-glucoside	C25H28O12
38	C00004477	5,4'-Dihidroxy-7,8,2',3'-tetramethoxy flavone 5-glucoside	C25H28O13
		Sterol	
39	C00003672	(-)-beta-Sitosterol	C29H50O
		Phenolic acid	
40	C00029961	Cinnamic acid	C9H8O2
41	C00000615	Caffeic acid	C9H8O4
42	C00002743	Ferulic acid	C10H10O4
43	C00002724	3-O-Caffeoylquinic acid	C16H18O9
		Sesquiterpene	
44	C00011907	Paniculide A	C15H20O4
45	C00011909	Paniculide C	C15H18O5
46	C00011908	Paniculide B	C15H20O5

3.2 Pharmacokinetics characteristics and toxicity potencies of AP compounds

When a drug is taken orally, it travels from the stomach to the small intestine, where most of it is absorbed [19]. To evaluate drug absorption, pKCSM predicts the percentage of the absorbed compound in human intestinal (% HIA) and intestinal mucosa permeability (Caco-2 permeability). In the pkCSM predictive model, a compound with an absorption value >80% is well-absorbed, while <30% is poorly-absorbed. Moreover, a compound is predicted to have high intestinal mucosa permeability if it is predicted to have Caco-2 permeability values >0.90 [18]. According to pKCSM predictive models (see Table 2), in absorption features, compounds **1-10, 16, 23-30, 39, 40, 42,** and **44** have absorption values (A1) >80%. Compounds **1-3, 6, 10, 15, 16, 23-29, 39, 40,** and **44** have Caco-2 permeability values (A2) >0.90. It means that these compounds are well-absorbed and have high intestinal mucosa permeability.

The volume of distribution (VDss) is an important indicator for estimating the proportion of a drug's total amount in the body versus its plasma concentration at a given time [20]. pKCSM developed the human VDss predictive model. The low VDss if log VDss < -0.15 and the high VDss if log VDss > 0.45 [18]. Drugs can also be distributed to the brain. However, the blood-brain barrier (BBB) prevents drugs from entering the brain. To predict whether a drug would cross the BBB and cause effects on the central nervous system, pKCSM provides BBB and CNS permeability predictions. A compound with logBB >0.3 is assumed to penetrate the BBB easily, whereas a compound with logBB < -1 is assumed to be poorly distributed to the brain. Moreover, a compound with logPS >-2 is considered to penetrate the central nervous system (CNS), while those with logPS <-3 are considered unable to penetrate the CNS [18]. According to the pKCSM prediction (see Table 2), compounds **1, 2, 6, 16, 23-24, 26, 36, 38-39,** and **43-46** have log VDss (D1) ≥ -0.15, but none of them have log VDss > 0.45, meaning that they can be distributed moderately to yield higher concentrations in tissue than in plasma. Compounds **1-17, 23-27, 29-30, 39-42,** and **44-46** have logBBB value (D2) ≥ -1, meaning that these compounds can readily cross the BBB. Compounds **1-10, 15, 16, 21-25, 27-28, 39-42,** and **44-45** have logPS value (D3) >-3, which means that they can penetrate the CNS. There have been safety concerns in the development of drugs that can easily cross the BBB and penetrate the CNS due to unforeseen neurotoxicity [21].

In the liver, Cytochrome P450 (CYP) deactivates some drugs, and it can also activate several drugs [22]. A CYP inhibitor is a molecule that inhibits the detoxification activity of CYP. The CYP inhibition activity of the molecule likely mediated many drug interactions. Therefore, it becomes essential to assess the CYP substrates and inhibitors of drug candidates. pKCSM provides predictive models of five CYP isoforms that are responsible for drug metabolisms, such as CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 [18]. In metabolism features, compounds **6, 23-25, 27,** and **29-30,** were predicted as CYP1A2 inhibitors (M1). Compounds **23-30** were predicted as CYP2C19 inhibitors (M2). Compounds **23-24, 26, 29,** and **30** were predicted as CYP2C9 inhibitors (M3). None of the 46 AP compounds were predicted as CYP2D6 inhibitors (M4). Compounds **23-24** and **28-30** were predicted as CYP3A4 inhibitors (M5).

Organic cation transporter 2 (OCT2) is a transporter in the human kidney that controls drug reuptake from the blood. It plays a key role in the disposition and renal clearance of drugs [23]. Thus, assessing a potential molecule candidate to be re-uptaken by OCT2 (OCT2 substrates) provides useful information regarding not only its clearance (excretion) but also potential contraindications [18].

According to pKCSM results in elimination features (E), compounds **15-16, 23-24**, and **27-28** were predicted to be re-uptaken by renal OCT2 (OCT2 substrates).

Toxicity evaluation is important to assure that drug candidates are safe. pKCSM also predicted the potential mutagenicity (based on AMES toxicity) and hepatotoxicity of AP compounds. In addition, the lethal dosage value (LD50) is a standard measurement to assess the acute toxicity of compounds. LD50 is the amount of a compound given all at once that causes the death of 50% of a group of test animals [18]. As a result, compounds **3, 28,** and **44-46** were predicted to have the potential to be mutagenic agents (T1). Compounds **11, 17,** and **43** were predicted to have potencies to be hepatotoxic agents (T2). The predicted lethal dosage values (LD50) of 46 AP compounds range from 241 to 3204 (mol/kg).

Based on the results above, compounds **1, 2,** and **39** were predicted to have good absorption and distribution features. None of these compounds were predicted to be CYP1A2 inhibitors, CYP2C19 inhibitors, CYP2C9 inhibitors, CYP2D6 inhibitors, CYP3A4 inhibitors, or OCT2 substrates. In toxicity evaluation, none of compounds **1, 2,** or **39** were predicted to have potencies to be mutagenic or hepatotoxic agents. The predicted LD50 values of these compounds were 1935, 2053, and 2424 (mol/kg), respectively. The molecular structures of compound 1 (14-Deoxy-11,12-didehydroandrographolide), compound 2 (14-Deoxyandrographolide), and compound 39 ((-)-beta-sitosterol) are depicted in **Figure 1.**

The therapeutic effects of AP are attributed to four major active diterpenoids, including andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, and 14-deoxyandrographolide. The highest content of 14-deoxyandrographolide was found in leaves at the transfer stage (between the seedling and vegetative stages). Meanwhile, 14-deoxy-11,12-didehydroandrographolide was at its highest level during the vegetative stage [24]. Compound (-)-beta-sitosterol is a phytosterol that is widely distributed in the plant kingdom and possesses many bioactivities [25]. In 2011, Xu et al. isolated (-)-beta-sitosterol along with 27 other compounds from the roots of AP [26].

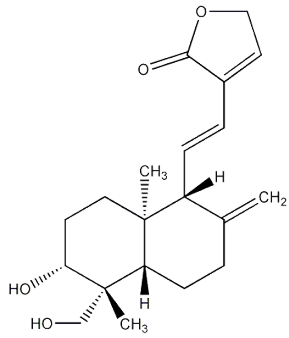
Table 2. Pharmacokinetics characteristics and toxicity potencies of AP compounds

Com / Req.	A1	A2	D1	D2	D3	M1	M2	M3	M4	M5	E	T1	T2	T3
	≥80	≥0.9	≥-0.15	≥-1	≥-3	-	-	-	-	-	-	-	-	
1	96.69	1.03	-0.13	0.04	-2.42	-	-	-	-	-	-	-	-	1935
2	96.65	0.99	-0.10	0.00	-2.47	-	-	-	-	-	-	-	-	2053
3	96.84	1.22	-0.29	-0.36	-2.68	-	-	-	-	-	-	+	-	2211
4	96.09	0.88	-0.25	-0.73	-2.76	-	-	-	-	-	-	-	-	1937
5	96.09	0.88	-0.25	-0.73	-2.76	-	-	-	-	-	-	-	-	1937
6	95.78	1.39	0.17	0.05	-2.08	+	-	-	-	-	-	-	-	2041
7	96.09	0.88	-0.25	-0.73	-2.76	-	-	-	-	-	-	-	-	1937
8	96.09	0.88	-0.25	-0.73	-2.76	-	-	-	-	-	-	-	-	1937
9	96.09	0.88	-0.25	-0.73	-2.76	-	-	-	-	-	-	-	-	1937
10	94.44	0.90	-0.31	-0.71	-2.86	-	-	-	-	-	-	-	-	2135
11	22.54	0.60	-1.21	-0.90	-3.32	-	-	-	-	-	-	-	+	2218
12	60.62	0.05	-0.40	-0.79	-3.43	-	-	-	-	-	-	-	-	2269

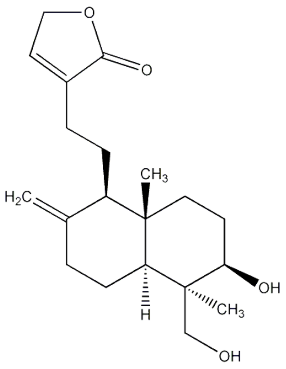
Continued Table 2…

13	60.62	0.05	-0.40	-0.79	-3.43	-	-	-	-	-	-	-	-	2269
14	60.62	0.05	-0.40	-0.79	-3.43	-	-	-	-	-	-	-	-	2269
15	74.84	0.96	-0.41	-0.60	-2.68	-	-	-	-	-	+	-	-	2126
16	98.74	1.04	0.05	-0.51	-2.24	-	-	-	-	-	+	-	-	2211
17	62.26	0.47	-0.87	-0.99	-3.64	-	-	-	-	-	-	-	+	2337
18	50.31	0.38	-0.40	-1.17	-3.85	-	-	-	-	-	-	-	-	251
19	30.31	0.30	-0.64	-1.11	-4.10	-	-	-	-	-	-	-	-	2361
20	30.31	0.30	-0.64	-1.11	-4.10	-	-	-	-	-	-	-	-	2361
21	0.50	-5.02	-1.45	-1.18	-2.88	-	-	-	-	-	-	-	-	3204
22	0.50	-5.02	-1.45	-1.18	-2.88	-	-	-	-	-	-	-	-	3204
23	95.45	1.11	-0.10	-0.46	-2.14	+	+	+	-	+	+	-	-	2085
24	95.45	1.11	-0.10	-0.46	-2.14	+	+	+	-	+	+	-	-	2085
25	94.12	1.34	-0.25	0.18	-2.23	+	+	-	-	-	-	-	-	241
26	92.00	1.36	0.13	0.01	-3.07	-	+	+	-	-	-	-	-	2324
27	94.15	0.98	-0.31	-0.56	-2.30	+	+	-	-	-	+	-	-	2345
28	96.35	1.23	-0.37	-1.16	-2.86	-	+	-	-	+	+	+	-	2478
29	94.17	1.28	-0.50	-0.84	-3.02	+	+	+	-	+	-	-	-	2276
30	83.16	0.08	-0.17	-0.93	-3.16	+	+	+	-	+	-	-	-	2422
31	52.46	0.26	-0.34	-1.39	-4.52	-	-	-	-	-	-	-	-	2822
32	61.89	0.24	-0.68	-1.43	-4.36	-	-	-	-	-	-	-	-	2918
33	62.11	0.14	-0.59	-1.42	-3.90	-	-	-	-	-	-	-	-	281
34	47.30	0.23	-0.27	-1.67	-3.94	-	-	-	-	-	-	-	-	2723
35	66.38	0.46	-0.33	-1.64	-3.99	-	-	-	-	-	-	-	-	2745
36	44.05	0.20	0.10	-1.84	-4.36	-	-	-	-	-	-	-	-	2601
37	60.62	0.45	-0.44	-1.80	-4.22	-	-	-	-	-	-	-	-	2775
38	58.14	0.11	-0.02	-2.01	-4.78	-	-	-	-	-	-	-	-	2667
39	93.82	1.19	0.14	0.80	-1.73	-	-	-	-	-	-	-	-	2424
40	97.20	1.73	-0.81	0.36	-1.51	-	-	-	-	-	-	-	-	2132
41	69.41	0.63	-1.10	-0.65	-2.61	-	-	-	-	-	-	-	-	2383
42	93.49	0.64	-1.32	-0.25	-2.58	-	-	-	-	-	-	-	-	2204
43	24.08	-0.95	-0.05	-1.39	-3.97	-	-	-	-	-	-	-	+	2181
44	96.56	1.27	0.15	-0.27	-2.93	-	-	-	-	-	-	+	-	2419
45	73.72	0.45	0.06	-0.31	-2.99	-	-	-	-	-	-	+	-	2756
46	64.15	0.32	0.06	-0.31	-3.04	-	-	-	-	-	-	+	-	2821

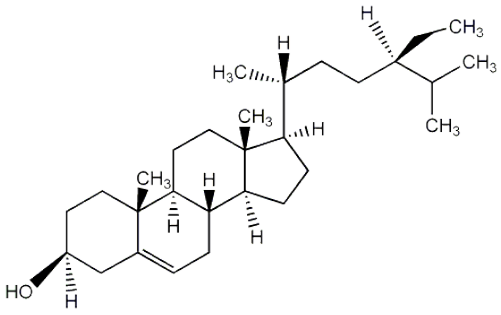
Abbreviation: Com (Compound); Req. (requirement value of good pharmacokinetic properties); A1 (% absorbed compound in HIA); A2 (Caco2 permeability); D1 (VDss or volume distribution); D2 (BBB permeability); D3 (CNS permeability); M1 (CYP1A2 inhibitor); M2 (CYP2C19 inhibitor); M3 (CYP2C9 inhibitor), M4 (CYP2D6 inhibitor), and M5 (CYP3A4 inhibitor); E1 (Renal OCT2 substrate); T1 (AMES toxicity or mutagenicity); T2 (hepatotoxicity); T3 (LD50).



14-Deoxy-11,12-didehydroandrographolide



14-Deoxyandrographolide



(-)-beta-Sitosterol

Figure 1. structures of compound **1** (14-Deoxy-11,14-didehydroandrographolide), compound **2** (14-Deoxyandrographolide), and compound **39** ((-)-beta-Sitosterol)

4. CONCLUSION

This study predicted the pharmacokinetic properties and toxicity of 46 AP compounds, including compounds from the diterpene lactone group, flavonoid group, phenolic group, and sterol group, using the pKCSM online server. According to the pKCSM prediction, compounds **1** (14-Deoxy-11,12-didehydroandrographolide)**, 2** (14-Deoxyandrographolide)**,** and **39** ((-)-beta-Sitosterol) have good pharmacokinetic features, and non-toxic. This computational method is a helpful approach to testing a large number of compounds. However, further research is still needed to confirm these predictions.

Conflicts of interest: The authors declare no conflict of interest.

References

[1] Hossain, S.; Urbi, Z.; Karuniawati, H.; Mohiuddin, R. B.; Moh Qrimida, A.; Allzrag, A. M. M.; Ming, L. C.; Pagano, E.; Capasso, R. *Andrographis Paniculata* (Burm. f.) Wall. Ex Nees: An Updated Review of Phytochemistry, Antimicrobial Pharmacology, and Clinical Safety and Efficacy. *Life (Basel)* **2021**, *11* (4), 348. https://doi.org/10.3390/life11040348.

[2] Kumar, S.; Singh, B.; Bajpai, V. *Andrographis Paniculata* (Burm.f.) Nees: Traditional Uses, Phytochemistry, Pharmacological Properties and Quality Control/Quality Assurance. *J Ethnopharmacol* **2021**, *275*, 114054. https://doi.org/10.1016/j.jep.2021.114054.

[3] Melchior, J.; Palm, S.; Wikman, G. Controlled Clinical Study of Standardized Andrographis Paniculata Extract in Common Cold - a Pilot Trial. *Phytomedicine* **1997**, *3* (4), 315–318. https://doi.org/10.1016/S0944-7113(97)80002-5.

[4] Widjajakusuma, E. C.; Jonosewojo, A.; Hendriati, L.; Wijaya, S.; Ferawati, null; Surjadhana, A.; Sastrowardoyo, W.; Monita, N.; Muna, N. M.; Fajarwati, R. P.; Ervina, M.; Esar, S. Y.; Soegianto, L.; Lang, T.; Heriyanti, C. Phytochemical Screening and Preliminary Clinical Trials of the Aqueous Extract Mixture of Andrographis Paniculata (Burm. f.) Wall. Ex Nees and Syzygium Polyanthum (Wight.) Walp Leaves in Metformin Treated Patients with Type 2 Diabetes. *Phytomedicine* **2019**, *55*, 137–147. https://doi.org/10.1016/j.phymed.2018.07.002.

[5] Agarwal, R.; Sulaiman, S. A.; Mohamed, M. Open Label Clinical Trial to Study Adverse Effects and Tolerance to Dry Powder of the Aerial Part of Andrographis Paniculata in Patients Type 2 with Diabetes Mellitus. *Malays J Med Sci* **2005**, *12* (1), 13–19.

[6] Makmur, T.; Siregar, F. A.; Siregar, S.; Lubis, I. A.; Bestari, R.; Zein, U. Open Clinical Trial of Sambiloto (Andrographis Paniculata) Ethanolic Extract Capsules in Treatment of Malaria Patients in Batubara District, Indonesia. *Med Arch* **2022**, *76* (6), 419–425. https://doi.org/10.5455/medarh.2022.76.419-425.

[7] Majeed, M.; Nagabhushanam, K.; Shah, K.; Mundkur, L. A Randomized, Double-Blind, Placebo-Controlled Study to Assess the Efficacy and Safety of a Nutritional Supplement (ImmuActiveTM) for COVID-19 Patients. *Evid Based Complement Alternat Med* **2021**, *2021*, e8447545. https://doi.org/10.1155/2021/8447545.

[8] Nugroho, A. E.; Andrie, M.; Warditiani, N. K.; Siswanto, E.; Pramono, S.; Lukitaningsih, E. Antidiabetic and Antihiperlipidemic Effect of *Andrographis Paniculata* (Burm. f.) Nees and Andrographolide in High-Fructose-Fat-Fed Rats. *Indian J Pharmacol* **2012**, *44* (3), 377–381. https://doi.org/10.4103/0253-7613.96343.

[9] Rachmani, E. P. N. Aktivitas Hipoglikemik Dan Hipolipidemik Fraksi Flavonoid Herba Sambiloto (*Andrographis Paniculata* (Burm.f.) Ness.) : Efek in Vivo Pada Tikus Yang Diinduksi Fruktosa Dan Lemak Tinggi Serta Kajian in Vitro Pada Enzim Alfa-Amilase, Alfa-Glukosidase Dan Antioksidan, Universitas Gadjah Mada, 2019. http://etd.repository.ugm.ac.id/penelitian/detail/170944 (accessed 2021-05-24).

[10] Jaiyesimi, K. F.; Agunbiade, O. S.; Ajiboye, B. O.; Afolabi, O. B. Polyphenolic-Rich Extracts of *Andrographis Paniculata* Mitigate Hyperglycemia via Attenuating β-Cell Dysfunction, pro-Inflammatory Cytokines and Oxidative Stress in Alloxan-Induced Diabetic Wistar Albino Rat. *J Diabetes Metab Disord* **2020**, *19* (2), 1543–1556. https://doi.org/10.1007/s40200-020-00690-2.

[11] Intharuksa, A.; Arunotayanun, W.; Yooin, W.; Sirisa-ard, P. A Comprehensive Review of *Andrographis Paniculata* (Burm. f.) Nees and Its Constituents as Potential Lead Compounds for COVID-19 Drug Discovery. *Molecules* **2022**, *27* (14), 4479. https://doi.org/10.3390/molecules27144479.

[12] Atanasov, A. G.; Zotchev, S. B.; Dirsch, V. M.; Supuran, C. T. Natural Products in Drug Discovery: Advances and Opportunities. *Nat Rev Drug Discov* **2021**, *20* (3), 200–216. https://doi.org/10.1038/s41573-020-00114-z.

[13] Kinghorn, A. D.; Pan, L.; Fletcher, J. N.; Chai, H. The Relevance of Higher Plants in Lead Compound Discovery Programs. *J Nat Prod* **2011**, *74* (6), 1539–1555. https://doi.org/10.1021/np200391c.

[14] Sun, D.; Gao, W.; Hu, H.; Zhou, S. Why 90% of Clinical Drug Development Fails and How to Improve It? *Acta Pharm Sin B* **2022**, *12* (7), 3049–3062. https://doi.org/10.1016/j.apsb.2022.02.002.

[15] Dowden, H.; Munro, J. Trends in Clinical Success Rates and Therapeutic Focus. *Nat Rev Drug Discov* **2019**, *18* (7), 495–496. https://doi.org/10.1038/d41573-019-00074-z.

[16] Ya'u Ibrahim, Z.; Uzairu, A.; Shallangwa, G.; Abechi, S. Molecular Docking Studies, Drug-Likeness and in-Silico ADMET Prediction of Some Novel β-Amino Alcohol Grafted 1,4,5-Trisubstituted 1,2,3-Triazoles Derivatives as Elevators of P53 Protein Levels. *Sci. Afr.* **2020**, *10*, e00570. https://doi.org/10.1016/j.sciaf.2020.e00570.

[17] Nakamura, Y.; Afendi, F. M.; Parvin, A. K.; Ono, N.; Tanaka, K.; Hirai Morita, A.; Sato, T.; Sugiura, T.; Altaf-Ul-Amin, M.; Kanaya, S. KNApSAcK Metabolite Activity Database for Retrieving the Relationships between Metabolites and Biological Activities. *Plant Cell Physiol* **2014**, *55* (1), e7. https://doi.org/10.1093/pcp/pct176.

[18] Pires, D. E. V.; Blundell, T. L.; Ascher, D. B. PkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. *J Med Chem* **2015**, *58* (9), 4066–4072. https://doi.org/10.1021/acs.jmedchem.5b00104.

[19] Stillhart, C.; Vučićević, K.; Augustijns, P.; Basit, A. W.; Batchelor, H.; Flanagan, T. R.; Gesquiere, I.; Greupink, R.; Keszthelyi, D.; Koskinen, M.; Madla, C. M.; Matthys, C.; Miljuš, G.; Mooij, M. G.; Parrott, N.; Ungell, A.-L.; de Wildt, S. N.; Orlu, M.; Klein, S.; Müllertz, A. Impact of Gastrointestinal Physiology on Drug Absorption in Special Populations—An UNGAP Review. *Eur J Pharm Sci* **2020**, *147*, 105280. https://doi.org/10.1016/j.ejps.2020.105280.

[20] Mansoor, A.; Mahabadi, N. Volume of Distribution. In *StatPearls*; StatPearls Publishing: Treasure Island (FL), 2022.

[21] Mikitsh, J. L.; Chacko, A.-M. Pathways for Small Molecule Delivery to the Central Nervous System Across the Blood-Brain Barrier. *Perspect Medicin Chem* **2014**, *6*, 11–24. https://doi.org/10.4137/PMC.S13384.

[22] Esteves, F.; Rueff, J.; Kranendonk, M. The Central Role of Cytochrome P450 in Xenobiotic Metabolism—A Brief Review on a Fascinating Enzyme Family. *J Xenobiot* **2021**, *11* (3), 94–114. https://doi.org/10.3390/jox11030007.

[23] Motohashi, H.; Inui, K. Organic Cation Transporter OCTs (SLC22) and MATEs (SLC47) in the Human Kidney. *AAPS J* **2013**, *15* (2), 581–588. https://doi.org/10.1208/s12248-013-9465-7.

[24] Pholphana, N.; Rangkadilok, N.; Saehun, J.; Ritruechai, S.; Satayavivad, J. Changes in the Contents of Four Active Diterpenoids at Different Growth Stages in Andrographis Paniculata (Burm.f.) Nees (Chuanxinlian). *Chinese Medicine* **2013**, *8* (1), 2. https://doi.org/10.1186/1749-8546-8-2.

[25] Babu, S.; Jayaraman, S. An Update on β-Sitosterol: A Potential Herbal Nutraceutical for Diabetic Management. *Biomed Pharmacother* **2020**, *131*, 110702. https://doi.org/10.1016/j.biopha.2020.110702.

[26] Xu, C.; Wang, Z.-T. [Chemical constituents from roots of Andrographis paniculata]. *Yao Xue Xue Bao* **2011**, *46* (3), 317–321.