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Phytochemical identification and *in silico* study of ethanolic extract of white cabbage as a phosphodiesterase 1B inhibitor

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ABSTRACT

Introduction: Memory dysfunction has remained a challenging issue globally. Nootropics have proven fruitful in managing cognitive dysfunction but because of their side effects, opportunities exist to explore alternatives. White cabbage is a cost-effective natural source of phytochemicals without side effects and has remained uninvestigated as a nootropic agent. This study sought to identify secondary metabolites in white cabbage extract (WCE) and to predict the molecular interaction between the phytochemical constituents of cabbage and phosphodiesterase-1B (PDE1B) using *in silico* studies.

Methods: The WCE was prepared by macerating crushed fresh white cabbage with ethanol for 24 h with continuous stirring. The phytochemical profile of WCE was analyzed using thin layer chromatography (TLC)-densitometry, and molecular docking studies were performed to predict the underlying mechanism action of the phytochemicals with PDE1B.

Results: The TLC-densitometry analysis showed that WCE was a rich source of sinigrin, whereas quercetin, chlorogenic acid, and rutin were not detected. *In silico* studies identified neobrassicin as having the highest affinity (ΔG_{bind}: −19.3358 kcal/mol) for PDE1B. However, quercetin (ΔG_{bind}: −13.1813 kcal/mol) and chlorogenic acid (ΔG_{bind}: −14.8706 kcal/mol) exhibited moderate interaction with PDE1B.

Conclusion: These results suggest that WCE has the potency to improve memory function by blocking PDE1B, and this preliminary study implies upcoming *in vitro* and *in vivo* research.

Implication for health policy/practice/research/medical education:
This study suggests that WCE has the potency to improve memory function by blocking PDE1B and can contribute to the development of pharmaceutical agents derived from natural resources to improve memory function.

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Introduction

Memory dysfunction, a neurodegenerative disorder, has remained a serious issue globally (1). Thus, the discovery of nootropics or memory enhancers, which are also known as "smart drugs", continues to gain attention. Nootropics are natural or synthetic substances that typically improve memory function in neurodegenerative diseases, such as Alzheimer's disease (AD), and can enhance vigilance, learning, attention, and executive functions (2-5). Nootropics target different molecules in the cell, thereby increasing cognitive function (3). Although cognitive improvement has received wide attention, understanding the effects of white cabbage extract (WCE) as a smart drug is still lacking (6). Many synthetic drugs approved by the

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American Food and Drug Administration (FDA) for the recovery of AD have failed to demonstrate efficacy in clinical trials by interacting with amyloid-β and tau protein and only provide short-term comfort from symptoms. Hence, they could not effectively treat the disease. Therefore, new substitute therapeutic agents need to be identified (7). Nootropics that target phosphodiesterases (PDEs) in the brain have been shown to help neuronal cell function (8,9). PDEs, a large family of enzymes (10), are widely distributed in tissues and break the phosphodiester linkages of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) (11) to produce metabolites such as AMP and GMP, respectively (10), which are indicated to have a role in cognitive function. The PDE family comprises 11 PDEs (PDE1-11) containing >60 isoenzymes with 21 genes and >100 gene variants (12,13). Among these PDEs, phosphodiesterase 1 (PDE1) significantly contributes to controlling memory activity, as corroborated by specific PDE1 inhibitors (14-16). PDE1 has been identified as a potential target for cognitive enhancement (17). The most prevalent isoform, PDE1B, is located in various areas of the brain, such as hippocampus, temporal cortex, frontal cortex, stratum, and parietal cortex, and plays a significant role in cognitive function (2,18). Hence, targeting PDE1 presents a therapeutic potential for nootropics (13).

Currently, the market is still lacking PDE1 inhibitors, and only vinpocetine (PubChem CID 443955), a synthetic alkaloid of apovincamine and ethyl ester derivative of lesser-periwinkle leaves (19), is currently being used as PDE1 inhibitor to treat memory dysfunction. However, vinpocetine has several limitations, including non-selectivity with PDE1 enzyme (20) and side effects, such as nausea, flushing, dizziness, headaches, dry mouth, heartburn, transient hypo- and hypertension, fetal harm or miscarriage, and induction of agranulocytosis (21). Though vinpocetine is widely used, this has not yet approved by FDA (22). For these reasons, a substitute PDE1 inhibitor, particularly from plant species would be of value. Organic compounds in "higher plants" have demonstrated potential in treating new and existing ailments (2). Therefore, PDE1 inhibitors, which can influence synaptic function may be preferred as helpful and accessible candidates to treat AD (23).

White cabbage (*Brassica oleracea* L. var. *capitata f. alba*) is one of the most popular vegetables worldwide (24). Cabbage has historically been applied as a medicinal herb for various conditions, including constipation, mushroom poisoning, hangover, sunstroke, fevers, headaches, sore feet, children croup, arthritis, hoarseness, melancholy, colic (25), and tender breasts (26). White cabbage has various reported pharmacological activities, including anticancer, antihypertensive, anticholesterolemic, antiobesity (27), antipsychotic (28), anticholinesterase (29), hepatoprotective (30-32), antidiabetic (33), antihyperlipidemic (34), anti-inflammatory (35,36), wound healing (37), gastroprotective (38,39), analgesic (40), antioxidant (41), antibacterial (42), antifungal (43), and anticoagulant effects (44). Cabbage is used in human diet mainly because of bioactive compounds. White cabbage contains phenolic acids, such as gallic acid (1.69 ± 0.02 mg/g), caffeic acid (8.05 ± 0.01 mg/g), p-coumaric acid (7.53 ± 0.04 mg/g) (45), and chlorogenic acid (8.75 mg/g) (46); flavonoids (47), including catechin (4.93 ± 0.01 mg/g), cyanidin (1.64 ± 0.01 mg/g), luteolin (0.76 ± 0.02 mg/g), quercetin (4.98 0.03 mg/g), kaempferol (3.71 ± 0.01 mg/g) (45), and rutin (0.037 ± 0.021 mg/g DW) (48); glucosinolates, namely progoitrin (6.71 ± 0.29 μmol/g dry matter (d.m.)), sinigrin (14.15 ± 0.33 μmol/g d.m.), gluconapin (0.61 ± 0.02 μmol/g d.m.), glucobrassicin (1.04 ± 0.04 μmol/g d.m.), 4-hydroxyglucobrassicin (0.80 ± 0.13 μmol/g d.m.), 4-methoxyglucobrassicin (0.64 ± 0.10 μmol/g d.m.), neoglucobrassicin (0.32 ± 0.02 μmol/g d.m.) (49), glucobrassicanapin, and glucoalyssin (50). It also contains β-carotene (2546 ± 191 μg/100 g wet weight basis) (51), vitamin C (329.45 ± 8.95 mg/100 g d.w.) (52), vitamin E (0.107 mg/100 g fresh weight) (53), neoxanthin, and violaxanthin (54). As per this data, chlorogenic acid, quercetin, and sinigrin are higher than other components. These three bioactive compounds have been claimed to play a major role in the inhibition of PDE1B activity (47). Therefore, it would be of value to predict the interaction of WCE with PDE1B by using *in silico* studies and to investigate its chemical composition.

Currently, no published study has identified the major bioactive compounds of WC in an ethanol extract or predicted the binding affinity of its metabolites with PDE1B by *in silico* studies. White cabbage is a common vegetable that has not been extensively explored as a nootropic agent targeting PDE1B. The aim of this study was to identify major bioactive compounds in WCE and predict the binding interaction of the phytochemical constituents of WCE with PDE1B via molecular docking. This study provides new insight into the discovery of a new agent targeting PDE1B for combating neurodegenerative disorders, including AD. Further *in vitro* and *in vivo* studies are required to strengthen the scientific evidence for this.

Materials and Methods

Materials and instruments

Fresh WC (Magelang District, Middle Java, Indonesia), aquabidest (Surabaya, Indonesia), deionized water (PT Bratachem, Yogyakarta, Indonesia), 0.9% saline (PT Braun Pharmaceutical Indonesia), ethanol (Merck, Germany), Whatman No. 1 filter paper (GE HealthCare, USA), silica gel F254 (Cat. 1055540001; Merck, Germany), quercetin (Merck, Germany), chlorogenic acid (Sigma-Aldrich, USA), sinigrin (Sigma-Aldrich, USA), rutin (Sigma-Aldrich, USA), formic acid (Merck, Germany), ethyl

acetate (Merck, Germany), n-hexane (Merck, Germany), chloroform (Merck, Germany), acetone (Merck, Germany), toluene (Merck, Germany), and TLC spray reagents consisted of Cerium sulfate, FeCl3 and citroboric (Merck, Germany) were used in this study. Among equipment, electric blender (Airlux BL-3022 Electric Blender), rotary evaporator (Shenzen POCE Technology Co., Ltd.), desiccator (NORMAX Glass Ware Desiccator, Indonesia), UV lamp (254 and 366 nm), CAMAG® TLC Scanner 3 (Muttenz, Switzerland) were used.

Plant material collection and identification

Fresh WC leaves were collected from Magelang district, Jawa Tengah, and authenticated at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia. A voucher specimen was deposited there (20.25.1 UN1/FFA.2/BF/PT/2022).

Extract preparation

Fresh WC leaves were thoroughly washed, and 1 kg of leaves was crushed with an electric blender to improve extraction. The crushed WC leaves were macerated with 5 L of 100% ethanol overnight for minimum of 24 hours and then filtered. The WC extract was evaporated using a rotary evaporator at 50°C, and the extract was stored in a desiccator at room temperature until dry.

Thin layer chromatography-densitometry analysis

Phytochemical analysis was performed using thin layer chromatography (TLC)-densitometry. The presence of quercetin, chlorogenic acid, sinigrin, and rutin in WCE (10 mg/mL) were investigated using TLC-densitometry dilutions of quercetin, chlorogenic acid, sinigrin, and rutin as reference agents (1 mg/mL). An aluminum plate precoated with silica gel F254 was applied as the stationary phase. In addition to this, a mixture of n-hexan: ethyl acetate: formic acid (6:4:0.5; 19 minutes), formic acid: ethyl acetate: aquabidest water (1:8:1.5; 25 minutes), ethyl acetate: methanol: water: formic acid (6:2:1:1 drops; 20 minutes) and ethyl acetate: formic acid: water (7:1.5:1.7; 29 minutes) was separately employed as the mobile phase. The time taken by the mobile phase was recorded by a stopwatch. Chromatographic detection of compounds was performed under UV light at 254 and 366 nm. The maximum wavelengths of 380, 330, 399, and 266 nm were assessed with a TLC scanner (CAMAG® TLC Scanner 3). Furthermore, the spots on TLC plates of WCE along with its standards (quercetin, chlorogenic acid, sinigrin, and rutin) were visualized after spraying with citroboric, $FeCl_3$, and cerium sulfate (for sinigrin and rutin), respectively.

In silico molecular docking studies

Molecular docking was performed using the Molecular Operating Environment (MOE) software (MOE 2022.10) to find the binding and to define the interaction of the phytochemical constituents of WCE with PDE1B. The experimental 3D structures of PDE1B_HUMAN enzyme with UniProt ID (Q01064) were searched first using UniProt (https://www.uniprot.org/) and later obtained from the Protein Data Bank (https://www.rcsb.org/), and PDB ID: 5UP0; the crystal structure of human PDE1B in complex with 8HP (6-[(4-chlorophenyl)methyl]-8,9,10,11-tetrahydro[1]benzothieno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-5(6H)-one) was selected for docking studies.

Known ligand dataset preparation

Known ligands (inhibitors of PDE1B) were downloaded from ChEMBL (open database of molecule with drug-like properties maintained by the European Molecular Biology Laboratory) based on their IC_{50} values. The known ligands were constructed as a dataset. This dataset was prepared using MOE with default settings (energy minimization by setting gradient as 0.1 kcal/mol and constraints as rapid water molecules) and it was used later in scoring function validation.

Test ligands preparation

The 2D structures of 24 phytochemical constituents of WCE or test ligands were obtained via their SMILES ID in PubChem, and their three-dimensional (3D) structures were prepared using MOE by minimizing energy.

Protein-ligand complex preparation

The 3D structure of PDE1B (5UP0) was prepared by removing metal atoms and by minimizing energy using MOE with the default parameters using QuickPrep tool; this complex was saved as mdb file for further docking validation.

Docking protocol validation

The docking protocol validation was carried out through redocking and scoring function validation. Induced fit method was preferred to perform the flexible docking on pocket atoms of protein. Triangle Matcher and London dG were selected as placement method and ASE was selected as scoring function. In redocking, the root mean square deviation (RMSD) was computed for evaluating position validation, and a good value of RMSD was considered within the threshold limit (<2 Å). The scoring function validation was analyzed by calculating the relationship between docking score and IC_{50} values of known ligands.

Docking of test ligands with protein

The prepared 3D structures of all 24 test ligands were docked with the prepared PDE1B (5UP0) using validated docking protocol. The binding affinities showed by docking score were calculated for ligand-enzyme complexes as kcal/mol. The docking results of 24 test ligands were analyzed and visualized in both 2D and 3D interactions.

Results

The ethanol extraction yield of WC was 3.017%. TLC-densitometry analysis was performed to identify the presence of major compounds (quercetin, chlorogenic acid, sinigrin, and rutin) in WCE. WCE was prepared in methanol and analyzed on precoated silica gel TLC plates and observed under UV light (254 and 366 nm) as well as visible light. We also performed TLC-densitometer analysis to obtain complementary evidence regarding the presence of these major compounds. The results indicated that quercetin had an Rf value of 0.35. At this Rf, no quercetin was detected in WCE (Figure 1a, b, c, d, e). To verify the presence of quercetin in WCE, the TLC plates were analyzed using a TLC-densitometer. The analysis was performed under the maximum wavelength at 380 nm, which confirmed that quercetin in WCE could not be detected, although the quercetin standard was detected (Figure 2A).

We could not detect chlorogenic acid in WCE, although the chlorogenic acid standard was clearly detected at an Rf of 0.63 (Figure 1f, g, h, i, j). An intensive spot was present on the TLC plate but this had a higher Rf compared with that of the chlorogenic acid standard. This spot was predicted as caffeic acid, another hydroxycinnamoyl ester of quinic acid with high structural similarity to chlorogenic acid. Thorough analytical work is required to confirm the structure. To confirm the presence of chlorogenic acid in WCE, the compound spots on the TLC plates were analyzed with a TLC-densitometer under a maximum wavelength at 330 nm. Figure 2B demonstrated that the chlorogenic acid standard had a sharp spectrum at 330 nm, although this spectrum could not be detected in the WCE. These results indicated that the WCE does not contain quercetin and chlorogenic acid.

In this study, sinigrin, a major glucosinolate present in *Brassica* species, was detected in WCE. The presence of sinigrin in WCE was analyzed using TLC, and the spots were visualized at 254 and 366 nm after spraying with cerium sulfate (Figure 2C). Further analysis using TLC-densitometer under a maximum wavelength at 399 nm confirmed the presence of sinigrin in WCE (Figure 2C). The last compound investigated in this study was rutin. Rutin is a glycoside form of quercetin, which represents a polar glucoside compound. Figure 1p, q, r, s, t showed that rutin (Rf 0.32) was not detected in WCE. Further investigation using TLC-densitometer under maximum wavelength at 266 nm confirmed the absence of rutin in WCE (Figure 2D).

Figure 3A displays a 3D crystal structure of protein (PDB: 5UP0) and its pocket together with the docking of all known ligands (Figure 3B), which indicates that all the known ligands adopt a similar position as that of the native ligand. The redocked configuration of target protein (PDB: 5UP0) with the native ligand (6-(4-chlorobenzyl)-8,9,10,11-tetrahydrobenzo[4,5]thieno[3,2-e][1,2,4]triazolo[1,5 c]pyrimidin-5(6H)-one)/(PubChem CID: 2243267) with the best position was used as reference for all known as well as test ligands (Figure 3C).

The 3D configurations of the native ligand with the positions of the native (redocked), known, and test ligands

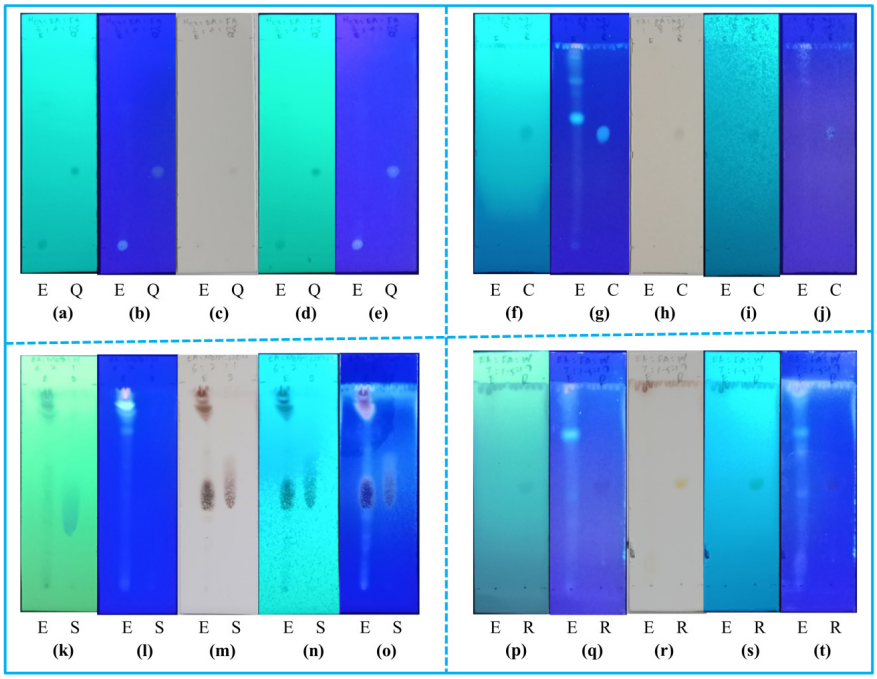


Figure 1. Thin layer chromatography (TLC) profile of white cabbage extract (WCE), quercetin, chlorogenic acid, sinigrin, and rutin. TLC plates (a), (f), (k), (p)-before spray (254 nm); (b), (g), (l), (q)-before spray (366 nm); (c), (h), (m), (r)-after spray; (d), (i), (n), (s)-after spray (254 nm); (e), (j), (o), (t)-after spray (366). E: extract; Q: quercetin; C: chlorogenic acid; S: sinigrin; R: rutin.

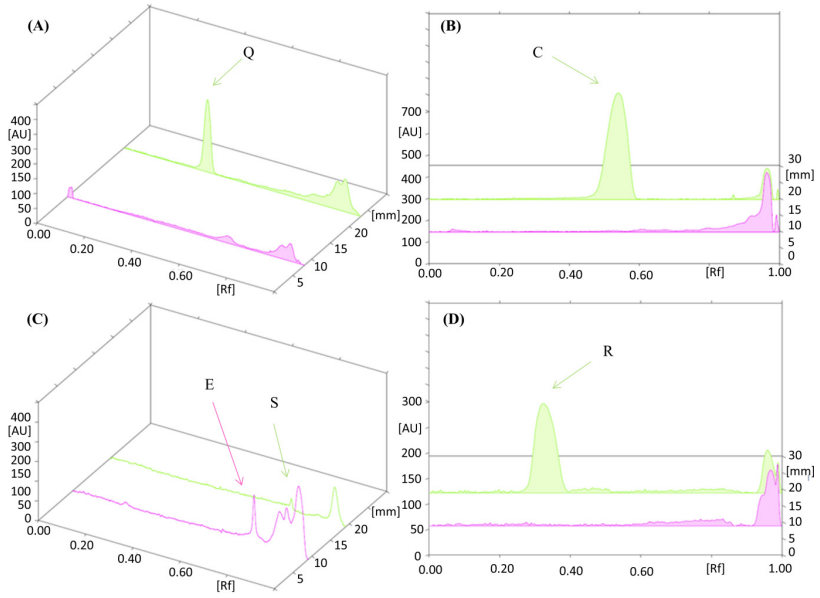


Figure 2. Spectrum of white cabbage extract (WCE), quercetin, chlorogenic acid, sinigrin, and rutin on all tracks. (A) Quercetin, (B) chlorogenic acid, (C) sinigrin, and (D) rutin. E: extract; Q: quercetin; C: chlorogenic acid; S: sinigrin; R: rutin.

alongside the native ligand are shown in Figures 4–6 (panels A & D with pocket shown and panels B & E without pocket shown), and their interaction with the receptor in 2D mode (C & F). The docking study found that the pyrimidine and thiophene rings of the native ligand have strong bifurcated π-π binding with Phe^{414}; whereas the chlorobenzene ring displayed a π-hydrogen interaction with Val^{439}. Interestingly, His^{395} and Gln^{443} act as sidechain donors; His^{395} interacts with nitrogen in the imidazole ring; whereas Gln^{443} interferes with oxygen of pyrimidine ring with a bond length of 0.5294 Å (Figure 4C). In addition, the pyrimidine, thiophene, and chlorobenzene rings of the

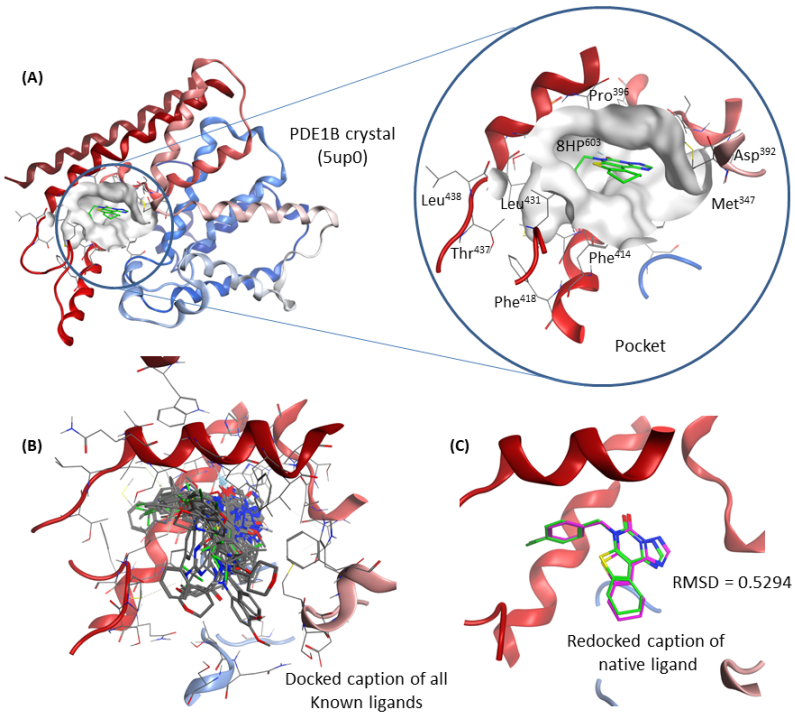


Figure 3. 3D view of crystal structure of phosphodiesterase-1B (PDE1B), positions of all known ligands and redocked configuration of native ligand. (A) 3D structure of PDE1B (5UP0) and its active site with pocket shown, which contains Leu^{438}, Pro^{396}, 8HP^{603}, Leu^{431}, Phe^{414}, Phe^{418}, Met^{437}, Asp^{392}, and Thr^{437} (they can be clearly seen in zoom out pocket). (B) 3D configuration of docking of all known ligands, which is important for test ligands. (C) 3D configuration of redocked native ligand showing the favorable RMSD (Root mean square deviation) value.

known ligand (CHEMBL4095097) exhibited a very strong π-π interaction with Phe^{446}. Gln^{A443} and Gln^{B443} showed sidechain interactions with the oxygen of pyrimidine ring with a bond length of 1.5128 Å (Figure 4F).

The 2D and 3D structures of vinpocetine and chlorogenic acid and their atomic interconnection with specific amino acid residues at active site of PDE1B are shown in Figure 5. The benzene ring of vinpocetine had a π-hydrogen interaction with Phe^{414} while the pyrrole ring had a π-π interaction with Phe^{446}. In addition, the nitrogen ring in piperidine acted as a donor with the sidechain of Gln^{443} with a bond length of 1.2075 Å (Figure 5C). The benzene-1,2-diol ring in chlorogenic acid formed a π-π bond with Phe^{446}, and two hydroxyl groups interacted with the Gln^{443} sidechain. However, the hydroxyl groups on cyclohexane bound to the sidechains of Asp^{392} and Met^{347}.

While Thr^{345} acted as backbone donor on cyclohexane, His^{278} acted as sidechain donor on oxygen ion of the carboxylic group, which is present on cyclohexane with a bond length of 1.6566 Å (Figure 5F).

The 2D and 3D structures of quercetin and neoglucobrassicin and the atoms of specific amino acids in the active site of protein are shown in Figure 6. The benzene-1,2-diol ring in quercetin interacted with Phe^{446}, and the His^{395} sidechain interacted with the oxygen present on 4H-pyran ring while a hydroxyl group interacted with Asp^{392} and Met^{347} via water molecules. Furthermore, two hydroxyl groups present on the benzene-1,2-diol ring interacted with the sidechains of His^{234} and Glu^{304}, respectively, with a bond length of 1.2627 Å (Figure 6C).

The indole ring in neoglucobrassicin made a bifurcated π-π bond with Phe^{446}, and sulfur trioxide interacted with His234 and Tyr^{233} via water molecules while Tyr^{233} donated an atom to the oxygen present in sulfur trioxide. However, Asn^{283} acted as a backbone acceptor with a bond length of 2.4520 Å (Figure 6F). The names and structures of all 24 test compounds along with vinpocetine (as standard) and their free bind energies (ΔG_{bind} [kcal/mol]), as well as their pIC50 values are detailed in Table 1.

Discussion

WC is a rich source of flavonoids, phenolic acids, and glucosinolates, and these phytochemicals participate in controlling various diseases, including neurodegenerative ones. Previous reports have shown that *Brassica* species and their bioactive compounds reduce the risk of neurodegenerative development in multiple animal and clinical studies (55-57). In this study, only ethanol was considered as an extraction vehicle for WCE. Ethanol is considered relatively safe compared to other organic solvent and it is the most common solvent used pharmaceutical industry (58-60). Additionally, it effectively extracted most of flavonoids, phenolic acids, and glucosinolates compounds (2,61).

Previous studies have identified the phytochemical constituents of white cabbage. It was shown that chlorogenic acid, quercetin, and sinigrin are the major compounds in the white cabbage. We hypnotized that these three bioactive compounds might play a major role in the inhibition of PDE1B activity. In this study, we provided a prediction of the molecular interaction between the phytochemical constituents of white cabbage

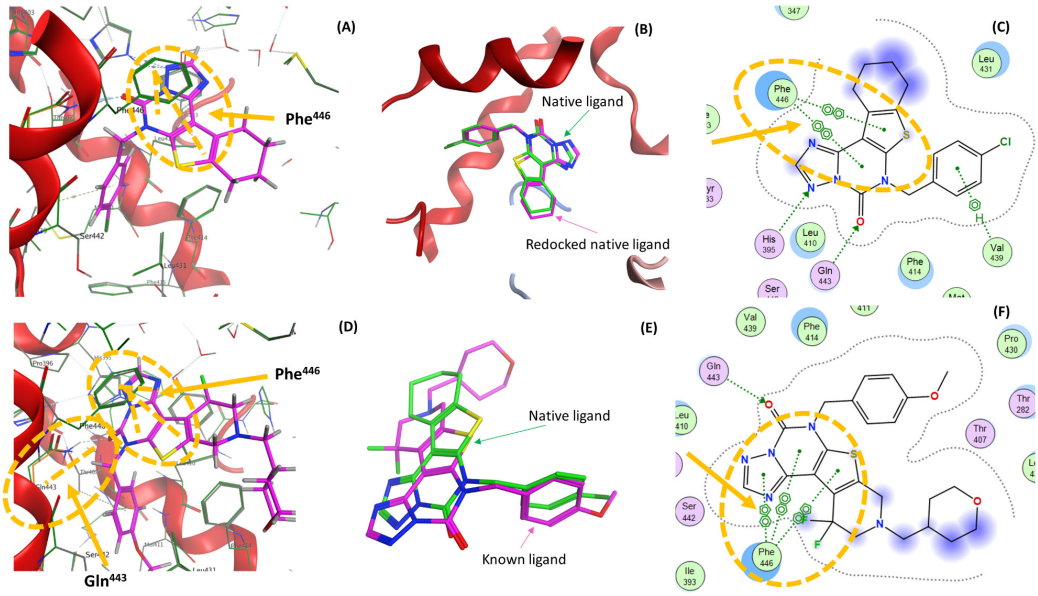


Figure 4. 3D configuration of native and known ligands with native ligand, and 2D interaction of amino acids of both native and known ligands. (A) Redocked native ligand 3D interaction with amino acids; (B) superposed binding orientation of native ligand with native ligand, (C) 2D interaction of native ligand with amino acids; (D) 3D interaction of docked known ligand (ChEMBL4095097) with amino acids; (E) known ligand superposed with native ligand (ChEMBL4095097); and (F) known ligand (ChEMBL4095097) 2D interaction with amino acids. Phe: Phenylalanine; Gln: Glutamine; Pro: Proline; Thr: Threonine; Ser: Serine; Ile: Isoleucine; Val: Valine; Leu: Leucine; His: Histidine.

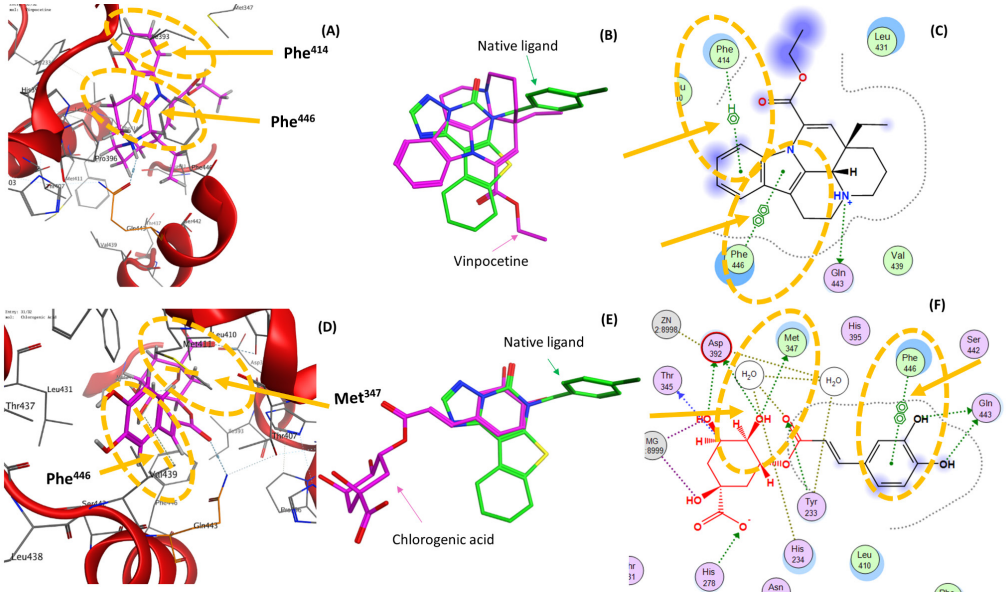


Figure 5. 3D configuration of vinpocetine and chlorogenic acid with native ligand, and 2D interaction of both vinpocetine and test ligands with amino acids. (A) Docked vinpocetine 3D interaction with amino acid; (B) superposed binding orientation of vinpocetine with native ligand; (C) vinpocetine 2D interaction with amino acids; (D) docked chlorogenic acid 3D interaction with amino acids; (E) chlorogenic acid superposed with native ligand; and (F) chlorogenic acid 2D interaction with amino acids. Phe: Phenylalanine; Gln: Glutamine; Pro: Proline; Thr: Threonine; Ser: Serine; Ile: Isoleucine; Val: Valine; Leu: Leucine; His: Histidine; Tyr: Tyrosine; Met: Methionine.

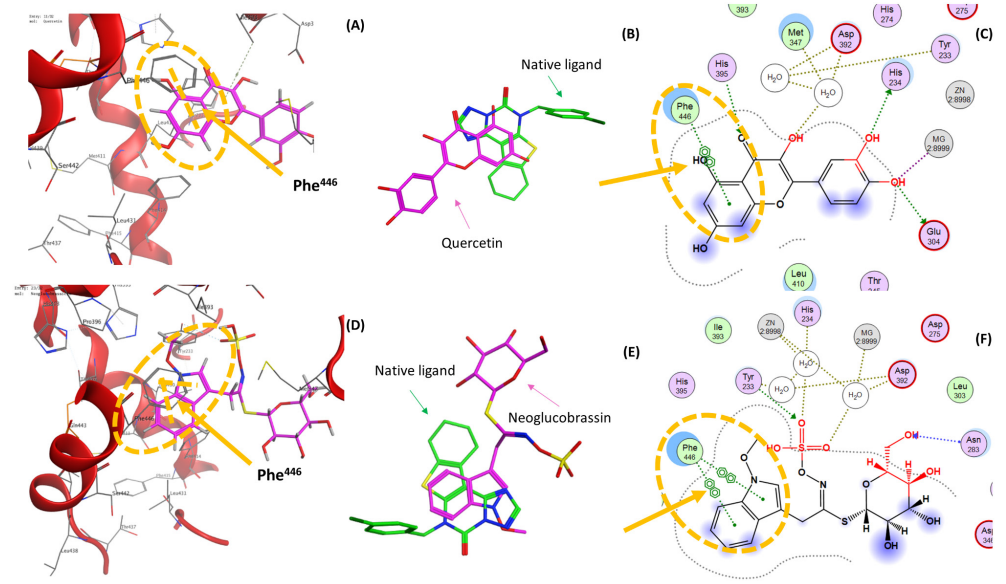


Figure 6. 3D configuration of quercetin and neoglucobrassicin with native ligand, and 2D interaction of both test ligands with amino acids. (A) Docked quercetin 3D interaction with amino acids; (B) superposed binding orientation of quercetin with native ligand; (C) quercetin 2D interaction with amino acids; (D) docked neoglucobrassicin 3D interaction with amino acids; (E) neoglucobrassicin superposed with native ligand; and (F) neoglucobrassicin 2D interaction with amino acids; Phe: Phenylalanine; Gln: Glutamine; Pro: Proline; Thr: Threonine; Ser: Serine; Ile: Isoleucine; Val: Valine; Leu: Leucine; His: Histidine; Tyr: Tyrosine; Met: Methionine.

(obtained from literatures) and PDE1B using the *in silico* study. To confirm the presence of the major phytochemical constituent in the WCE, the TLC-densitometry analysis was performed. We found that sinigrin was the major compound in the WCE extract. The presence of quercetin, chlorogenic acid, and rutin could not be confirmed. This finding differs from those of previous studies. Different extracts of *Brassica* species (white cabbage, Chinese cabbage, cauliflower, broccoli, and red cabbage) have been analyzed using reverse phase-high pressure chromatography (RP-HPLC) to detect phenolic and flavonoid compounds in previous studies. Quercetin and chlorogenic acid, which represent flavonoid and phenolic compounds, respectively, were identified as the major

Table 1. Structures of all 24 test compounds along with vinpocetine and their free bind energies (ΔGbind [kcal/mol]), as well as their pIC50 values

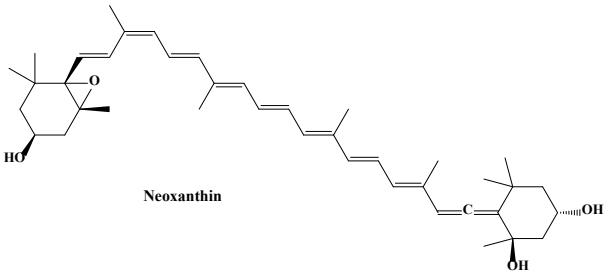
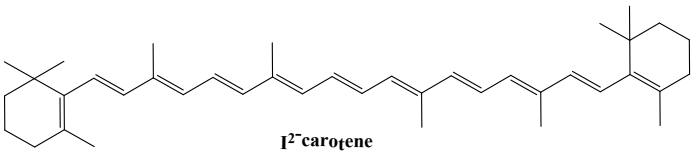
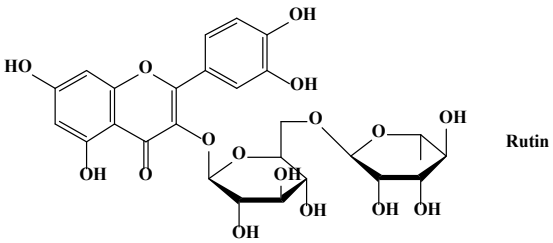
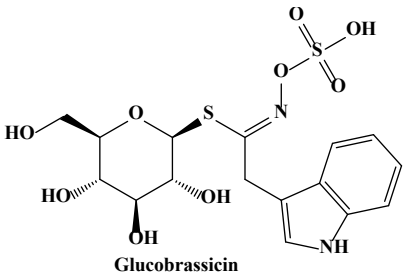
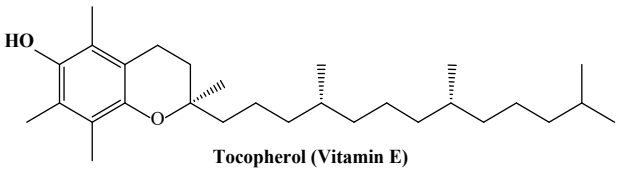
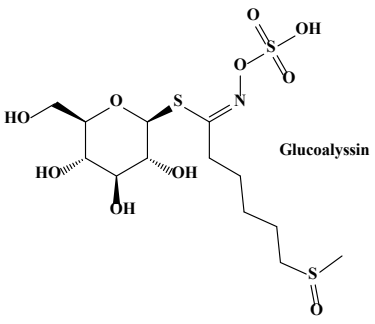
Test compounds	ΔG_{bind} (kcal/mol)	pIC_{50}
 Neoxanthin	−31.2207	13.9603
 I²⁻carotene	−28.8121	12.3107
 Rutin	−25.8001	10.2478
 Glucobrassicin	−24.6112	9.4335
 Tocopherol (Vitamin E)	−23.7416	8.8380
 Glucoalyssin	−20.2074	6.4174

Table 1. Continued

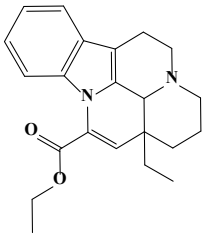
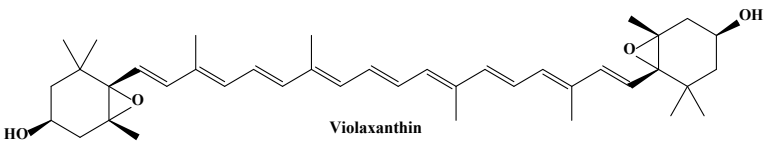
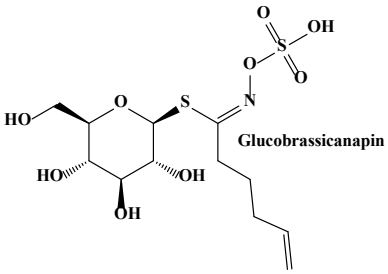
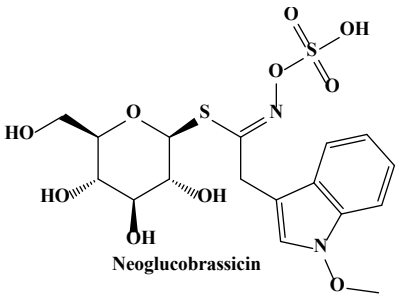
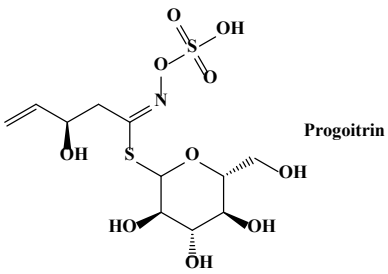
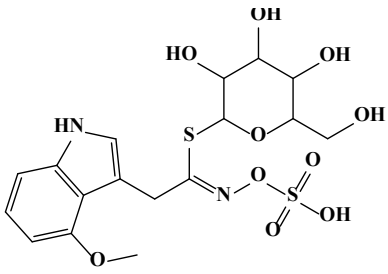
Test compounds	ΔG_{bind} (kcal/mol)	pIC_{50}
 Vinpocetine	−20.0572	6.3145
 Violaxanthin	−19.9154	6.2174
 Glucobrassicanapin	−19.6985	6.0689
 Neoglucobrassicin	−19.3358	5.8205
 Progoitrin	−18.5230	5.2638
 4-methoxyglucobrassicin	−18.3059	5.1151

Table 1. Continued

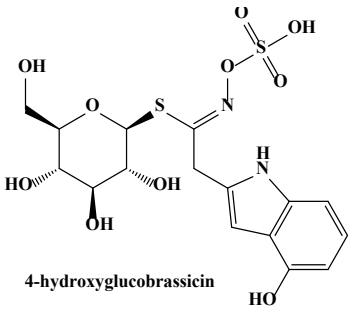
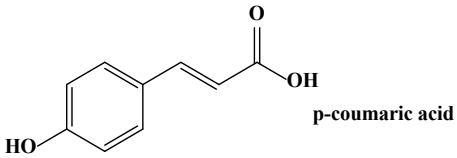
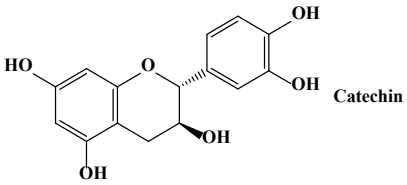
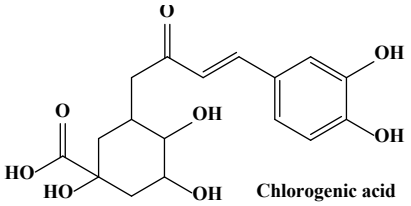
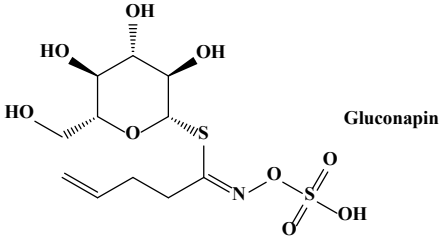
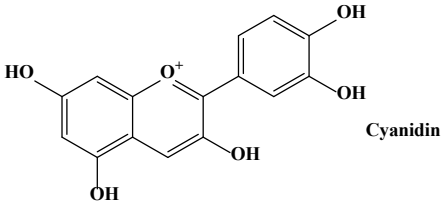
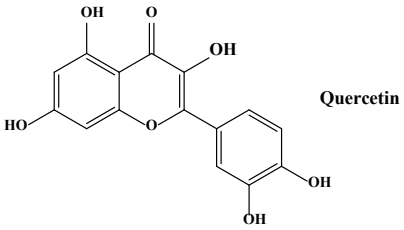
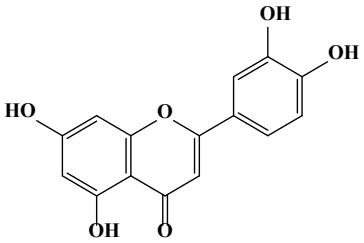
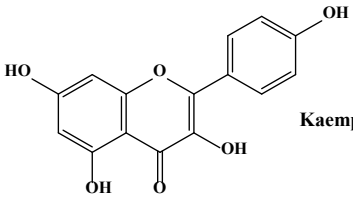
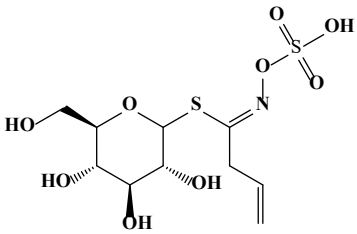
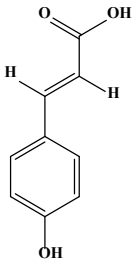
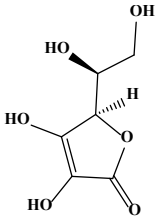
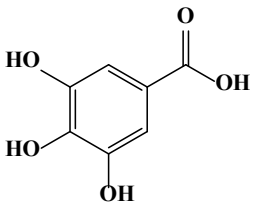
Test compounds	ΔG_{bind} (kcal/mol)	pIC_{50}
 4-hydroxyglucobrassicin	−18.0871	4.9652
 p-coumaric acid	−14.9431	2.8119
 Catechin	−14.9275	2.8012
 Chlorogenic acid	−14.8706	2.7623
 Gluconapin	−14.3643	2.4155
 Cyanidin	−13.3437	1.7165
 Quercetin	−13.1813	1.6053

Table 1. Continued

Test compounds	ΔG_{bind} (kcal/mol)	pIC_{50}
 Luteolin	−12.7149	1.2858
 Kaempferol	−12.3212	1.0162
 Sinigrin	−11.6280	0.5414
 Caffeic acid	−8.0784	−1.889
 Ascorbic Acid	−7.7118	−2.1407
 Gallic acid	−6.2417	−3.1475

compounds (62,63). This result was consistent with that reported by Orfali et al (64).

WC was shown to contain sinigrin (2-propenyl glucosinolate) (65), which is a precursor of allyl isothiocyanate. This compound has diverse biological activities (66), including anticancer, anti-inflammatory, antibacterial, antifungal, antioxidant, and wound healing effects (67). In line with this study, the presence of sinigrin in WCE was previously reported by Dighe and Charegaonkar (68) and Amir et al (69). TLC-densitometry analysis demonstrated that sinigrin was the main compound detected in WCE. Previous studies have indicated that an aqueous extract of WC contains flavonoids (quercetin) and phenolic acids (chlorogenic acid) (45). However, in this study, quercetin and chlorogenic acid were not found in ethanol extract of WC. Chlorogenic acid, a hydroxycinnamoyl ester of quinic acids, was previously reported as one of the most abundant natural polyphenols in WC (70). TLC is a selective, easy to perform, and inexpensive method as compared with other sensitive chromatographic techniques for identification of compounds (71); however, TLC analysis was not sufficient to quantify the minute quantities of compounds that may be present in WCE. Therefore, we recommend that compounds present in minimal amounts be assessed using more sensitive techniques for compound quantitation, such as RP-HPLC, gas chromatography-mass spectrometry, and liquid chromatography-high resolution mass spectrometry.

Before conducting the docking study, the protein targets were validated (redocked) and RMSD values were used as a parameter. RMSD is a distinguishing feature that exhibits the duplicability of protein and native ligand complex in the development of a fitting configuration; an ideal RMSD value is <1 Å, but <2 Å is also acceptable (72). Molecular docking was then performed to obtain insight into the possible interaction and binding affinity of PDE1B with the phytochemical constituents of WC. The catalytic domain and the binding mode pf PDB1 were derived from the crystal structure of 5UP0, where the binding pocket was comprised of Leu^{438}, Pro^{396}, $8HP^{603}$, Leu^{431}, Phe^{414}, Phe^{418}, Met^{437}, Asp^{392}, and Thr^{437} amino acids (Figure 3) (73). These *in silico* studies of PDE1B are consistent with those in previous investigations (55,72,73). In this study, by using TLC we identified sinigrin as one of the major compounds in WCE. However, the presence of quercetin, chlorogenic acid, and rutin could not be detected. The *in silico* studies showed that sinigrin did not interacted with PDE1B, whereas quercetin and chlorogenic acid exhibited moderate binding with PDE1B. The strongest binding interaction was shown by neoglucobrassicin PDE1B. Further in vitro and in vivo bioactivity guided isolation is required to decipher the most active compound as nootropic and cognitive function enhancing agent.

Helmi et al reported that *Caesalpinia sappan* L. ethanol extract had more PDE1 inhibitory activity than that of other fractions. They also reported that the free-bond energy (ΔGbind) of the tested compounds did not differ among them, with the lowest free-bond energy shown by vinpocetine (2). ΔG_{bind} is the critical factor responsible for the receptor-ligand binding strength between the targeted PDE1B and the WC test compounds. A low ΔG_{bind} score indicates the stability and strength of the interaction between an enzyme (e.g., PDE1B) and its ligands. These factors contribute to the pharmacological effects. WCE can potentially target PDE1 (molecular docking has shown its constituent compounds can interact with PDE1B). Therefore, WC could be a useful source of a natural cognitive enhancer to combat memory dysfunction (72) and should be investigated further with *in vitro* and *in vivo* studies.

Conclusion

This study showed that WCE was a rich source of sinigrin as demonstrated via TLC-densitometry. In contrast, we did not detect quercetin, chlorogenic acid, or rutin in WCE. However, the *in silico* studies showed that among the 24 compounds evaluated, sinigrin did not show any interaction with PDE1B, whereas neoglucobrassicin exhibited the strongest binding interaction with PDE1B. In addition, quercetin and chlorogenic acid exhibited moderate binding with PDE1B. Thus, additional investigations should be performed on WCE as a nootropic and cognitive function enhancing agent. TLC analysis could not quantify minute concentrations of bioactive compounds present in WCE, and consequently, we suggest that highly sensitive approaches, such as HPLC or LC-HRMS should be considered for this purpose.

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Author contributions

ZI and NF contributed to the experimental design. NA contributed to writing of the manuscript, performed the experiments, and analyzed the data. KNL drew chemical structures, reviewed and checked Eglish grammar. NSOU reviewed and edited. ZI, NF, and AS supervised the project. All authors read and approved the final manuscript.

Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

Ethical issues including plagiarism, double publication, and data fabrication have been completely observed by the authors.

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