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Phytochemical profiles and novel biofunctions of *Dillenia ovata* Wall. ex Hook.f. et Thomson: A Vietnamese indigenous medicinal plant

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Abstract

Dillenia ovata Wall. ex Hook. f. et Thomson (DOWHT)—an indigenous medicinal plant collected in the Central Highland of Vietnam—has been utilized by medical folks for ages. However, almost related scientific evidence still has not been clarified. This study aimed to report the phytochemical profiles and novel potent biofunctions of DOWHT. The testing bioactivities of different parts used of DOWHT indicated this herbal demonstrated moderated inhibition against α-amylase and α-glucosidase, while it showed potent anti-oxidant and anti-acetylcholinesterase (AChE). Of these, the inhibition against the key enzymes targeting anti-diabetes and Alzheimer's disease was notably reported for the first time. The phytochemical profiles of all the parts used of DOWHT were investigated via GC–MS and UHPLC analysis. Totally, 46 compounds, including 30 volatiles (compounds 1–30) and 16 phenolics (compounds 31–46), were newly identified from DOWHT extracts. In this work, various volatiles and phenolics were newly found in this herbal species. In the docking study, some major phenolics including Epigallocatechin gallate (34), Epicatechin gallate (36), Vitexin (37), and Apigetrin (41) possess DS values (− 12.5 to − 13.3 kcal/mol) better than Berberine chloride (− 12.1 kcal/mol). Moreover, almost tested metabolites comply with the drug-likeness properties of Lipkin's rules prediction and nontoxicity via the ADMET test. The results suggest the herb DOWHT may be a potential source for drug discovery and these in silico results may be good reference information for further in vitro and in vivo experiments.

Keywords *Dillenia ovata* · Anti-oxidant · Anti-diabetes · Anti-Alzheimer · Phytochemical profile · Drug discovery · Medicinal plants

Extended author information available on the last page of the article

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Introduction

Medicinal plants and their isolated compounds have been considered the best choice for controlling diseases and health enhancement to replace chemical agents showing numerous negative side effects for users. Various potent medicinal benefits of plant extracts and their isolated compounds have been investigated for their potent medicinal benefits via in vitro and in vivo studies [1–5]. The vast array of studies indicated that alkaloids, flavonoids, tannins, and phenolics are major components of plants [2, 6], and some plant species contain unique saponins [7]. However, among the estimated 250,000–400,000 plant species, only 6% have been studied for biological activity, and about 15% have been investigated for phytochemicals. This shows a need for phyto-pharmacological evaluation of herbal drugs [7].

Vietnam is a tropical country with a great biodiverse currently ranked sixteenth place in the world. More than 10,000 reported plant species were identified from Vietnam, of these around 4,000 herbal species have been cost-effective used by medical folks [8], and it is estimated that 75% of Vietnamese people use traditional medicine as their primary source of treatment for common health problems [8]. However, lots of herbals are being used by folks with little or nonscientific evidence. Thus, studies based on biological effects and phytochemical profiles for further investigation in various animal models, and pre-clinical and clinical research are in need.

Dillenia species have been used as traditional medicines in South and Southeast Asian countries for the management of various diseases (arthritis, dysentery, diabetes, blennorrhagia, hepatitis, gastrointestinal disorders, inflammation, hemorrhoids, wounds, and leishmanial ulcers) [9, 10]. The modern pharmacological investigations revealed that the extracts from genus *Dillenia* demonstrated potential biological activities: anti-oxidant, anti-microbial, anti-hemorrhagic, anti-inflammatory, anti-ulcer, immunological, anti-cancer, etc. [9, 10]. Notably, almost all parts of *Dillenia* plants, including leaves, fruit, stem bark, root, and also their Latex, are traditionally utilized for therapeutic purposes [10].

Dillenia ovata Wall. ex Hook. f. et Thomson (DOWHT) is a medicinal plant belonging to the genus *Dillenia*. DOWHT has also been utilized by medical folks for ages [9–11]. However, very few studies concerning the investigation of medical effects, chemical profiles as well as the molecular mechanism action of bioactive compounds were identified from DOWHT [12–15]. Notably, up to date, no reports on biological and phytochemicals of DOWHT growing in the Central Highland of Vietnam have been announced. This study accessed medical effects, including anti-oxidant activity, anti-diabetes, and anti-Alzheimer. The chemical profiles of the extracts of some parts used of DOWHT were investigated. The interaction and potential drug development of the bioactive compounds identified from DOWHT were also conducted using computational study in this work. The designation and works conducted in this report are summarized in Fig. 1.

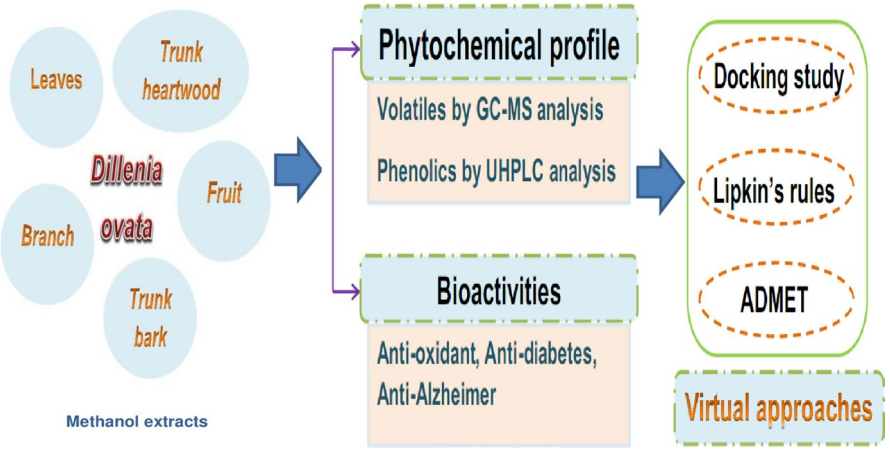


Fig. 1 The designation and works conducted in this report

Experimental section

Materials

The samples of *Dillenia ovata* Wall. ex Hook.f. et Thomson, including leaves, trunk heartwood, trunk bark, branch, and fruit of the medicinal plant, were collected in Yok Don National Park, Dak Lak Province, Vietnam, in 2022. This medicinal plant was identified by a botanist (Thi Huong Tran), and the voucher specimen DOWHT-YD-85 (Dillenia ovata Wall. ex Hook. f. et Thomson—Yok Don—Sample 85) was deposited at the Natural Products Lab of Institute of Biotechnology and Environment, Tay Nguyen University. The dry template was stored at Institute of Biotechnology and Environment, Tay Nguyen University, Buon Ma Thuot, Vietnam. The images of *Dillenia ovata* Wall. ex Hook.f. et Thomson (DOWHT) at natural conditions and the dry template are shown in the supplementary section (Figure S1, Figure S2). Porcine pancreatic (type VI-B) α-amylase was bought from Sigma-Aldrich, USA. Rat α-glucosidase, acetylcholinesterase, and 2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co., USA. Acarbose was obtained from Sigma-Aldrich (St. Louis City, MO, USA). The solvents, reagents, and other commonly used chemicals were of the highest grade available.

Method preparation of methanol (MeOH) extracts from different parts used of DOWHT using ultrasound-assisted extraction method

The ultrasound-assisted extraction (UAE) method: The preparation of MeOH extracts from different parts used of DOWHT was done according to the method presented in our previous report [16]. The powder of different parts (2 g) including leaves, trunk heartwood, branch, trunk bark, and fruit was soaked in 20 mL of methanol for 2 min and then sonicated for 20 min at 25 kHz with a micro-tipped probe

(diameter 10 mm) immersed 1 cm into above 20 mL of methanol extract (Vietsonic, VS28H, Vietnam). The extracts were centrifuged at 10,000 × g for 15 min, and the supernatants were filtered through a Whatman filter paper. The residues were extracted twice, as mentioned above. Then the extracts were combined, and methanol was added to make a volume of 100 mL in a volumetric flask. The samples were stored at 0-4^{0}C until further use.

Biological assays

Anti-oxidant activity was evaluated using the DPPH radical scavenging assay and ABTS assay previously described in detail by Nguyen et al. [17]. The mixtures in a 96-well plate of 100 µL samples (in various concentrations) or distilled water (blank sample) with 25 µL DPPH 0.75 mM (soluted in methanol) were kept for 30 min in the dark and measurement of the optical density at a wavelength of 517 nm. For ABTS radical-scavenging activity assay, tested samples were soluted in various concentrations and mixed with pre-diluting ABTS solution (reaching an optical density of 0.7). Kept the mixture for 10 min at room temperature and measured absorption at a wavelength of 734 nm. Trolox, a commercial anti-oxidant compound, was also tested for anti-oxidant effect for comparison. All tests were conducted in triplicate. The control sample using a commercial compound (Trolox) was tested at the same condition for comparison purposes. The anti-oxidant ability was measured via formulation: inhibitory activity (%) = (A–B)/A × 100%. Of those: A is the optical density of a blank sample and B is the optical density of tested samples. The IC_{50} value (half-maximal inhibitory concentration of samples) was determined via a standard equation constructed by inhibitory activity (%) and respective concentration of the tested sample.

In vitro anti-diabetic effect was accessed using anti-α-glucosidase and anti-α-amylase assays presented by Nguyen et al. [16] and Nguyen TH et al. [18], respectively. A mixture of 50 µL sample solution, 50 µL α-glucosidase solution, and 100 µL potassium phosphate buffer was incubated for 20 min at 37 °C. Then added 50 µL p-nitrophenyl glucopyranoside and kept for 40 min at 37 °C. The optical density was measured at a wavelength of 410 nm. For anti-α-amylase assay, 50 µL sample (at different concentrations) was mixed with 150 µL α-amylase solution (0.25 U/mL) and kept for 10 min at 37 °C. Then, added 200 µL soluble starch 0.25% and measured optical density at 540 nm after being kept for 20 min at 37 °C. The control sample using a commercial compound (acarbose) was tested at the same condition for comparison purposes. The inhibitory activity (%) and IC_{50} value also were defined the same as above.

The anti-Alzheimer ability was evaluated via inhibition of acetylcholinesterase—one of the key enzymes related to Alzheimer's disease, and the assay was presented in the previous work [19]. Kept a mixture of 120 µL phosphate buffer, 60 µL tested sample, and 60 µL enzymatic solution (0.5 mM) for 15 min at 25 °C in a 96-well plate. Then, added 30 µL 5, 5′-dithiobis-2-nitrobenzoic acid (0.003 M) and 40 µL acetylthiocholine iodide (0.002 M) and kept the mixture for 10 min at 25 °C. Measurement of the absorbance is at the wavelength of 415 nm. The control sample using

a commercial compound (Berberine chloride) was tested at the same condition for comparison purposes. The inhibitory activity (%) and IC_{50} value also were defined the same as above.

GC–MS analysis

The herbal extracts were soluted in MeOH and then using the QuEChERS method of solid-phase extraction to purify. GC (Thermo Trace GC Ultra, USA) and ITQ900 (Thermo, USA) were conducted for analysis. A TG-SQC capillary column (30 m × 0.25 mm × 0.25 µm) was used for the GC–MS equipment. Helium (99.999%)—a carrier gas —was used at a flow rate of 1 mL/min. The sample solution (1µL) with a split ratio of 10:1 was injected into the system. The ion-source temperature and the injector temperature were set at 230 °C and 250 °C, respectively. The oven temperature program was set increasingly from 70 °C (isothermal for 2 min) up to 280 °C with a velocity of 15 °C/min. MS data were collected at 70 eV in a scanning interval time of 0.5 s, and fragments from 50 to 650 Da. The compounds were identified via reference with reported compounds from the Mass Spectra Library (NIST 17.L and Wiley).

UHPLC analysis

The extract samples were dissolved in MeOH at a concentration of 10 mg/mL and filtered by a 0.45-µm PVDF membrane filter (Millipore Sigma, USA). The volume of 2 µL of the sample was injected into UHPLC (Thermo Ultimate 3000) system. The component in the sample was separated by a column (Hypersil GOLD aQ, 3 µm, 150 × 2.1 mm) which was kept at a temperature of 30 °C. A mobile phase including methanol (MeOH) and water along with 0.1% phosphoric acid was applied, and the program was set following as 5% MeOH (0.0–0.5 min), 5–30% MeOH (0.5–8.0 min), 30–45% MeOH (8.0–13 min), 45–65% MeOH (13.0–18.0 min), 65–95% MeOH (18.0–22.0 min), 95–5% MeOH (22.0–23.0 min). The flow rate was used at 0.2 mL/min, and the constituents were detected at 265 nm [20].

Virtual studies

Docking study

A docking study was carried out by using Molecular Operating Environment software (MOE-2015.10) to predict the active compound concerning inhibition against the respective enzyme targeting. The protocol was performed according to steps presented in the former reports [18, 21–24].

Enzyme structure preparation: The acetylcholinesterase structure data were obtained from RCSB Protein Data Bank. Their 3D structures and the most active sites on each enzyme were prepared by MOE-2015.10 software. All of it was conducted at the same virtual pH of 7.

Ligand structures preparation: The inhibitor structures (ligands) from the herbal extracts were constructed using ChemBioOffice 2018 software and MOE software. The parameter condition was set up at Force field MMFF94x; R-Field 1: 80; cell shape 90, 90, 90; and gradient 0.01 RMS kcal.mol^{-1}A^{-2}; virtual pH 7; cutoff, Rigid water molecules, space group p1, cell size: 10, 10, 10.

Docking ligands into enzymes and the collection of output data: The ligands were docked into the active site of enzymes by MOE software. Some output data are harvested to analyze including RMSD (root-mean-square deviation), DS (docking score), linkage types, the linkages distances, and compositions of amino acids.

The five Lipkin's rules and pharmacokinetic properties prediction

The five Lipkin's rules were conducted by the online software accessed at (http://www.scfbioiitd.res.in/software/drugdesign/lipinski.jsp (accessed on June 10, 2023)). Some pharmacokinetic properties were analyzed via ADMET assay by a web tool SwissADME. The output data of pharmacokinetic parameters (Water solubility, CaCO2 permeability, Intestinal absorption, Skin permeability, P-glycoprotein substrate, P-glycoprotein I inhibitor, P-glycoprotein II inhibitor, VDss of human, Fraction unbound, BBB permeability, CNS permeability, CYP2D6 substrate, CYP3A4 substrate, CYP1A2 inhibition, CYP2C19 inhibitor, CYP2C9 inhibitor, CYP2D6 inhibitor, CYP3A4 inhibitor, Total clearance, Renal OCT2 substrate, AMES toxicity, Max. tolerated dose, hERG I inhibitor, hERG II inhibitor, Oral rat acute toxicity, Oral rat chronic toxicity, Hepatotoxicity, Skin sensitization, T.Pyriformis toxicity, Minnow toxicity) have been detailed in a previous report [25] and used as a public reference accessed online at (http://biosig.unimelb.edu.au/pkcsm/theory (accessed on June 10, 2023)).

Statistical analysis

Experimental results were conducted with triplicates and statistically processed on IBM SPSS Statistics Version 25. The data represent for the mean of 3 replicates $\pm$ standard deviation with significance $p < 0.05$.

Results and Discussion

Biological activities evaluation of extracts from different parts used of DOWHT

For investigating the medical effects of DOWHT, some parts used of this herbal, including leaves, trunk heartwood, trunk bark, branch, and fruit, were extracted with MeOH and the extracts were tested for anti-oxidant, anti-diabetes, and anti-Alzheimer activities. The bioactivities of these extracts were presented under IC_{50} values (half-maximal inhibitory concentration of samples) in Table 1.

Free radicals may attack all types of cells in the body and lead to oxidant stress which is a vital reason cause to other serious diseases [26]. The elimination of DPPH (a free radical) and ABTS cation radicals are popular methods for anti-oxidant

Table 1 The bioactivities of different parts of DOWHT

No	Part used	Anti-oxidant (μg/mL)		Anti-diabetes (μg/mL)		Anti-Alzheimer (μg/mL)
		Anti-DPPH	Anti-ABTS	aGI	aAI	AChEI
1	Leaves	18.9 ± 0.3^{e}	$1.03 \pm 0.06^{d,e}$	187.3 ± 0.4^{e}	34.5 ± 0.4^{d}	0.11 ± 0.01^{d}
2	Trunk heartwood	48.1 ± 0.4^{c}	4.67 ± 0.39^{c}	275.9 ± 0.5^{c}	102.9 ± 0.4^{b}	0.11 ± 0.01^{d}
3	Branch	10.0 ± 0.3^{g}	0.8 ± 0.01^{e}	93.5 ± 0.5^{f}	26.7 ± 0.4^{e}	0.40 ± 0.01^{c}
4	Trunk bark	22.4 ± 0.3^{d}	1.3 ± 0.01^{d}	194.4 ± 0.9^{d}	49.1 ± 0.4^{c}	0.71 ± 0.01^{b}
5	Fruit	271.1 ± 0.6^{a}	20.60 ± 0.4^{b}	537.4 ± 0.6^{b}	379.9 ± 1.4^{a}	0.11 ± 0.005^{d}
6	Trolox	60.0 ± 0.3^{b}	33.2 ± 0.3^{a}			
7	Acarbose			1249.9 ± 0.3^{a}	5.6 ± 0.2^{f}	
8	Berberine chloride					301.02 ± 0.15^{a}
9	Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
10	P	0.05	0.05	0.05	0.05	0.05
11	CV%	1.13	3.30	1.32	0.25	5.10
12	LSD	0.64	0.46	0.96	1.15	0.11

aGI: α-glucosidase inhibition; *aAI*: α-amylase inhibition; *AChEI*: acetylcholinesterase inhibition; *CV*: coefficient of variation; *LSD*: least significant difference; a,b,c,d,e,f: values in the same column with the different letters are significantly different

ability measurement. Thus, the anti-oxidant activity was tested via assessment of the DPPH and ABTS radical scavenging ability in this study. Based on the results in Table 1, the extracts of DOWHT showed high ABTS and DPPH radical scavenging ability with the low IC_{50} values in the range of 0.8–20.6 μg/mL and 10.0–271.1 μg/mL, respectively. Among them, three parts including branch, leaves, and trunk bark showed good effects on both DPPH and ABTS activities. The IC_{50} values for DPPH and ABTS are respective of 10.0 μg/mL and 0.8 μg/mL for branch; 18.9 μg/mL and 1.0 μg/mL for leaves; 22.4 μg/mL and 1.3 μg/mL for trunk bark. The fruit presents the weakest activity with IC_{50} values of 271.1 μg/mL and 20.6 μg/mL, respectively. Trolox, a commercial anti-oxidant compound, was also tested for anti-oxidant effect, showing moderate ABTS and DPPH radical scavenging ability with IC_{50} values of 60 μg/mL and 33.2 μg/mL, respectively. Thus, in the comparison, almost the extracts of DOWHT demonstrated more potential anti-oxidant effect than that of Trolox. In addition, DOWHT's bark part was reported in another research for anti-oxidant ability via DPPH assay with a good inhibition of 2–10 μg/mL [15]; as such, DOWHT may be suggested as rich sources for anti-oxidant compounds.

Anti-diabetes activity was tested via inhibition on two main types of enzymes related to increasing blood sugar including α-glucosidase [27] and α-amylase [28]. These samples possess moderate enzymes inhibition activity, and the inhibition of α-amylase activity (IC_{50} values of 26.7–379.9 μg/mL) seems higher compared to α-glucosidase (IC_{50} values of 93.5–537.4 μg/mL). The branch demonstrated the best effect (IC_{50} = 26.7–93.5 μg/mL) and the following are leaves (IC_{50} = 34.5–187.4 μg/mL) and trunk bark (IC_{50} = 49.1–194.4 μg/mL), while fruit also showed the weakest activity (IC_{50} = 379.9–537.4 μg/mL). Acarbose, an anti-diabetic compound, was also tested inhibition against α-glucosidase and

α-amylase and showed IC_{50} values of 1250 µg/mL and 5.6 µg/mL, respectively. Thus, the DOWHT extracts showed potential effects, comparable to or higher than acarbose.

Alzheimer's inhibition was tested via resistance of acetylcholinesterase (AchE)—a key enzyme that causes decreased choline neurotransmitters [29]. The extracts of DOWHT showed potent inhibition for AChE with low IC_{50} values under 0.7 µg/mL. Comparison of different parts, three parts including leaves, trunk heartwood, and fruit, possesses the same high effect with IC_{50} values of 0.1 µg/mL. The branch part also presented good activity with an IC_{50} value of 0.4 µg/mL. The IC_{50} of trunk bark reached 0.7 µg/mL. Berberine chloride, a commercial AchE inhibitor, was tested for comparison and showed a weaker effect (IC_{50} values of 301 µg/mL) than that of DOWHT extracts.

In general, extracts of DOWHT herb showed good medical effects on tested bioactivities compared to control compounds; this may be due to the extract of this herb possessing abundant bioactive compounds belonging to popular groups such as phenolic compounds, alkaloids, saponins, tannins, coumarins, polypeptides, cardiac glycosides, resins, flavonoids, and terpenoids [13, 15]. Of those, anti-diabetes and anti-Alzheimer activities of DOWHT extracts were notably reported for the first time in this work. Up to date, the studies on elucidating the bioactivities of different parts used of this herb have few reported; as such, this is also a unique point of this work. Notably, this herb's leaf extract was recorded as nontoxicity for normal human cells [12]; thus, DOWHT is a potential herb to keep researching further. In the bioactivity comparison of herbal parts together, the fruit showed weak activity in almost tests (except for AChE inhibition). Branch extract presented the most potent effects on almost tested bioactivities. Leaves and trunk bark also showed high inhibition, and they are also common parts that were used in research [30–34]. While the branch part is rarely used in research, it also seems potential in fact exploitation and application. The branch part can harvest significant amounts annually without cutting trees. Thus, it is also a promising part to discover for further studies.

Natural phenolic compounds from herbs have recorded a lot of potential bioactivities for promising applications in medicine [35, 36]. In fact, some of these components were used in functional food for human health promotion [35]. Based on these potential applications, the demand to discover new natural phenolic compounds from medicinal plants is still being carried on. *Dillenia* species were also reported for many bioactivities in medicine, even showing potential effects in preclinical research [37]. Notably, the activities of DOWHT have only been tested for anti-bacterial, anti-fungal activity, and anti-oxidant [14].

Some reports also evaluated bioactivities for different parts of medicinal plants. The anti-oxidant effect of *Zanthoxylum armatum*'s parts was conducted [38]. The results showed that fruit inhibited the best and following is bark, and seed with IC_{50} values of 45.6, 67.8, and 86.75 µg/mL, respectively. The different parts of *Euphorbia neriifolia* Linn. herb were tested for anti-oxidant activity by some assays such as DPPH, FRAP, and H_2O_2 assays [39]. Of those, in MeOH extract, the leaves part showed the best effects in FRAP and H_2O_2 tests with the lowest IC_{50} values of 153.4 µg/mL and 2.0 µg/mL, respectively. The latex part presented the highest activity in the DPPH test with an IC_{50} value of 1.0 µg/mL. Each herb will have different

potential parts used. In this study, the branch part of DOWHT is considered a potential part used and promising for use in further research.

Chemical profiles and the contents of major phenolic compounds from herbal DOWHT

In this study, the volatile compounds in different parts of herbal DOWHT were detected via GC–MS analysis (results in Table 2). In total, 30 volatile compounds (1–30) were detected in five different parts of this herb. Compounds were arranged, respectively, in order of the detected peak based on the recorded retention time. There are differences in composition of volatile constituents and their content in each part of the herb. Almost volatile compounds (20 compounds) are concentrated on the DOWHT's leaves relative contents around 0.6–20.6%. In this part, six major volatiles were first identified with high content, including 4-Pregnen-20-&-ol-3-one (6.1%), trans-α-bergamotene (6.6%), shizukanolide (5.6%), tetradecanoic acid, methyl ester (20.6%), octadecanoic acid (9.0%), sebacic acid, 2-(2-chlorophenoxy) ethyl pentyl ester (7.1%). No volatile compounds were found on the trunk bark and fruit parts. In total, 15 volatile compounds in total 30 compounds appear in the trunk heartwood of DOWHT with relative percent contents in the range of 2.8–13.3%. The branch part has 13 compounds with a content in the range of 3.3–14.7%. The chemical structures of these volatile compounds are shown in Fig. 2, and the GC–MS profiles are presented in supplementary section (Figure S3-S5).

The phenolic compounds and their content were also determined by the UHPLC method. The result is presented in Table 3. The data show that all parts of herbal DOWHT are rich in phenolic compounds with high content. Sixteen phenolic compounds (31–46) were detected in DOWHT extracts, and almost compounds belonged to the flavonoid group; only three compounds are polyphenols including gallic acid (31), chlorogenic acid (33), and salicylic acid (38). Of those, salicylic acid (38) is a phenolic compound accounting for the highest content in almost parts of this herb up to 19,937.3 μg/g. In five of the herbal parts used, the branch and fruit possess rich phenolic compounds; the range of respective contents is low at 5.7–482.6 μg/g and 21.2–2466.2 μg/g. In contrast, the leaves part only has nine per total of sixteen phenolic compounds, but it Is a high range of content around 110.6–15,237.1 μg/g. In all parts, trunk heartwood and trunk bark are potential parts for exploitation that possess abundant phenolic compounds with high contents of 10.7–19,937.3 μg/g and 68.8–13,536.7 μg/g, respectively. The chemical structures of these phenolics are shown in Fig. 3, and the UHPLC fingerprinting is presented in the supplementary section (Figure S6-S11).

Among species belonging to *Dillenia* family, few studies related to the phytochemical of DOWHT have been reported [12–15]. Research by Thooptianrat et al. [12] analyzed the phytochemical profile in the leaves of various *Dillenia* species via the GC–MS method. This work used hexane solvent for extraction and in the extract of DOWHT detected some different compounds compared to our research (using methanol solvent) including oleamide (36.9%), squalene (3.5%), β-sitosterol (5.0%), vitamin E (18.5%), quinone methide (4.7%), palmitic acid (4.6%), stigmasterol

Table 2 Profile of volatile compounds in different parts of DOWHT detected by GC–MS

No	Name	RT (min)	Relative contents of volatile compounds in DOW's parts (%)				
			Leaves	Trunk heartwood	Branch	Trunk bark	Fruit
1	o-Xylene	5.1	2.8 ± 0.3^{b}	–	13.1 ± 0.3^{a}	–	–
2	Phenethyl butenoate	5.5	2.1 ± 0.2^{c}	3.5 ± 0.3^{b}	9.2 ± 0.3^{a}	–	–
3	Oxalic acid, isobutyl pentyl ester	7.4	1.1 ± 0.1^{c}	2.8 ± 0.2^{b}	4.6 ± 0.2^{a}	–	–
4	Trans-3-chloroallyl alcohol	9.2	0.60 ± 0.04^{c}	2.8 ± 0.2^{b}	4.0 ± 0.2^{a}	–	–
5	Allyl 3-indoleglyoxylate	10.0	1.4 ± 0.2	–	–	–	–
6	N-Benzyl-N'-phenyl-urea	10.6	–	–	3.3 ± 0.2	–	–
7	Picric acid	11.4	2.5 ± 0.1	–	–	–	–
8	1,14-Dihydroxyhexadecane	13.9	2.6 ± 0.2^{b}	5.3 ± 0.2^{a}	–	–	–
9	Succinic acid, 2-chloro-6-fluorophenyl 2-chlorophenyl ester	14.4	5.1 ± 0.2	–	–	–	–
10	Phthalic acid, 4-isopropylphenyl methyl ester	14.7	2.2 ± 0.2^{c}	13.3 ± 0.4^{b}	14.7 ± 0.7^{a}	–	–
11	4-Phenyl-morpholine-2,6-dione	15.4	–	–	5.1 ± 0.3	–	–
12	N-(2-Hydroxy-3-phenoxypropyl)benzamide	16.0	–	10.7 ± 0.4	–	–	–
13	Myrtenol	17.2	–	6.0 ± 0.2^{b}	8.8 ± 0.3^{a}	–	–
14	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	17.3	–	5.3 ± 0.2	–	–	–
15	γ-Cadinene	17.4	5.8 ± 0.4	–	–	–	–
16	Methyl [2]Staffane-3-carboxylate	17.6	2.2 ± 0.2^{b}	–	3.2 ± 0.2^{a}	–	–
17	2,3-Dehydro-α-ionone	17.7	–	–	3.7 ± 0.2	–	–
18	2-Cyclohexen-1-one, 3-(3-hydroxybutyl)-2,4,4-trimethyl-	18.1	–	4.5 ± 0.1	–	–	–
19	Pentadecanoic acid	18.3	–	7.4 ± 0.2	–	–	–
20	(-)-Curcuphenol	18.7	3.6 ± 0.2	–	–	–	–
21	4-Pregnen-20-&-ol-3-one	18.9	6.1 ± 0.1^{b}	8.0 ± 0.2^{a}	–	–	–
22	Lactose	19.1	5.9 ± 0.3	–	–	–	–
23	Isophthalic acid, 2-chloroethyl pentyl ester	19.2	-	–	12.9 ± 0.4	–	–

Table 2 (continued)

No	Name	RT (min)	Relative contents of volatile compounds in DOW's parts (%)				
			Leaves	Trunk heartwood	Branch	Trunk bark	Fruit
24	Trans-α-bergamotene	19.3	6.6 ± 0.2	–	–	–	–
25	Shizukanolide	20.0	5.6 ± 0.2^{b}	4.5 ± 0.3^{c}	11.1 ± 0.3^{a}	–	–
26	Tetradecanoic acid, methyl ester	20.2	20.6 ± 0.9^{a}	8.0 ± 0.4^{b}	–	–	–
27	Octadecanoic acid	20.5	9.0 ± 0.4^{b}	10.8 ± 0.5^{a}	5.6 ± 0.3^{c}	–	–
28	γ1-cadinene aldehyde	20.6	–	6.2 ± 0.3	–	–	–
29	Sebacic acid, 2-(2-chlorophenox) ethyl pentyl ester	22.0	7.1 ± 0.3	–	–	–	–
30	Stigmasta-5,24(28)-dien-3-ol, (3β,24Z)-	22.1	3.1 ± 0.3	–	–	–	–

Values in the same row with the different letters are significantly different

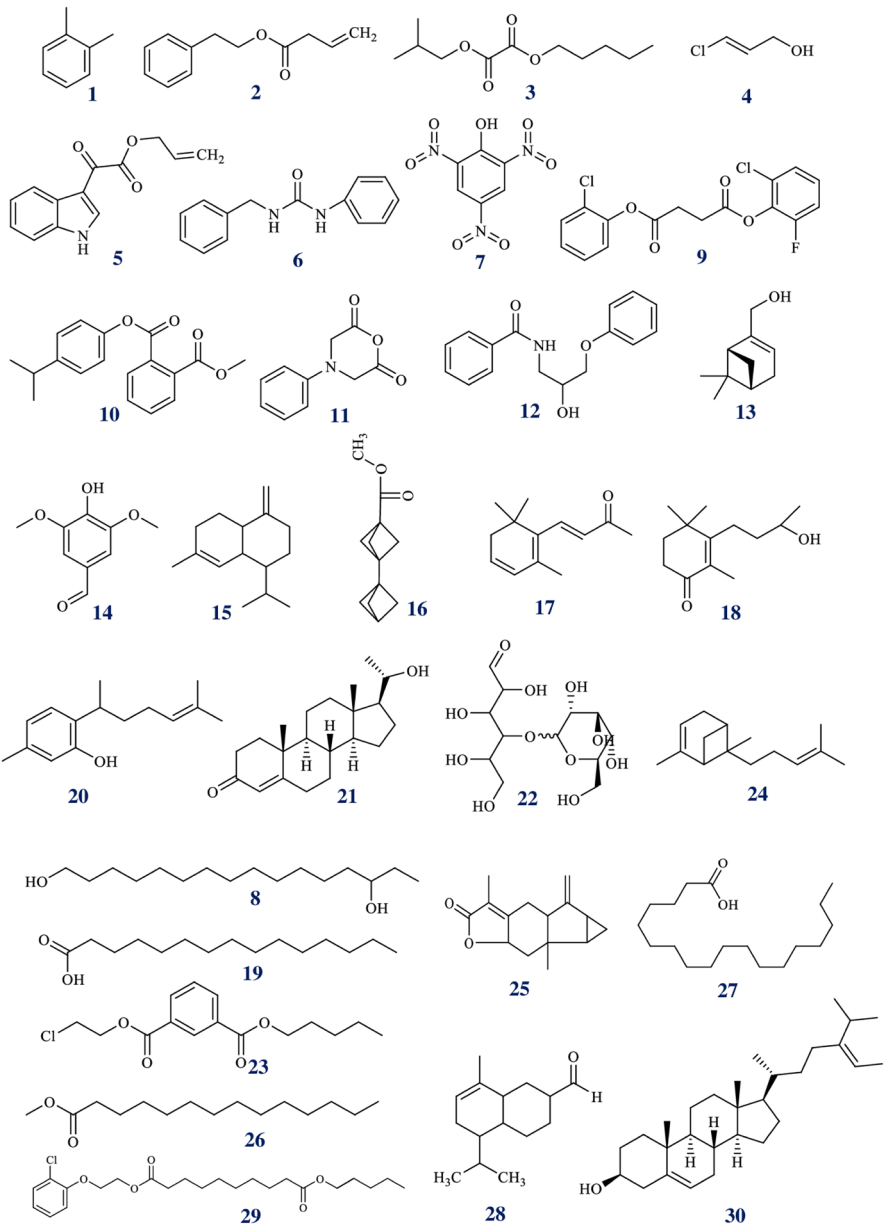


Fig. 2 The chemical structures of volatile compounds were identified from the DOWHT extracts by GC–MS analysis

(2.0%), palmitamide (3.4%), eicosane (1.4%), stearic acid (2.2%), butylated hydroxytoluene (0.8%), 2,4-di-tert-butylphenol (2.3%), unknown compounds (14.1%). Besides, differences in varieties, climatic conditions, and soils also lead to these

Table 3 The content of phenolics in the MeOHs extract from five parts of DOWHT

No	Compound	Family	The content of phenolic compounds (μg/g)					Pr > F	P	CV	LSD
			Leaves	*Trunk heartwood*	*Branch*	*Trunk bark*	Fruit				
31	Gallic acid	Polyphenol	950.4 ± 0.9^{a}	639.8 ± 1.4^{b}	94.6 ± 1.2^{e}	309.6 ± 0.8^{c}	255.7 ± 2.4^{d}	< 0.0001	0.05	0.5	2.6
32	Catechin	Flavonoid	–	950.1 ± 1.9^{b}	385.0 ± 1.4^{d}	1347.6 ± 1.7^{a}	622.7 ± 1.6^{c}	< 0.0001	0.05	0.2	2.7
33	Chlorogenic acid	Polyphenol	–	1457.1 ± 1.8^{a}	107.0 ± 1.5^{d}	427.2 ± 1.9^{b}	281.7 ± 2.2^{c}	< 0.0001	0.05	0.5	3.0
34	Epigallocatechin gallate	Flavonoid	3063.8 ± 2.4^{a}	1958.9 ± 1.3^{b}	111.1 ± 1.4^{e}	788.2 ± 0.9^{c}	299.5 ± 1.3^{d}	< 0.0001	0.05	0.4	2.8
35	Epicatechin	Flavonoid	–	7719.3 ± 1.1^{a}	268.3 ± 0.9^{d}	1344.4 ± 1.2^{b}	435.9 ± 1.2^{c}	< 0.0001	0.05	0.1	1.8
36	Epicatechin gallate	Flavonoid	2713.3 ± 1.9^{a}	2166.3 ± 0.9^{b}	95.4 ± 1.0^{d}	2162.7 ± 1.4^{c}	89.9 ± 1.4^{e}	< 0.0001	0.05	0.5	2.5
37	Vitexin	Flavonoid	1317.7 ± 1.6^{a}	1155.5 ± 1.8^{b}	87.6 ± 1.5^{e}	996.4 ± 1.2^{c}	272.5 ± 0.9^{d}	< 0.0001	0.05	0.5	2.6
38	Salicylic acid	Polyphenol	$15{,}237.1 \pm 1.8^{b}$	$19{,}937.3 \pm 1.8^{a}$	482.6 ± 1.9^{e}	$13{,}536.7 \pm 1.5^{c}$	2466.2 ± 1.1^{d}	< 0.0001	0.05	0.1	3.0
39	Isovitexin	Flavonoid	3517.1 ± 1.4^{a}	820.8 ± 1.1^{c}	46.7 ± 1.4^{e}	996.8 ± 1.3^{b}	289.2 ± 1.2^{d}	< 0.0001	0.05	0.7	2.3
40	Rutin	Flavonoid	–	591.5 ± 1.4^{b}	51.9 ± 0.6^{c}	1038.3 ± 1.9^{a}	–	< 0.0001	0.05	0.3	1.9
41	Apigetrin	Flavonoid	6738.4 ± 2.1^{a}	1409.0 ± 2.1^{b}	256.4 ± 0.9^{d}	1037.2 ± 1.9^{c}	72.6 ± 1.1^{e}	< 0.0001	0.05	0.4	3.1
42	Myricetin	Flavonoid	–	270.6 ± 1.9^{b}	36.9 ± 1.4^{d}	803.9 ± 1.6^{a}	264.7 ± 1.4^{c}	< 0.0001	0.05	1.0	2.5
43	Quercetin	Flavonoid	271.4 ± 1.9^{b}	110.5 ± 1.7^{c}	11.4 ± 0.7^{d}	334.1 ± 1.5^{a}	110.6 ± 2.0^{c}	< 0.0001	0.05	2.1	2.9
44	Luteolin	Flavonoid	110.6 ± 2.0^{c}	225.0 ± 1.2^{b}	8.9 ± 0.1^{e}	702.8 ± 1.4^{a}	69.7 ± 0.9^{d}	< 0.0001	0.05	0.8	2.3
45	Kaempferol	Flavonoid	–	43.7 ± 1.5^{b}	5.7 ± 0.2^{d}	82.3 ± 1.2^{a}	21.2 ± 0.4^{c}	< 0.0001	0.05	1.9	1.6
46	Apigenin	Flavonoid	–	10.7 ± 0.7^{b}	–	68.8 ± 1.9^{a}	–	< 0.0001	0.05	1.9	1.7

CV: coefficient of variation; LSD: least significant difference; a,b,c,d,e,f: values in the same row with the different letters are significantly different

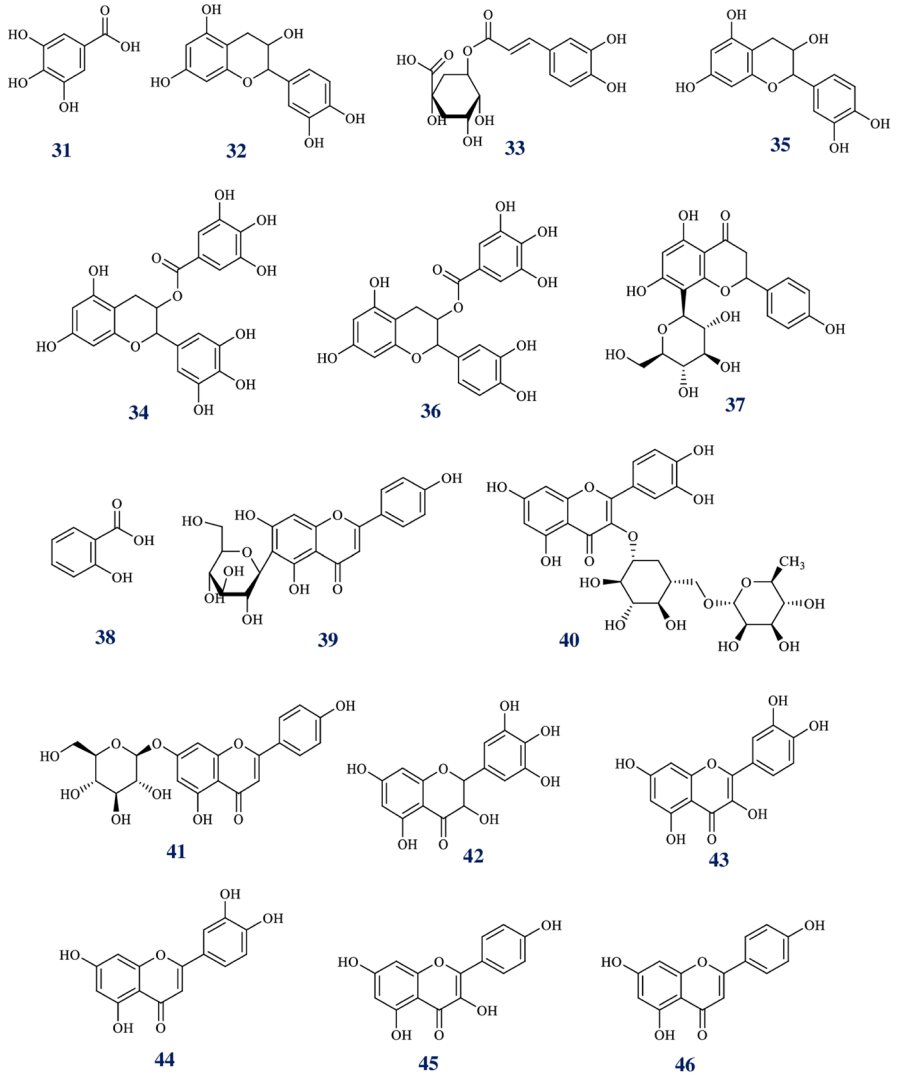


Fig. 3 The chemical structures of phenolic compounds were identified from the DOWHT extracts by UHPLC analysis

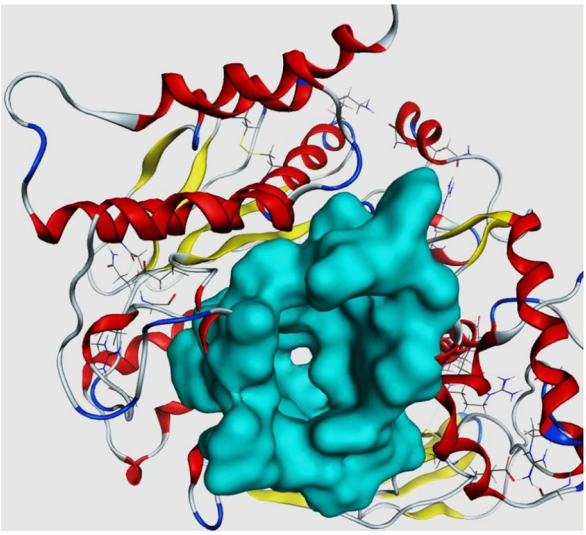
differences in phytochemicals. Some research tested qualitative some phytochemical compounds in ethanol bark extract of DOWHT and confirmed that this herbal part contains phenolic compounds, alkaloids, saponins, tannins, coumarins, polypeptides, cardiac glycosides, resins, flavonoids, and terpenoids [13, 15]. Based on the literature, the phytochemical information on DOWHT still is very limited. Notably, the phytochemical compounds in this work were detected from DOWHT for the first time. Moreover, normally research only focuses on the exploitation of one part of the herb. Thus, this study has been conducted to identify the chemical profile

including the volatile and phenolic compounds and their relative contents in different parts of this medicinal plant. This information has provided useful scientific data to orient further experimental studies.

Potential energy binding and interaction of bioactive compounds toward enzyme acetylcholinesterase targeting anti-Alzheimer via *docking study*

In this part, a virtual study was conducted for acetylcholinesterase due to this enzyme target which was inhibited the most potent. In docking performance, the inhibitor (ligand) may interact with various sites on the enzyme (named binding site), and the most active binding site was chosen for investigation in detail. Based on the output data of MOE using the site finder function, 25 binding sites were found and binding suite 1 (BS1) was suggested as the most active BS. This BS contains 39 residues. The 3D structure of BS1 and its residues are shown in Fig. 4.

Among forty-six compounds identified from herbal DOWHT, nine phenolic compounds (31, 34, 36, 37, 38, 39, 41, 43, and 44) appear in all parts of DOWHT, six volatile compounds (21–27, 29) with high content in the leaves part (where almost volatiles were detected), and berberine chloride—a commercial AchE inhibitor—was used for docking into the BS1 of AchE. The results are presented in Table 4. The effective interaction was displayed via some parameters such as RMSD (root-mean-square deviation), DS (docking score), linkage types, the linkages distances, and compositions of amino acids. The DS value of the ligand and respective target enzyme is under − 3.20 kcal/mol, and it is lower showing a greater enzyme-binding ability [40]. In fifteen compounds, some phenolics such as Epigallocatechin gallate (34), Epicatechin gallate (36),



(a)

39 amino acids containing in the binding site:
GLN69 TYR70 VAL71
ASP72 GLN74 SER81
TRP84 ASN85 PRO86
TYR116 GLY117 GLY118
GLY119 TYR121 SER122
GLY123 SER124 LEU127
TYR130 GLU199 SER200
TRP233 TRP279 LEU282
PHE284 ASP285 SER286
ILE287 PHE288 ARG289
PHE290 PHE330 PHE331
TYR334 GLY335 HIS440
GLY441 TYR442 ILE444

(b)

Fig. 4 The 3D structure of BS1 on AChE **a** the 39 amino acids contained in the binding site **b**

Table 4 The results of docking study of metabolites from *DOWHT* with acetylcholinesterase

Ligands	RMSD (Å)	DS (kcal/mol)	Linkages	Amino acids interacting with the ligands [Distance (Å) / E (kcal/mol)/ linkage type]
4-Pregnen-20-&-ol-3-one (**21**)	1.2	− 8.3	1 linkage (1 H-acceptor)	Tyr130 (2.59/-2.1 H-acceptor)
Trans-α-Bergamotene (**24**)	0.9	− 6.2	2 linkages (2 H-pi)	Trp84 (3.66/-1.0 H-pi) Tyr334 (4.22/-0.6 H-pi)
Shizukanolide (**25**)	1.0	− 6.4	2 linkages (1 H-acceptor, 1 H-pi)	Tyr130 (2.77/-1.3 H-acceptor) Phe330 (3.60/-0.6 H-pi)
Tetradecanoic acid, methyl ester (**26**)	0.9	− 7.1	2 linkages (1 H-acceptor, 1 H-pi)	Arg289 (3.34/-1.9 H-acceptor) Tyr334 (4.01/-0.8 H-pi)
Octadecanoic acid (**27**)	1.5	− 10.0	3 linkages (1 H-donor, 1 H-acceptor, 1 H-pi)	Phe284 (294/-3.6 H-donor)Ser286 (3.17/-0.6 H-acceptor) Trp84 (3.92/-0.7 H-pi)
Sebacic acid, 2-(2-chlorophenoxy)ethyl pentyl ester (**29**)	1.7	− 8.6	1 linkage (1 H-donor)	Tyr70 (3.25/-0.6 H-donor)
Gallic acid (**31**)	1.3	− 12.1	2 linkages (1 H-donor, 1 H-pi)	Glu199 (2.84/-3.8/H-donor) Phe330 (3.27/-0.7/H-pi)
Epigallocatechin gallate (**34**)	1.2	− 12.5	4 linkages (4 H-donor)	Ser81 (2.79/-3.7/H-donor) Ser81 (3.18/-2.6/H-donor) Glu199 (3.60/-0.5/H-donor) Glu199 (2.80/-5.0/H-donor)
Epicatechin gallate (**36**)	1.5	− 13.3	7 linkages (4 H-donor, 1 H-pi, 2 pi-pi)	His440 (2.59/-1.5/H-donor) Glu199 (2.87/-2.7 H-donor) Tyr70 (2.60/-2.5 H-donor) Asn85 (2.56/-1.9 H-donor) Phe330 (3.91/-1.1 H-pi) Trp84 (3.92/-0.0 pi-pi) Tyr334 (3.74/-0.0 pi-pi)
Vitexin (**37**)	1.1	− 12.8	4 linkages (2 H-donor, 1 H-acceptor, 1 H-pi)	Asp72 (3.29/-1.0/H-donor) Glu199 (2.84/-4.3 H-donor) Ser200 (2.62/-1.1 H-acceptor) Phe330 (4.32/-1.3 H-pi)
Salicylic acid (**38**)	1.9	− 9.1	2 linkages (1 H-donor, 1 pi-H)	Ser81 (2.94/-3.5 H-donor) Asp72 (4.30/-0.8 pi-H)
Isovitexin (**39**)	1.5	− 12.0	4 linkages (1 H-donor, 1 H-acceptor, 2 pi-H)	Phe331 (3.07/-1.5 H-donor) Ser286 (3.28/-1.3 H-acceptor) Trp279 (4.75/-0.7 pi-H) Asn280 (3.79/-0.8 pi-H)
Apigetrin (**41**)	1.1	− 12.7	4 linkages (3 H-donor, 1 H-acceptor)	Phe331 (3.13/-1.3 H-donor) Asp72 (2.74/-1.5 H-donor) Glu199 (2.90/-3.4 H-donor) Phe288 (2.90/-2.8 H-acceptor)
Quercetin (**43**)	0.7	− 11.3	5 linkages (3 H-donor, 2 pi-pi)	Ser122 (2.92/-1.1 H-donor) Glu199 (2.70/-2.6 H-donor) Typ70 (2.72/-1.6 H-donor) Trp84 (3.76/-0.0 pi-pi) Trp84 (3.40.-0.0 pi-pi)
Luteolin (**44**)	1.5	− 11.0	3 linkages (1 H-donor, 1 H-acceptor, 1 pi-H)	Glu199 (2.75/-5.8 H-donor) His440 (3.02/-1.5 H-acceptor) Gly118 (3.77/-0.7 pi-H)
Berberine chloride (**BC**)	1.65	− 12.1	3 linkage (1 H-donor, 1 H-acceptor, 1 H-pi)	Glu199 (2.75/-1.4 H-donor) Tyr130 (2.86/-1.3 H-acceptor) Trp84 (3.97/-0.7 H-pi)

Vitexin (37), and Apigetrin (41) possess DS values better than the commercial inhibitor (Berberine chloride) with DS values in a range of − 12.5 to − 13.3 kcal/mol. The remaining compounds have DS values of − 6.2 to − 12.1 kcal/mol. The RMSD value lower than 2.0 Å is widely accepted, and if it is over 3.0 Å that was predicted negligible enzyme inhibition ability [41]. All tested phenolics and volatiles possess the RMSD within the standard range with values of 0.7–1.9 Å. The number of linkages with the enzyme denotes closer interaction of the inhibitor with the enzyme target. The interaction of AChE's amino acids with ligands is detailed in Table 4 and Fig. 5. The commercial control inhibitor only possesses three linkages with AchE (1 H-donor, 1 H-acceptor, 1 H-pi). The phenolic Epicatechin gallate (36) interacts with AChE by the most linkages (seven bonds) including four H-donor bonds with respective amino acids as His440, Glu199, Tyr70, Asn85; one H-pi bond with Phe330; and two pi-pi bonds with Trp84 and Tyr334. Following, quercetin (43) with five linkages includes three H-donor bonds with respective amino acids Ser122, Glu199, Typ70, and two pi-pi bonds with two Trp84. There are four phenolics (34, 37, 39, 41) possessing four linkages with the target enzyme, of those, compound 34 with four H-donor bonds; compound 37 showing four bonds of two H-donor, one H-acceptor, one H-pi; compound 39 has one H-donor, one H-acceptor, two pi-H bonds; compound 41 displays three H-donor bonds and one H-acceptor bond. Phenolic 44 has three linkages (H-donor, H-acceptor, and pi-H) with respective amino acids of AChE including Glu199, His440, and Gly118. The remaining compounds (21, 24, 25, 26, 29, 31, and 38) only bind with this enzyme by one or two linkages.

For further understanding of the more potent inhibitory effect of Epigallocatechin gallate (34), Epicatechin gallate (36), Vitexin (37), and Apigetrin (41) than Berberine chloride, their frontier molecular orbitals were further investigated. As shown in Fig. 6, all these compounds possess an insulation-to-semiconduction energy gap value in the accepted range (3.2 eV < EG < 9 eV), indicating that they have good intermolecular binding capability toward targeting protein [42]. However, compounds (**34**, **36**, **37**, and **41**) demonstrated more inhibitory effect (DS values of − 12.5 to − 13.3 kcal/mol) than Berberine chloride (DS value of − 12.1 kcal/mol). Inside into the interactions of these ligands toward AChE, these compounds (**34**, **36**, **37**, and **41**) were found to interact with AChE via creating more interactions (4–7 linkages) than Berberine chloride (3 linkages). In addition, almost the linkages of compounds **34**, **36**, **37**, and **41** are H-donors. Thus, the data of the highest occupied molecular orbital (HOMO) related to the inhibitory effect were also examined. The compound processed a higher E_{HOMO} value indicating it had a better inhibitory activity [43]. As shown in Fig. 6, all the compounds **34**, **36**, **37**, and **41** had higher E_{HOMO} values in the range of − 6.17.07 to − 5.66 eV than that of Berberine chloride with E_{HOMO} value of − 8.65 eV. In general, these results and the docking study (DS values) are in agreement.

The prediction of drug-likeness properties and pharmacokinetic properties of some potential inhibitors of herbal DOWHT

The prediction of drug-likeness properties was performed using Lipkin's rules if it satisfies at least two in five rules [44]. Five rules include (rule 1)—molecular

mass < 500 Da; (rule 2)—LogP value (high lipophilicity) ≤ 5; (rule 3)—hydrogen bond donors ≤ 5; (rule 4)—hydrogen bond acceptors ≤ 10; (rule 5)—molar refractivity in range of 40–130. Based on the results shown in Table 5, all fifteen compounds complied with Lipkin's rules that suggestion of they possess drug-likeness properties. Of those, seven compounds including five volatiles (21, 24, 25, 26, and 27) and two phenolics (43 and 44) satisfy all rules of this test same as the control compound. Only compound 34 fits with three rules, while the remaining compounds (29, 31, 36, 37, 38, 39, and 41) all comply with four in five rules.

The ability for drug development will be limited by unexpected pharmacokinetic properties. Currently, pharmacokinetic properties (absorption, distribution, metabolism, excretion) and toxicity are presentive for efficacy and safety in drug discovery and development that can be predicted via the ADMET test [45]. The ADMET results of fifteen compounds are presented in Tables 6 and 7. Phenolics show good water solubility, while volatiles possess moderate water solubility. The capacity of $CaCO_2$ permeability of all volatiles is significant, and among phenolics, only compound 5 complies with this property. All compounds have good intestinal absorption, and almost, all can have skin permeability (except volatiles 24 and 26). Besides, only phenolics (34, 36, 37, 39, 41, 43, and 44) are P-glycoprotein substrates. Phenolics (34, 36, 37, 39, 43, and 44) present a high volume of distribution in the human body (VDss). Phenolics (34, 36, 37, 39, 41, 43, and 44) and volatiles (24, 26, and 27) indicate unbound (free) drugs. All phenolics cannot cross the blood–brain barrier (BBB) and have noneffect on the nervous system (except compound 44), however, almost volatiles can impact the nervous system, and volatiles 24–26 can pass over BBB. Although phenolics do not be substrates of cytochrome P450 enzyme systems, but almost these compounds also do not inhibit this enzyme system, in which all volatiles are considered as the CYP3A4 substrate. Almost compounds have a good half-life with relatively low total clearance. Almost phenolics do not be the substrate of OCT2, except compound 25. All compounds showed no AMES toxicity, and only compounds 21, 29, 34, and 36 are hERG II inhibitors. The oral rat acute toxicity expressed as LD50 and dosing > 2 mol/kg is considered safe; of those, only volatiles 24–27 have doses under 2 mol/kg.

In general, drug-likeness and pharmacokinetic properties of some metabolites from DOWHT herb were predicted to suggest this herb may be a potential source for drug discovery. These in silico results may be good reference information for further in vitro and in vivo experiments.

Conclusions

The extracts of herbal *Dillenia ovata* Wall. ex Hook. f. et Thomson (DOWHT) were found potent anti-oxidant, anti-anti-acetylcholinesterase, and moderate inhibition against α-amylase and α-glucosidase. Of these, the inhibition against the key enzymes targeting anti-Alzheimer's and anti-diabetes disease was the new records in this work. The phytochemical profiles of all the parts used of DOWHT were investigated using GC–MS and UHPLC analysis, and various volatile and phenolic were

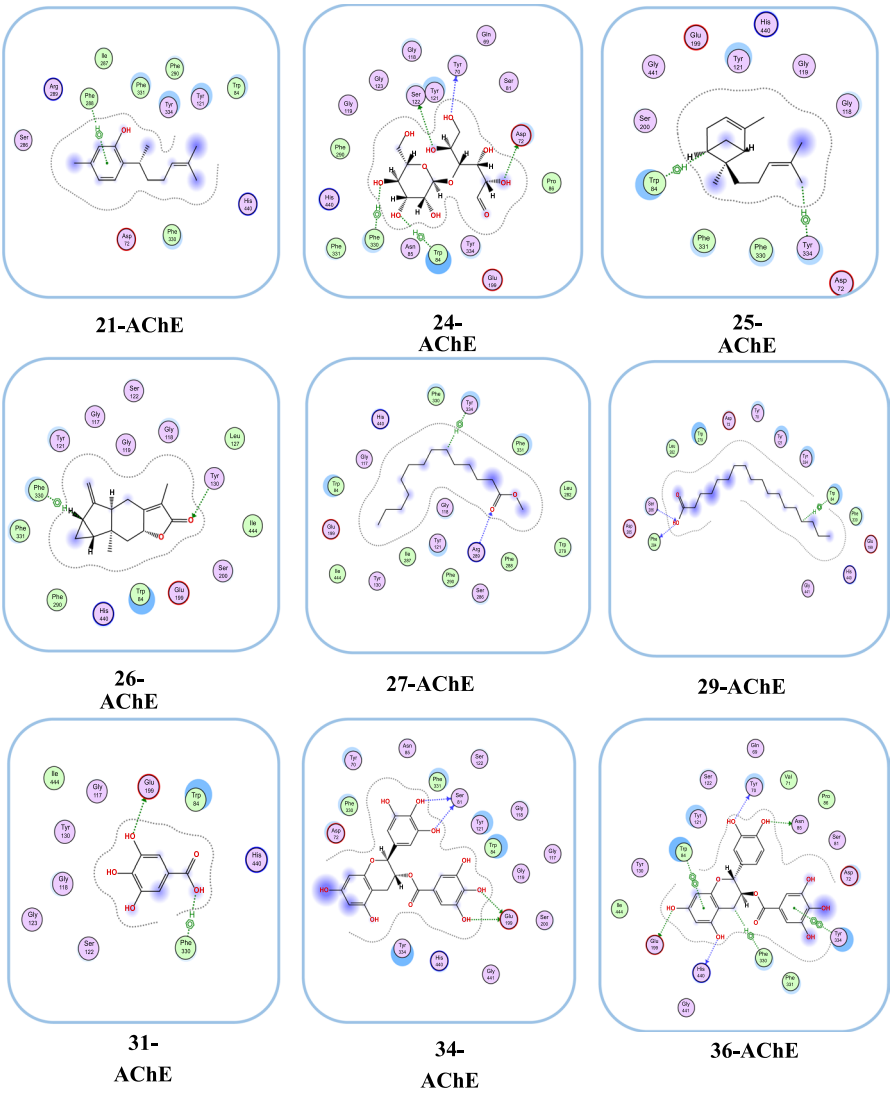


Fig. 5 The interaction of ligands with the binding site of acetylcholinesterase

found for the first time in this herb. Further, the virtual study was applied to search inside the interaction and energy binding of major bioactive compounds toward the targeting enzyme AChE. The docking study indicated that some major phenolics, including Epigallocatechin gallate (34), Epicatechin gallate (36), Vitexin (37), and Apigetrin (41), showed better energy binding (DS values in the range of 12.5 to − 13.3 kcal/mol) than Berberine chloride (DS value of − 12.1 kcal/mol). Based on Lipkin's rules of five and ADMET prediction, almost the identified compounds from DOWHT showed drug-likeness properties and none toxic for human use. These

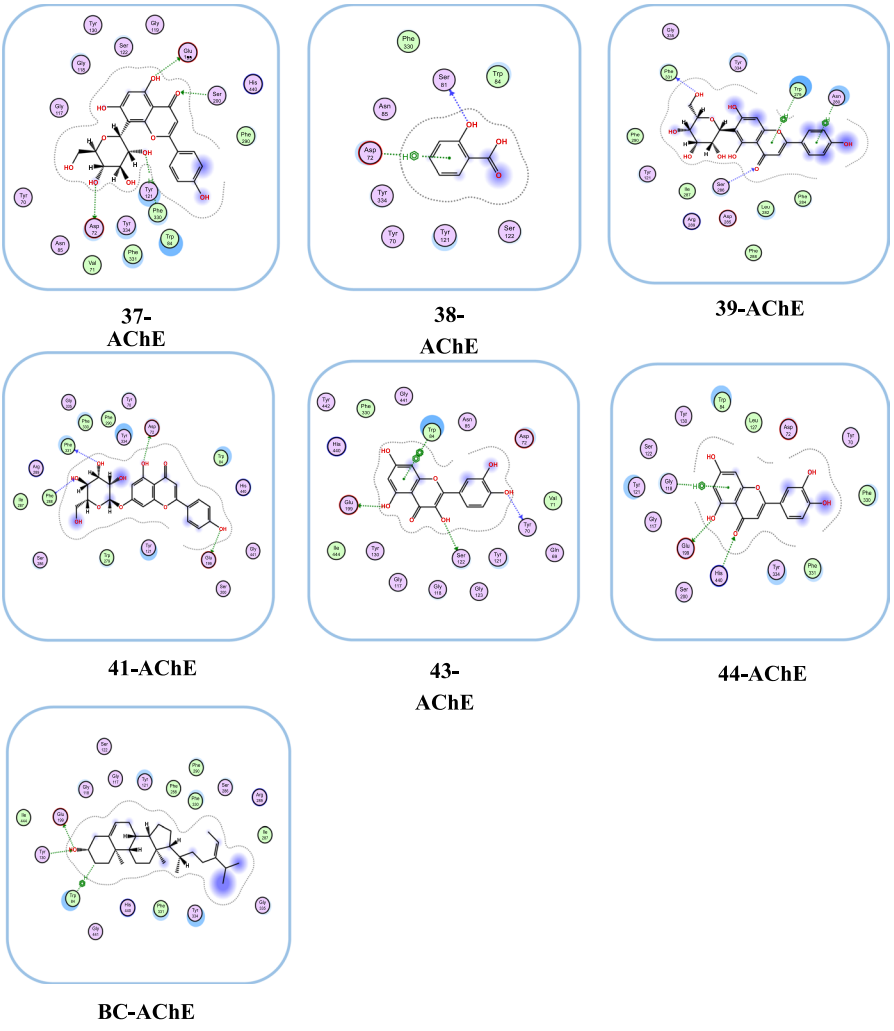


Fig. 5 (continued)

results suggest that DOWHT may be a promising natural source of anti-oxidant, anti-Alzheimer's and anti-diabetes drugs.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11164-023-05126-z.

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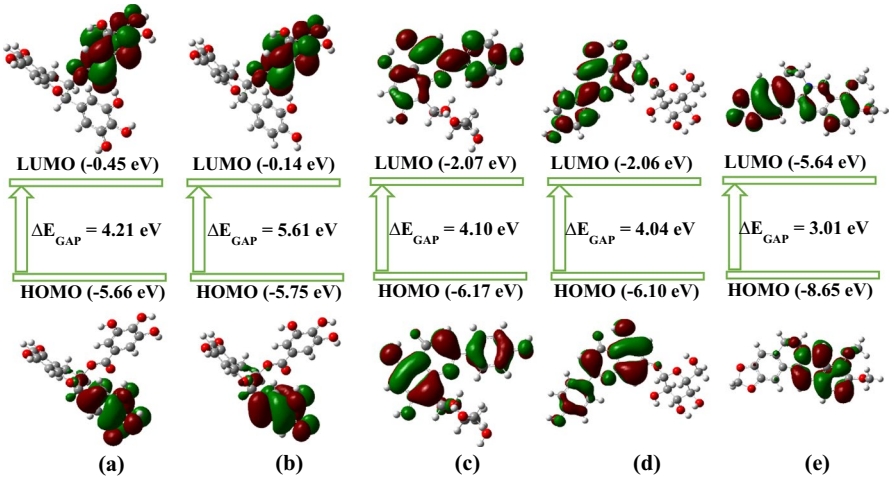

Fig. 6 The frontier molecular orbitals of Epigallocatechin gallate **a**, Epicatechin gallate **b**, Vitexin **c** and Apigetrin **d**, and Berberine chloride **e** analyzed by DFT at level of theory B3LYP/6–31G. HOMO: highest occupied molecular orbital; LUMO: lowest unoccupied molecular orbital; DFT: density functional theory

Table 5 Prediction of development into drugs via five Lipkin's rules of metabolites from *DOWHT*

Compound	Mass (Da)	Hydrogen bond donor	Hydrogen bond acceptors	LogP	Molar refractivity
4-Pregnen-20-&-ol-3-one (**21**)	316	1	2	4.515	91.88
Trans-α-Bergamotene (**24**)	204	0	0	4.725	66.74
Shizukanolide (**25**)	230	0	2	2.851	64.148
Tetradecanoic acid, methyl ester (**26**)	242	0	2	4.860	73.09
Octadecanoic acid (**27**)	283	0	2	4.998	84.55
Sebacic acid, 2-(2-chlorophenoxy) ethyl pentyl ester (**29**)	406	0	5	5.771	115.05
Gallic acid (**31**)	169	3	5	−0.833	35.77
Epigallocatechin gallate (**34**)	458	8	11	2.233	108.92
Epicatechin gallate (**36**)	442	7	10	2.528	107.26
Vitexin (**37**)	432	7	10	−0.066	103.53
Salicylic acid (**38**)	137	1	3	−0.244	32.44
Isovitexin (**39**)	432	7	10	−0.066	103.53
Apigetrin (**41**)	432	6	10	−0.107	103.54
Quercetin (**43**)	302	5	7	2.011	74.05
Luteolin (**44**)	286	4	6	2.125	72.48
Berberine chloride	337	0	4	2.733	93.03
Lipkin's rules	**≤500**	**≤5**	**≤10**	**≤5**	**40–130**

Table 6 The prediction of pharmacokinetic properties and toxicity of volatiles via ADMET simulation

Properties	Compounds						
	Control	21	24	25	26	27	29
Absorption							
Water solubility[(1)]	−3.973	−5.174	−5.968	−3.906	−6.109	−5.973	−5.956
$CaCO_2$ permeability[(2)]	1.734	1.715	1.395	1.628	1.602	1.556	1.43
Intestinal absorption (human)[(3)]	97.147	98.096	96.229	98.295	93.022	91.317	90.655
Skin permeability[(4)]	−2.576	−2.921	−1.677	−2.647	−2.244	−2.726	−2.691
P-glycoprotein substrate[(5)]	Yes	No	No	No	No	No	No
P-glycoprotein I inhibitor[(5)]	No	Yes	No	No	No	No	Yes
P-glycoprotein II inhibitor[(5)]	Yes	No	No	No	No	No	Yes
Distribution							
VDss (human)[(6)]	0.58	0.293	0.861	0.517	0.311	−0.528	0.106
Fraction unbound (human)[(6)]	0.262	0.001	0.149	0.288	0.142	0.051	0
BBB permeability[(7)]	0.198	0.004	0.86	0.638	0.711	−0.195	−0.029
CNS permeability[(8)]	−1.543	−2.11	−1.988	−2.262	−1.788	−1.707	−2.713
Metabolism							
CYP2D6 substrate[(5)]	No	No	No	No	No	No	No
CYP3A4 substrate[(5)]	Yes	Yes	Yes	Yes	Yes	Yes	Yes
CYP1A2 inhibitor[(5)]	Yes	No	No	Yes	Yes	Yes	No
CYP2C19 inhibitor[(5)]	No	No	No	Yes	No	No	Yes
CYP2C9 inhibitor[(5)]	No	No	No	No	No	No	No
CYP2D6 inhibitor[(5)]	Yes	No	No	No	No	No	No
CYP3A4 inhibitor[(5)]	No	No	No	No	No	No	Yes
Excretion							
Total clearance[(9)]	1.27	0.671	1.176	0.461	1.793	1.832	0.515
Renal OCT2 substrate[(5)]	No	No	No	Yes	No	No	No
Toxicity							
AMES toxicity[(5)]	Yes	No	No	No	No	No	No
Max. tolerated dose (human)[(10)]	0.144	−0.634	0.084	−0.007	0.257	−0.791	0.954
hERG I inhibitor[(5)]	No	No	No	No	No	No	No
hERG II inhibitor[(5)]	No	Yes	No	No	No	No	Yes
Oral rat acute toxicity (LD50)[(11)]	2.571	2.018	1.68	1.589	1.636	1.406	2.249
Oral rat chronic toxicity[(12)]	1.89	2	1.367	1.869	2.851	3.33	1.366
Hepatotoxicity[(5)]	Yes	Yes	No	No	No	No	No
Skin sensitzation[(5)]	No	No	Yes	Yes	Yes	Yes	No
T.Pyriformis toxicity[(13)]	0.354	0.99	1.562	0.878	2.208	0.65	0.624
Minnow toxicity[(14)]	−0.277	−0.067	−0.103	0.673	−0.891	−1.565	−2.078

Unit: [(1)] log $mol.L^{-1}$; [(2)] log Papp (10^{-6} $cm.s^{-1}$); [(3)] %; [(4)] log Kp; [(5)] Yes/No; [(6)] log $L.kg^{-1}$; [(7)] log BB; [(8)] log PS; [(9)] log $mL.min^{-1}.kg^{-1}$; [(10)] log $mg.kg^{-1}.day^{-1}$; [(11)] $mol.kg^{-1}$; [(12)] log $mg.kg^{-1}\_bw.day^{-1}$; [(13)] log $\mu g.L^{-1}$; [(14)] log mM

Table 7 The prediction of pharmacokinetic properties and toxicity of phenolics via ADMET simulation

Properties	Compounds									
	Control	31	34	36	37	38	39	41	43	44
Absorption										
Water solubility[(1)]	− 3.973	− 2.56	− 2.894	− 2.911	− 2.845	− 1.808	− 2.812	− 2.559	− 2.925	-3.094
$CaCO_2$ permeability[(2)]	1.734	− 0.081	− 1.521	− 1.264	− 0.956	1.151	− 0.618	0.33	− 0.229	0.096
Intestinal absorption (human)[(3)]	97.147	43.37	47.37	62.09	46.69	83.88	64.72	37.60	77.20	81.13
Skin permeability[(4)]	− 2.576	− 2.735	− 2.735	− 2.735	− 2.735	− 2.723	− 2.735	− 2.735	− 2.735	− 2.735
P-glycoprotein substrate[(5)]	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
P-glycoprotein I inhibitor[(5)]	No	No	No	No	No	No	No	No	No	No
P-glycoprotein II inhibitor[(5)]	Yes	No	Yes	Yes	No	No	No	No	No	No
Distribution										
VDss (human)[(6)]	0.58	− 1.855	0.806	0.664	1.071	− 1.57	1.239	0.342	1.559	1.153
Fraction unbound (human)[(6)]	0.262	0.617	0.215	0.158	0.242	0.563	0.21	0.218	0.206	0.168
BBB permeability[(7)]	0.198	− 1.102	− 2.184	− 1.847	− 1.449	− 0.334	− 1.375	− 1.391	− 1.098	− 0.907
CNS permeability[(8)]	− 1.543	− 3.74	− 3.96	− 3.743	− 3.834	− 3.21	− 3.754	− 3.746	− 3.065	− 2.251
Metabolism										
CYP2D6 substrate[(5)]	No	No	No	No	No	No	No	No	No	No
CYP3A4 substrate[(5)]	Yes	No	No	No	No	No	No	No	No	No
CYP1A2 inhibitor[(5)]	Yes	No	No	No	No	No	No	No	Yes	Yes
CYP2C19 inhibitor[(5)]	No	No	No	No	No	No	No	No	No	No
CYP2C9 inhibitor[(5)]	No	No	No	No	No	No	No	No	No	Yes
CYP2D6 inhibitor[(5)]	Yes	No	No	No	No	No	No	No	No	No
CYP3A4 inhibitor[(5)]	No	No	Yes	No	No	No	No	No	No	No

Table 7 (continued)

Properties	Compounds									
	Control	31	34	36	37	38	39	41	43	44
Excretion										
Total clearance[9]	1.27	0.518	0.292	−0.169	0.444	0.607	0.442	0.547	0.407	0.495
Renal OCT2 substrate[5]	No	No	No	No	No	No	No	No	No	No
Toxicity										
AMES toxicity[5]	Yes	No	No	No	No	No	No	No	No	No
Max. tolerated dose (human)[10]	0.144	0.70	0.441	0.449	0.557	0.61	0.649	0.515	0.499	0.499
hERG I inhibitor[5]	No	No	No	No	No	No	No	No	No	No
hERG II inhibitor[5]	No	No	Yes	Yes	No	No	No	No	No	No
Oral rat acute toxicity (LD50)[11]	2.571	2.218	2.522	2.558	2.595	2.282	2.558	2.595	2.471	2.455
Oral rat chronic toxicity[12]	1.89	3.06	3.065	2.777	4.635	2.483	5.37	4.359	2.612	2.409
Hepatotoxicity[5]	Yes	No	No	No	No	No	No	No	No	No
Skin sensitzation[5]	No	No	No	No	No	No	No	No	No	No
T.Pyriformis toxicity[13]	0.354	0.285	0.285	0.285	0.285	0.263	0.285	0.285	0.288	0.326
Minnow toxicity[14]	−0.277	3.188	7.713	6.146	4.897	1.812	5.18	5.507	3.721	3.169

Unit: [1] log $mol.L^{-1}$; [2] log Papp (10^{-6} $cm.s^{-1}$); [3] %; [4] log Kp; [5] Yes/No; [6] log $L.kg^{-1}$; [7] log BB; [8] log PS; [9] log $mL.min^{-1}.kg^{-1}$; [10] log $mg.kg^{-1}.day^{-1}$; [11] $mol.kg^{-1}$; [12] log $mg.kg^{-1}\_bw.day^{-1}$; [13] log $\mu g.L^{-1}$; [14] log mM

and S.-L.W. validated the study; T.K.P.P., A.D.N, T.T.N., T.H.N., Q.V.N., and M.D.D. were involved in formal analysis; T.K.P.P., T.Q.P., and M.D.D. investigated the study; V.B.N and M.D.D. helped in resources; M.D.D. curated the data; V.B.N helped in supervision and writing—original draft preparation; V.B.N, A.D.N, M.D.D., and S.-L.W. performed writing—review and editing; M.D.D. and V.B.N administrated the project.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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