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# HPLC fingerprint of flavonoids, enzyme inhibition and antioxidant activity of *Newbouldia laevis* stem-bark: an in vitro and in silico study

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## Abstract

**Background** Medicinal plant contains multiple bioactive compounds with therapeutic potentials. Due to their availability, affordability, and minimal known side effects, they are widely practiced. Identification, quantification, and establishment of their interaction with physiological enzymes help in the standardization of plant-based medicinal extracts. In this study, gas chromatography/flame ionization detector (GC–FID) and high-performance liquid chromatography (HPLC) analysis were used to determine the bioactive components in the ethanol extract of *Newbouldia laevis* stem bark. The antioxidant activity of the extract was determined. Enzyme inhibitory potency of the flavonoids' components was investigated against acetylcholinesterase, butyrylcholinesterase, phospholipase A2, α-glucosidase, and α-amylase.

**Results** Analysis of ethanol extract of *N. laevis* stem-bark revealed alkaloids (0.37%), tannins (1.82 mg/TEq/g), flavonoids (5.85 mg/QEq), steroids (0.11 mg/10 g) and glycosides (0.08 mg/10 g). The HPLC fingerprint of flavonoids showed high concentrations (mg/100 g) of catechin (47.11), apigenin (15.68), luteolin (18.90), kaempferol (41.54), and quercetin (37.64), respectively. In vitro 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging ability of the extract was exhibited at 150 and 200 mg/mL, respectively. At 300 mg, most in vitro antioxidant potentials (lipid peroxidation, metal chelating ability, hydroxyl, nitric oxide, sulfide oxide radicals scavenging abilities) were obtained. The extract showed varying inhibitory abilities (> 50%) on acetylcholinesterase, butyrylcholinesterase, phospholipase A2, α-glucosidase and α-amylase at 300 mg/mL, $IC_{50}$ of 129.46, 237.10, 169.50, 251.04 and 243.06 mg/mL, respectively, with inhibition constants (Ki) of 3.92, 1.63, 1.11, 2.95 and 2.11. Results showed an affinity for the targeted enzymes with free energies higher than the standard drugs.

**Conclusion** The results revealed that the *N. laevis* stem bark possesses antioxidant activity and enzyme inhibitory activity on the physiological enzyme that has been implicated in diabetes. In vitro and in silico inhibition of these physiological enzymes by extract suggests that the stem bark can be effective in ameliorating the complications associated with diabetes mellitus.

**Keywords** Medicinal plant, Diabetes, Enzyme inhibition, Antioxidants, Flavonoids

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## Background

Diabetes (hyperglycemia) is characterized by a prolonged high concentration of blood glucose in the blood, resulting from defects with insulin or insulin receptors [1]. It is associated with several complications such as neuropathy and retinopathy, while excessive reactive oxygen species, reduced antioxidant status and hyperglycemia increase the diseased condition [2]. International Diabetes Federation [3] predicted about 642 million new diabetes cases by 2040. Type 2 diabetes mellitus has become a universal health crisis that threatens several aspects of nationhood, including the economy and the health care system (United Nations organization, 2001). The disease is associated with several complications including inflammation and Alzheimer's disease [4], where insulin resistance has been implicated to be a risk factor for Alzheimer disease development [5]. They are pieces of evidence that inflammation is an essential pathogenesis process of diabetes, and diabetes-related vascular complications [4], thus inhibiting or targeting inflammatory processes could manage complications associated with diabetes and its progression.

Phospholipase $A_2$ is responsible for the formation of inflammatory mediators that control physiological processes such as neuroinflammation and oxidative stress [6]. This enzyme is implicated in Alzheimer's disease pathogenesis [6]. Typically, diabetes is characterized by prolonged hyperglycemia, which occurs as a result of insulin insensitivity in the modulation of plasma glucose concentration. Thus, the pharmacological control of carbohydrate metabolizing enzymes could aid in ameliorating the rise in plasma glucose concentration. α-Glucosidase and phosphatase are important physiological enzymes that aid in the absorption of sugars by breaking complex carbohydrates to yield glucose, and their inhibition would lead to reduction in blood glucose [7].

Plants have been the source of several agents used in the management of different diseased conditions, diabetes inclusive [8], and *N. laevis* has been used [9], due to the high cost, adulteration and unavailability of the orthodox drugs. Though, there is a dearth of information on the using *N. laevis* stem-bark in *Diabetes mellitus* management.

*Newbouldia laevis* is a perennial plant found in several communities of Nigeria as a hedge to mark boundaries and for its ornamental qualities (Tropical Plant Database, 2019) and commonly known locally as Akoko (Yoruba) Ogilishi (Igbo) and Ikhimi (Edo). Several pharmacological activities that have been attributed to the plant extract include antibacterial [10, 11] and antioxidant activity [12]. Also, the extract is used in treating constipation and bile [13]. Researchers are employing computational techniques (in silico study) in drug discovery, authentication and development, due to their more convenience and economical, which has made it a widely accepted technique [14].

## Methods

### Chemicals

2,2-diphenyl-1-picrylhydrazyl, Butyrylcholine iodide, Ascorbate, Sulfanilamide, Acetylcholine iodide, 5,5′-dithio-bis (2-nitrobenzoic acid) (Sigma Aldrich, Germany) were some of the analytical chemicals used.

### Sample collection

The stem of *N. laevis* was plucked on 2 October 2021 from a farm in Edem-Ani, Nsukka Local government Area, Southeast Nigeria. The plant was identified by Mr Felix Nwafor, Plant Science and Biotechnology Department, as *Newbouldia laevis G. Don*. (*Sapotaceae)* with voucher number PCG/UNN/0359. This study was approved by the faculty of biological science ethical clearance committee with reference Number FBS/2022/00183.

### Extraction of *N. laevis* phytochemicals

The extract was prepared using 500 g of the dried, pulverized sample and 1000 mL of an absolute ethanol solution by cold maceration at room temperature. The mixture was shaken intermittently for 72 h and filtered through Whatman filter paper number 1 (pore size 11 mm). The final extract was concentrated on rotary vacuum evaporator (R215, Buchi, Flawil, Switzerland) at 45 °C and reduced pressure. The dried extract was kept in a refrigerator at − 4 °C.

### Phytochemicals analysis by GC-FID

The quantitative analysis of phytochemicals was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15-m MXT-1 column (15 m × 250 um × 0.15 um) was used. Phytochemicals were quantified using the area and the mass of internal standard.

### HPLC analysis of *N. laevis* flavonoids

The flavonoid content of *N. laevis* was determined using high-performance liquid chromatography (HPLC) (Hangzhou-LC-8518, Zhejiang, China). Specifically, the HPLC ultraviolet (UV) detector supported by N200 chromatography software helped to establish the chemical constituents of the flavonoid's contents. The HPLC instrument operated with a low-pressure gradient, solvent delivery pump, high-pressure switching valve, as well as high-sensitivity UV detector. Column size was 150 × 4.6 mm, with an injected sample volume of ~ 40 mL. Mobile phase was set for flavonoids (Acetonitrile/Methanol/Water/THF, 70:20:8:2),

using wavelength (Lamda maximum) of 280 nm, column temperature of ~ 40 °C, and run time of ~ 20 min. Results of flavonoids were expressed as µg/100 mL. The flavonoid components of the *N. laevis* extract was identified by comparing their retention time (RT) of the peaks with that of know reference standards.

### In vitro antioxidant activity

#### *2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging potential.*

DPPH scavenging power of *Newbouldia laevis* stem-bark was determined by the method [15]. The radical scavenging potential was estimated using the equation below;

$$\text{DPPH radical scavenging (\%)} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100.$$

#### *Determination of OH* scavenging power*

The determination of OH*(hydroxyl radical) scavenging power was described by Jin et al. [16]. The absorbance of the mixture was read at 536 nm. The OH* scavenging power was calculated as follows: Scavenging activity (%) = (Abs. sample − Abs. blank)/(Abs0 − Abs. blank) × 100. where Abs0 is the absorbance of the deionized water instead of $H_2O_2$ and sample in the assay system.

#### *SO* scavenging assay*

SO* (superoxide radical) scavenging power was determined according to [17] methods. The absorbance was read at 560 nm after 20 min. The percentage inhibition of superoxide generation was estimated.

#### *NO* scavenging power*

The determination of NO* (nitric oxide radical) scavenging power of the extract was as described by Sangameswaran et al. [18]. The reference standard used was ascorbic acid.

$$\text{Percentage of inhibition} = [(Ao - A1)/Ao] \times 100;$$

where $A_o$ is control absorbance and $A_1$ is sample absorbance.

#### *Ferric cyanide ($Fe^{3+}$) reducing antioxidant power assay*

Ferric cyanide reducing power of the extracts was measured by absorbance measurement of the formation of the Perl's Prussian Blue complex following the addition of excess $Fe^{3+}$ at 700 nm [18].

#### *Acetylcholinesterase and butyrylcholinesterase inhibitory activity assay*

Acetylcholinesterase (AchE) and butyrylcholinesterase (BuchE) inhibition were assayed using [19] methods. The absorbance was read at 412 nm. The percentage enzyme inhibition was calculated as follows; Acetylcholinesterase and butyrylcholinesterase activities were calculated with the formula.

$$\text{AChE activity \%} = \frac{Ao - A1}{Ao} \times 100;$$

$$\text{BChE activity \%} = \frac{Ao - A1}{Ao} \times 100$$

where $A_o$ is the control absorbance and $A_1$ is the sample absorbance.

#### *Assay of phospholipase $A_2$*

Phospholipase $A_2$ ($PLA_2$) activity was as described by (Agatemor et al., 2019). The enzyme was extracted from *Aspergillus flavus* cultured on nutrient agar for 7 days, homogenized and centrifuged at 6000 rpm for 30 min. The assay was initiated by adding 0.5 mL of the *Newbouldia laevis* stem-bark, 0.3 mL of the substrate and 0.2 mL of the enzyme preparation. The reaction mixture stood at 37 °C for 60 min, after which the resulting mixture was centrifuged at 6000 rpm for 30 min, and the absorbance was taken at 418 nm. The standard inhibitor used was Prednisolone. Enzyme activity inhibition was calculated:

$$\text{\% Inhibition} = 1 - \frac{\text{Enzyme activity of test sample}}{\text{Enzyme activity of Control}} \times 100.$$

#### *α-amylase inhibition*

Inhibition of α-amylase activity was carried out by [20] method, using 100, 200, 300 and 500 mg/mL of the extract. The absorbance was read and recorded at 540 nm. Acarbose (different concentrations) was used as a positive control.

$$\text{Percentage (\%) amylase inhibition} = [(\text{AControl540 ASamples540nm})/\text{AControl540nm})] \times 100.$$

#### *Assay of α-glucosidase activities*

Inhibition of α-glucosidase activity was determined using the procedure of [21] with some modifications. The amount of p-nitrophenyl produced after enzymatic dephosphorylation was estimated by measuring the absorbance at 405 nm using JENWAY 6404. The inhibition (%) was calculated as (Ac − As)/Ac 100%, where Ac is the absorbance of the control and As is the absorbance of the sample. Acarbose was used as a positive control.

### Bioinformatics tools

Bioinformatics tools such as AutoDock Vina [22], Open Babel GUI [23], PROTOX-II (http://tox.charite.de/protox_II/), Discovery Studio 2016 client and Chimera 1.8.1 [24] were utilized in this study.

### Retrieval and preparation of target protein

The crystallized forms of the proteins (enzymes), Phospholipase $A_2$ protein data bank identification number (PDB ID: 1G4I), Acetylcholinesterase (PDB ID: 2ACE), α-Glucosidase (PDB ID: 2JKP), α-amylase (PDB ID: 2QMK) and Butyrylcholinesterase (PDB ID: 6QAE) were retrieved from the Protein Data Bank (https://www.rcsb.org/). Bovia: Discovery studio 2016 client was used to prepare the proteins for molecular docking study. The active sites were predicted based on the co-crystallized ligand (internal ligand). The grid was also adjusted through manual visualization.

### Ligand preparation

The phytocompounds, Apigenin, Catechin, Isorhamnetin, Kaempferol, Quercetin, Naringin and Luteolin were selected from the results of the HPLC analysis and in vitro studies carried out using the extract. These phytocompounds exhibited a strong effect on our targeted proteins as shown in the *in vitro* studies. The two-dimensional structures of these phytocompounds and used standard drugs (Galantamine, Prednisolone and Acarbose) were downloaded from Pubchem (www.pubchem.com) in.sdf format and were converted to protein data bank (PDB) format using Open Babel Tool.

### Molecular docking of phytocompounds of *N. laevis* with targets proteins

Molecular docking studies of the selected ligands (phytocompounds) against the targeted proteins were performed. These studies were carried out using AutoDock vina tool 1.5.7 [22]. A detailed visualization and comparison of the docked sites of the target proteins with the selected ligands were carried out by Discovery Studio 2016 client and Chimera 1.8.1 [24].

**Table 1** Phytochemical analysis

| Compounds | Inference | Concentration |
|---|---|---|
| Alkaloids | Detected | 0.37% |
| Tannins | Detected | 1.82 mg/TEq/g |
| Flavonoids | Detected | 5.85 mg/QEq |
| Terpenoids | Not detected | – |
| Saponins | Not detected | – |
| Steroids | Detected | 0.11 mg/10 g |
| Glycosides | Detected | 0.08 mg/10 g |

### Toxicity prediction of phytocompounds and standard drugs

The drug-likeness and toxicity properties of the Phytocompounds were carried out by uploading their smile format on PROTOX-II webservers (Charite University of Medicine, Institute for Physiology, Structural Bioinformatics Group, Berlin, Germany).

### Data analysis

The statistical analysis was carried out using the statistical program GLM model (SAS Institute, Cary, NC, USA, 2001). The means of values were compared using independent *t*-test of significance ($p < 0.05$).

## Result

### Phytochemical analysis

Alkaloids (0.37%), tannins (1.82 mg/TEq/g), flavonoids (5.85 mg/QEq), steroids (0.11 mg/100 g) and glycosides (0.08 mg/10 g), respectively, were quantified in the stem-bark extract of *N. laevis*. Terpenoids and saponins were not identified (Table 1). The presence of flavonoids, alkaloids and tannins is an indication that the extract possesses some pharmacological activities.

### HPLC flavonoids fingerprint of *N. laevis* stem-bark extract

The HPLC profile of the flavonoids content of the stem-bark extract showed (+)-catechin (47.11 mg/100 g), apigenin (15.68 mg/100 g), resveratrol (3.80 mg/100 g), genistein (1.22 mg/100 g), daidzein (6.77 mg/100 g), butein (5.35 mg/100 g), naringenin (8.23 mg/100 g), biochanin (8.41 mg/100 g), luteolin (18.90 mg/100 g), kaempferol (41.54 mg/100 g), (−)-epicatechin (5.77 mg/100 g), (−)-epigallocatechin (1.63 mg/100 g), gallocatechin (1.45 mg/100 g), quercetin (37.64 mg/100 g), (−)-epicatechin-3-gallate (2.83 mg/100 g), (−)-epigallocatechin-3-gallate (2.73 mg/100 g), isorhamnetin (11.83 mg/100 g), robinetin (5.94 mg/100 g), myricetin (7.26 mg/100 g), baicalein (5.46 mg/100 g), nobiletin (3.72 mg/100 g), tangeretin (2.73 mg/100 g), artemetin (2.13 mg/100 g), silymarin (1.87 mg/100 g), naringin (15.49 mg/100 g) and hesperidin (2.47 mg/100 g). The extract had a high concentration of flavonoids as evidenced by the HPLC fingerprint. Especially (+)-catechin (47.11 mg/100 g), apigenin (15.68 mg/100 g), biochanin (8.41 mg/100 g), luteolin (18.90 mg/100 g), kaempferol (41.54 mg/100 g), (−)-epicatechin (5.77 mg/100 g), quercetin (37.64 mg/100 g), isorhamnetin (11.83 mg/100 g), robinetin (5.94 mg/100 g), myricetin (7.26 mg/100 g) and naringin (15.49 mg/100 g) (mg/100 g) as shown in Fig. 1. The HPLC data is presented in the supplementary material (Additional file 1: Table S1). Some of these flavonoids have been established to possess various pharmacological activities.

### DPPH scavenging, antioxidant activity and metal chelating ability of ethanol stem-bark extract of *N. laevis*

Another important potential of medicinal plants is their power to mop up radicals generated in vitro by DPPH. DPPH radical scavenging power of the extract of *N. laevis* exhibited a concomitant dose-dependent scavenging potential (Fig. 2), except at 500 mg when a drastic decrease in the scavenging ability was obtained. A non-significant increase in the scavenging power of the extract at 150 and 200 mg, when compared to Vitamin C. *N. laevis* extract, showed high DPPH radical scavenging power (Fig. 2).

Table 2 shows the antioxidant potential of the stem-bark extract of *N. laevis.* There was a significant difference

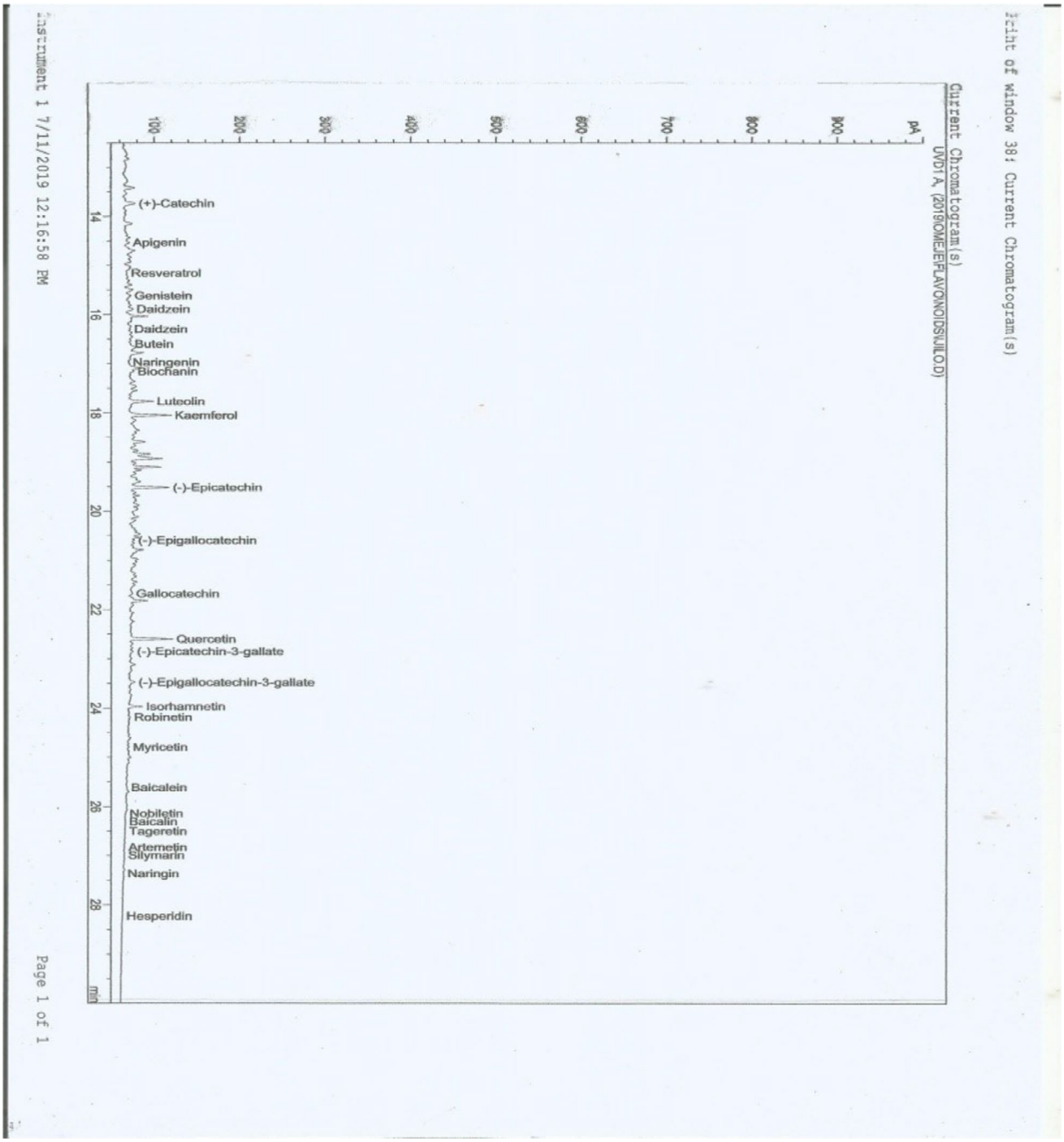


**Fig. 1** HPLC flavonoids fingerprint of *N. laevis* stem-bark extract

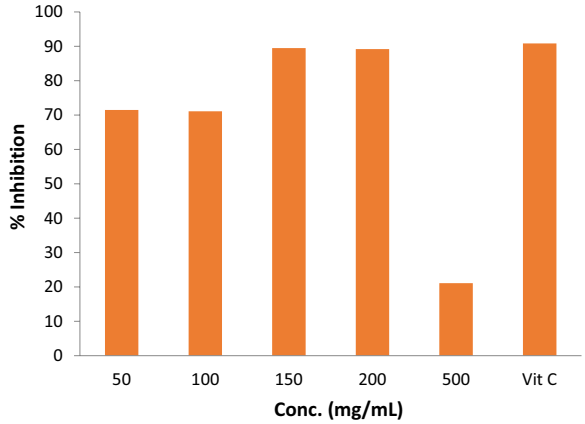


**Fig. 2** in vitro DPPH scavenging activity of ethanol stem-bark extract of *N. laevis*

in the metal chelating ability of this extract ($p > 0.05$) among 100, 200 and 500 mg/mL, when compared to 300 mg/mL (53.94) and Vit C (59.33%), respectively.

A similar result was obtained for the metal chelating ability of the extract, where 300 mg showed a significantly higher ($p > 0.05$) chelating ability than other concentrations studied.

Furthermore, the OH* scavenging ability and SO* radical scavenging ability showed similar activity. At 100, 200, 300 and 500 mg, the ferric reducing potential showed a concentration-dependent increase in electron transfer rate (Table 2). The percentage OH* scavenging power of the extract at lower concentrations of 100 and 200 mg/mL was 34.12 and 38.59%. At 300 mg/mL, 60.27% of scavenging ability was obtained, though the percentage scavenging ability of the extract reduced to 44.53% at 500 mg/mL of the extract. The percentage inhibition of hydroxyl radicals by the plant extract was low ($p < 0.05$) when compared to the standard (Ascorbic acid) gave 86.41% inhibition. Among the concentrations used, 300 mg/mL gave the highest scavenging ability, by mopping up more than 60% of the radicals generated in vitro.

Low concentrations of the extract gave a higher percentage nitric oxide radical scavenging ability of 75.18 and 77.20% at 100 and 200 mg/mL of the extract, respectively. The subsequent increase in the extract concentration to 300 and 500 mg/mL gave 68.40 and 62.50% inhibition of the nitric oxide radical generation (Table 2). There was a non-significant ($p < 0.05$) difference among the extract concentrations studied, though there was a significant increase ($p > 0.05$) in the percentage inhibition of nitric oxide radical scavenging ability at 300 and 500 mg/mL and the Ascorbic acid. Furthermore, the ability to inhibit lipid peroxidation in vitro was determined at 100, 200, 300 and 500 mg/mL. 44.50, 47.96, 53.94 and 36.39 percentage inhibition were obtained, respectively (Table 2). 300 mg/mL gave the highest inhibition of lipid peroxidation, with other concentrations producing lower than 50% of lipid peroxidation inhibition. 100 mg of the extract showed the highest scavenging ability of nitric oxide radical than other radical species, while for lipid peroxidation, hydroxyl radical and FRAP it could not produce 50% inhibition as shown in Table 2.

Similarly, NO* scavenging power of 77.20% was the highest inhibition of the radical species obtained by 200 mg of the extract, while $56.11 > 52.41 > 50.40$ were obtained for the scavenging of metal chelating ability, FRAP and SO* radical scavenging ability.

### Enzyme inhibitions potentials of *Newbouldia laevis* stem-bark extract

This study investigated the in vitro bioactivity of *Newbouldia laevis* stem-bark on acetylcholinesterase, butyrylcholinesterase, α-amylase, phospholipase $A_2$, antioxidant potential and α-glucosidase enzymes. The stem-bark extracts exerted varying degrees of inhibition on acetylcholinesterase (AchE), butyrylcholinesterase (BuchE), phospholipase $A_2$, α-glucosidase and α-amylase. The percentage inhibition of cholinesterases was between 58 and 89.71 at different concentrations of the extract as shown in Table 3. At 100 mg/

**Table 2** Antioxidant potentials of *Newbouldia laevis* stem-bark extract

| Antioxidant potentials | 100 mg/mL | 200 mg/mL | 300 mg/mL | 500 mg/mL | Vit.C (mg/mL) |
|---|---|---|---|---|---|
| Lipid peroxidation (%) | $44.50^{bA} \pm 1.04$ | $47.96^{bA} \pm 0.34$ | $53.94^{cA} \pm 0.44$ | $36.39^{dA} \pm 0.75$ | $99.33^{a} \pm 1.04$ |
| Metal chelating ability (%) | $54.86^{bB} \pm 0.90$ | $56.11^{bB} \pm 3.02$ | $68.33^{cB} \pm 2.53$ | $47.18^{dB} \pm 1.28$ | $89.20^{a} \pm 0.85$ |
| OH* Scavenging ability (%) | $34.12^{bA} \pm 0.66$ | $38.59^{bC} \pm 1.42$ | $60.27^{cB} \pm 1.74$ | $44.53^{dB} \pm 1.44$ | $86.41^{a} \pm 0.22$ |
| $NO^{*}$ Scavenging ability (%) | $75.18^{bA} \pm 0.43$ | $77.20^{bA} \pm 0.51$ | $68.40^{cB} \pm 0.77$ | $62.50^{cC} \pm 2.03$ | $80.69^{a} \pm 3.67$ |
| $SO^{*}$ Scavenging ability (%) | $55.37^{bB} \pm 0.17$ | $50.40^{bB} \pm 0.99$ | $62.79^{cB} \pm 0.40$ | $49.93^{dB} \pm 0.55$ | $89.38^{a} \pm 2.99$ |
| FRAP (%) | $49.05^{bA} \pm 2.40$ | $52.41^{bB} \pm 0.76$ | $57.33^{cA} \pm 0.11$ | $43.69^{bB} \pm 0.83$ | $97.52^{a} \pm 2.09$ |

Values are of triplicate tests, in mean ± standard error (SE); values with lowercase are statistically significant ($p < 0.05$) to Vit. C (Standard); values with different uppercase are statistically significant ($p < 0.05$) across Concentrations

mL, 58.12 and 60.38% of AchE and BuchE inhibitions were obtained, with *Ki* of 3.92 and 1.63, respectively, which were significantly low ($p < 0.05$), compared to the standard inhibitor (89.76%) galantamine. Subsequently, high inhibition of $PLA_2$ activity was obtained at all concentrations of the extract studied (Table 4), with an inhibition constant (*Ki*) of 1.11. The inhibition constant (*Ki*) measures the strength of an inhibitor, the smaller the constant, the stronger the inhibition [25]. There was a non-significant difference between the inhibition of extract and that of prednisolone (94.76%). At 100 mg/mL, 65.20 and 55.66 percentage inhibition of the enzymes were obtained, and 65.19, 69.84 and 71.21 percentage inhibition were obtained at the concentrations of 200, 300 and 500 mg/mL, with *Ki* of 2.95 and 2.11, respectively.

From the *Ki* obtained, *N. laevis* extract inhibited α-amylase more than α-glucosidase. At 100-300 mg, there was a significant increase when compared to Acarbose, a standard α-glucosidase inhibitor (87.51%). At 200, 300 and 500 mg/mL of the stem-bark extract, 59.85–66.82 percentage inhibition was obtained. There was a non-significant increase in the extract inhibition of the α-glucosidase and α-amylase at the concentrations of 100–300 mg/mL. At 500 mg/mL, the percentage inhibition of α-glucosidase (71.21) was significantly high ($p > 0.05$), when compared to the percentage inhibition of α-amylase at the same concentration.

### Bioinformatic studies of the interaction of *Newbouldia laevis* flavonoids with some enzymes

In this study, the compound library was docked against some of the targets for diabetic complications, including α-Glucosidase (PDB ID: 2JKP), α-amylase (PDB ID: 2QMK), Phospholipase $A_2$ (PDB ID: 1G4I), Acetylcholinesterase (PDB ID: 2ACE) and Butyrylcholinesterase (PDB ID: 6QAE) and the binding energies obtained in Kcal/mol for the best-docked position are reported in Table 5

**Table 4** Inhibitory kinetics of stem-bark extract on some enzymes

| Enzyme inhibition | $IC_{50}$ (mg/mL) | *Ki* |
|---|---|---|
| Acetylcholinesterase | $129.46^{A} \pm 1.60$ | $3.92^{a} \pm 1.04$ |
| Butyrylcholinesterase | $237.10^{B} \pm 0.43$ | $1.63^{b} \pm 0.18$ |
| Phospholipase $A_2$ | $169.50^{C} \pm 0.21$ | $1.11^{b} \pm 0.10$ |
| α-Glucosidase | $251.04^{B} \pm 1.05$ | $2.95^{a} \pm 0.35$ |
| α-Amylase | $243.06^{B} \pm 0.47$ | $2.11^{a} \pm 0.55$ |

Values with different uppercase are statistically significant ($p < 0.05$) compared down the enzymes

## Discussion

The antioxidant abilities of plants rich in phenolics are considered as a good source medication for various disease condition. [26, 27] had shown that the leaf extract of *N. laevis* contains high phenolics. This is in tandem with [9] reports. In the report of [26], alkaloids, flavonoids, saponins and steroids were not detected in the extract of *N. laevis* leaf. Also, Usman and Osuji [27] did not report that alkaloids and saponins were detected in their study. Whereas, tannins, flavonoids, alkaloids, saponins, and steroidal glycosides were detected in the study of Josiah and Bartholomew [28]. [26] had reported the type of solvents, geographical location, season and time of plant material harvest as some factors that affect the phytochemicals present in plant extracts. The medicinal properties such as the anti-diabetic activity of plant extracts have been attributed to the high concentration of alkaloids and flavonoids [20]. The high concentration of these phytochemicals in the *N. laevis* extract makes it a good antidiabetic agent. Flavonoids are shown to have

**Table 3** Enzyme inhibitions potentials of *Newbouldia laevis* stem-bark extract

| Enzyme inhibition | 100 mg/mL | 200 mg/mL | 300 mg/mL | 500 mg/mL | Standard inhibitors (mg/mL) |
|---|---|---|---|---|---|
| Acetylcholinesterase (%) | $58.12^{bA} \pm 0.15$ | $63.62^{bA} \pm 1.04$ | $52.59^{cA} \pm 1.08$ | $62.17^{dA} \pm 0.27$ | $89.76^{a} \pm 2.87$ (Galantamine) |
| Butyrylcholinesterase (%) | $60.38^{bB} \pm 0.90$ | $71.08^{bB} \pm 0.18$ | $64.30^{cB} \pm 0.32$ | $89.71^{dB} \pm 1.93$ | $91.59^{a} \pm 1.18$ (Galantamine) |
| Phospholipase $A_2$ (%) | $87.77^{bA} \pm 0.66$ | $91.83^{bC} \pm 1.60$ | $96.15^{cB} \pm 1.93$ | $84.53^{dB} \pm 1.44$ | $94.76^{a} \pm 1.00$ (Prednisolone) |
| α-Glucosidase (%) | $65.20^{bC} \pm 0.43$ | $65.19^{bD} \pm 0.51$ | $69.84^{cB} \pm 0.33$ | $71.21^{cC} \pm 0.09$ | $87.51^{a} \pm 0.39$ (Acarbose) |
| α-Amylase | $55.66^{bC} \pm 1.17$ | $59.85^{bC} \pm 2.13$ | $66.82^{bC} \pm 1.04$ | $60.82^{bC} \pm 0.39$ | $79.52^{a} \pm 0.51$ (Acarbose) |

Values were of triplicate tests, (mean ± standard error (SE); values with different lowercase are statistically significant ($p < 0.05$) compared to Standard inhibitors; values with different uppercase are statistically significant ($p < 0.05$) across concentrations

**Table 5** Binding energies/affinities of compounds (Kcal/mol)

| | Internal ligand | Standard drug | Apigenin | Catechin | Quercetin | Kaempferol | Isorhamnetin | Naringin | Luteolin |
|---|---|---|---|---|---|---|---|---|---|
| Acetyl-cholinesterase | − 4.8 | − 8.4 | − 9.7 | − 10.0 | − 10.2 | − 9.9 | − 10.2 | − 10.6 | − 10.0 |
| Butyryl-cholinesterase | − 8.9 | − 9.0 | − 9.1 | − 9.4 | − 9.5 | − 9.1 | − 9.1 | − 10.3 | − 9.5 |
| α-Glucosidase | − 6.9 | − 5.9 | − 8.7 | − 8.6 | − 8.9 | − 8.3 | − 9.2 | − 8.8 | − 9.3 |
| Phospholipase $A_2$ | − 9.9 | − 9.8 | − 10.1 | − 9.8 | − 10.5 | − 9.8 | − 10.6 | − 11.4 | − 10.3 |
| α-Amylase | − 6.9 | − 6.3 | − 9.4 | − 9.3 | − 9.3 | − 9.2 | − 9.1 | − 10.2 | − 9.5 |

pharmacological activities. Quercetin has important pharmacological functions such as the neuroprotective and cardioprotective ability [29]. One of the antioxidant compounds is catechin [30], which is involved in the protection of the skin [31]. Isorhamnetin and naringin were reported to possess anti-inflammatory and antioxidative functions, tumor-inhibiting and bone regeneration [32, 33]. The inhibitory activity could be due to a high concentration of flavonoids, including luteolin, myricetin and quercetin, which have been previously reported to possess α-amylase inhibitory ability [34]. The medicinal potential of plant-based material could be attributed to high flavonoid concentration [35]. Flavonoids have been reported to possess various pharmacological potentials. [35] reported epicatechin and epigallocatechin gallate possess known insulin-like properties and hypoglycemic agents. Thus, the presence of high flavonoid concentration exerts most of the pharmacological activities observed in the *N. laevis* extract.

Several plant extracts have been reported to possess reactive radical scavenging abilities, and this potential could be due to the presence of polyphenolic compounds [24, 34]. Here, the DPPH scavenging ability of the extract was comparable to reports on *N. laevis* leaf. Also, the presence of tannins in the extract would have aided in exerting the antioxidant potential obtained. Tannins have been earlier reported to possess free radicals scavenging power [36]. Similarly, alkaloids were reported to exert antioxidant activity [37], antimalaria and antihypertensive activities [38]. Flavonoids are a potent antioxidant agent [39] and anticarcinogenic agent [40].

Table 2 shows the antioxidant potential of the stem-bark extract of *N. laevis*. One of the significant indicators of the antioxidant activity of a compound is its ability to transfer electrons [41]. There was a significant difference in the metal chelating ability of this extract ($p > 0.05$) among 100, 200 and 500 mg/mL, when compared to 300 mg/mL (53.94) and Vit C (59.33%), respectively. A similar result was obtained for the metal chelating ability of the extract, where 300 mg showed a significantly higher ($p > 0.05$) chelating ability than other concentrations studied.

Though different parts of the *N. laevis* plant have been reported to possess antioxidant activity, the stem-bark extract studied proved to possess more antioxidant activity when compared to other parts of the plant. The OH* scavenging activity of *N. laevis* stem-bark (60.27%) is higher when compared to 44.5% obtained for N. laevis leaf extract as reported by [42] and 42% (0.42 mg/mL) reported by [43]. The FRAP potential of the stem-bark extract (57.33%) is higher compared to value obtained by [28] for *N. laevis* leaf extract. The data obtained for the stem-bark extract suggest it possess more antioxidant power than other parts of the plant.

The excessive expression of some physiological enzymes (α-amylase, phospholipase $A_2$ and α-glucosidase) is implicated in the etiology of diabetes Alzheimer's disease [40, 41], inflammation [44] and hypertension [40, 45]. Thus, this study investigated the in vitro bioactivity of *Newbouldia laevis* stem-bark on acetylcholinesterase, butyrylcholinesterase, α-amylase, phospholipase $A_2$, and α-glucosidase enzymes. The control of these enzymes (inhibition) has been reported as an effective method of managing or curing these diseases. Due to their availability, affordability and minimal known side effects, Phyto-therapies are becoming highly recommended and practiced.

Though, non-significant ($p > 0.05$) inhibition of the enzyme was obtained when compared between the cholinesterases. Generally, the extract showed more inhibition of BuchE than AchE at 200, 300 and 500 mg/mL (Table 3). 68–74% inhibition of AchE and BuchE was obtained by the flavonoid-rich extract as reported by [46] Also, the high inhibition ability of cholinesterase was reported by [47], of the extract of *C. albidum,* while a low inhibition value was obtained for AchE as reported by [48]. Flavonoids are known to exert anticholinesterase activity [49], which implies their implication in the management of neurodegenerative diseases.α-amylase is essential in the digestion of carbohydrates, which involves the hydrolysis of starch to oligosaccharide [34]. These are further metabolized by α-glucosidases to glucose which then enters the bloodstream. Rapid breakdown of dietary starch will lead to consistent elevated

post-prandial hyperglycemia. Thus, the inhibition of these enzymes will reduce the concentration of post-prandial glucose. World Health Organization during the Traditional Medicine Strategy 2002–2005 in Geneva, Switzerland, in 2002 had stated that one approach to controlling high blood glucose concentration is by inhibiting amylase and glucosidase.

Flavonoid is a known as potent inhibitors of carbohydrase [50]. Previous research reported $IC_{50}$of 0.36–50 mM of flavonoids on α-amylase [34], while the extract of guava leaf gave $IC_{50}$ of 4.3–4.8 mM for α-amylase inhibition. A low $IC_{50}$ of 74.35 mg/mL was obtained for the inhibition of α-amylase by *M. indica* [51], compared to 243.06 mg/mL obtained in this study. Tannins were also reported as potent inhibitors of α-amylase' [52], while $IC_{50}$ of 23.05 mg/mL was obtained for phenolic compounds [53]. Also, a lower $IC_{50}$ of 83.72 mg/mL was obtained for the inhibition of α-amylase by alkaloids [54]. Likewise, terpenoids have been reported to have α-amylase inhibitory properties [55]. A lower $IC_{50}$ (5.43–0.9 µg/mL) was reported for the inhibition of the enzymes [20]. High inhibition concentrations ($IC_{50}$) of 243.06 and 251.04 mg/mL were obtained for the extract on the α-amylase and α-glucosidase, respectively.

Molecular docking analysis fosters the prediction of the nature of binding interactions between a drug or phytochemical (ligand) and its protein targets (usually referred to as the receptors) [56]. *Newbouldia laevis* has been shown to possess diverse bioactivities, especially for managing diabetic conditions and their associative complexities. Some of these diabetic complexities include sensory disorders, neurological, systemic inflammation, retinopathy, and others. Using HPLC analysis, the phytoconstituents of the flavonoid extract of *N. laevis* were obtained and used to construct the phytochemical library. The compounds obtained are majorly flavonoids and polyphenolics, including apigenin, catechin, quercetin, kaempferol, isorhamnetin, naringenin, and luteolin. The 2D and 3D structures of the compounds were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). They were used for molecular docking to physiological enzymes and its complexities, including α-Amylase, α-Glucosidase, acetylcholinesterase (AChE) butyrylcholinesterase (BuChE), and Phospholipase $A_2$.

In detail, α-Amylase and α-Glucosidase are enzymes that foster carbohydrate digestion, thereby increasing the postprandial glucose level in diabetic patients; hence, inhibiting these enzymes can be a management option against diabetes [53, 54]. In the same vein, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are key enzymes in several neurological disorders. Their inhibition provides an option in the management of neurological disorders. Recent studies have reported the elevation of these enzymes in the blood of diabetic patients, just as in the neurological condition of Alzheimer's disease [49]. Moreover, lastly, Phospholipase $A_2$ (PLA2) is another key enzyme prominent in several inflammatory conditions, especially in diabetics, such as retinopathy, periodontitis, and others. The PLA2 enzymes specifically foster the release of arachidonic acid to generate lipid mediators of inflammation [57]. Therefore, the management of diabetes and its complexities can be better achieved through a multi-targeted approach to discovering a drug or drugs with multi-inhibitory activities against different enzymes involved in the etiology of diabetes.

The binding energy measures the relative affinities or strength of interaction between a ligand in its target. The higher the negative value of binding energy, the better the ligand-receptor interaction or binding affinities. The binding energies of the different phytochemicals against the various targets, as reported in Table 5, showed better interactions of the phytochemicals within the binding pocket of the receptors than both the standard drugs and co-crystallized ligands. Moreover, all the phytocompounds showed significant activities against AChE, α-Glucosidase, and α-Amylase. Whereas, against butyrylcholinesterase and Phospholipase $A_2$, the affinities were similar to the standard drugs. Although all the phytochemicals identified from the ethanol extract of *Newbouldia laevis* stem-bark showed interesting affinities to the different targets, naringenin had the best binding affinity among others toward all the targets except α-glucosidase. Based on the docking result from Table 5, the plant's phytochemicals can be ranked based on their potencies against diabetes complexities as naringenin > Luteolin > Quercetin > Isorhamnetin > catechin > apigenin > kaempferol. Examining the 2D interaction of the phytochemicals against binding pocket amino acids of the various targets showed the common existence of hydrogen bonding to residues like aspartate, glutamate, histidine, arginine and others. Moreover, other weak interactions such as Vander Waals interaction and different pi-pi interactions among aromatic rings existed among atoms of the phytochemical and amino acids in the active site of the target (Figs. 3, 4, 5, 6, 7). The 3D structures (Figs. 8, 9, 10, 11, 12) indicated that the phytocompounds from the *N. laevis* bind deeply into the enzyme's active site, rather than just a shallow or surface binding. Deeply bound compounds usually indicate a more stable interaction than shallow binders, although there is a need for more advanced molecular dynamic simulations and DFT analysis to ascertain the claim. In addition to the high binding affinity, all phytochemicals showed insignificantly or zero toxicity from the toxicity

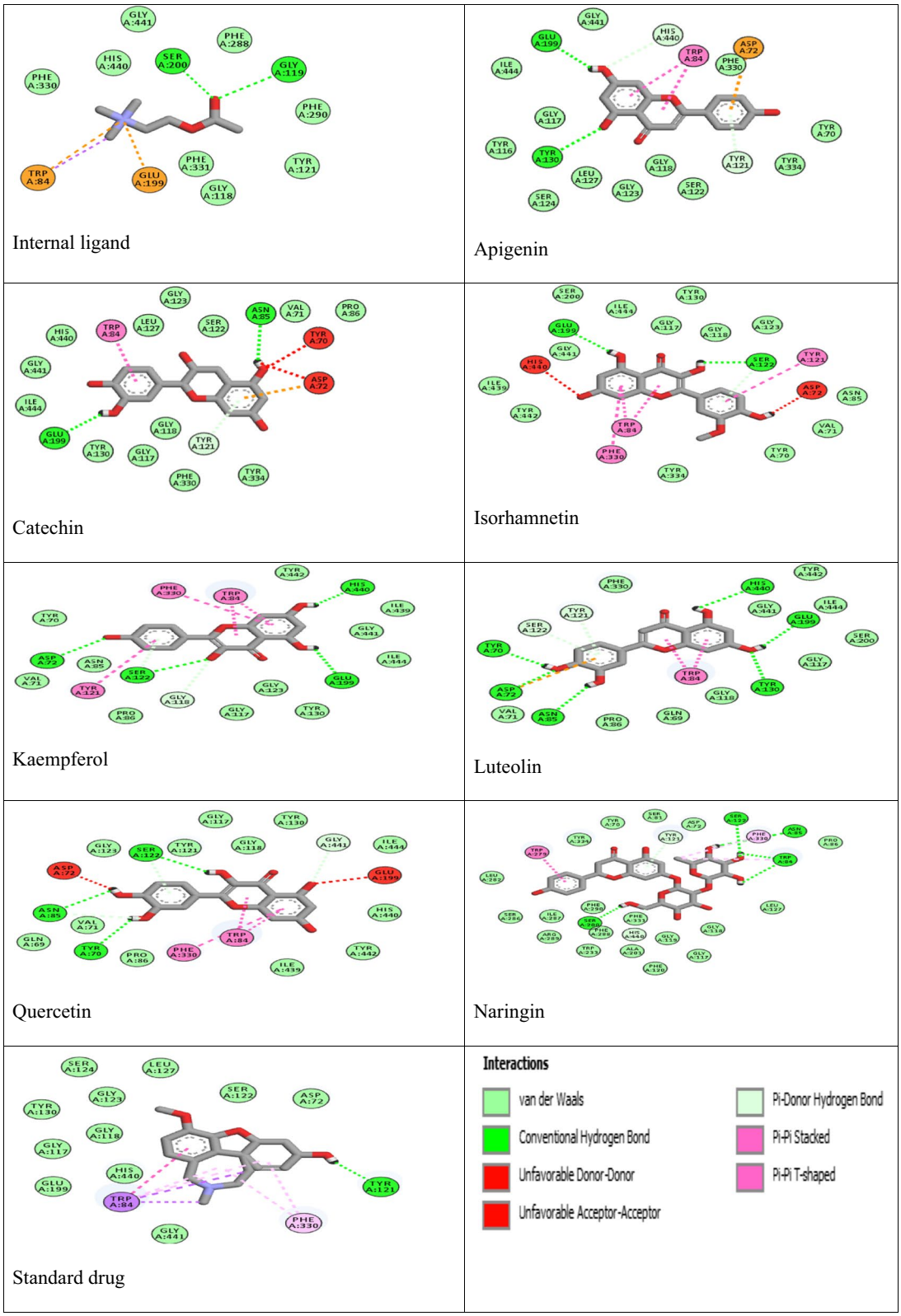


**Fig. 3** 2D views of interactions with acetylcholinesterase

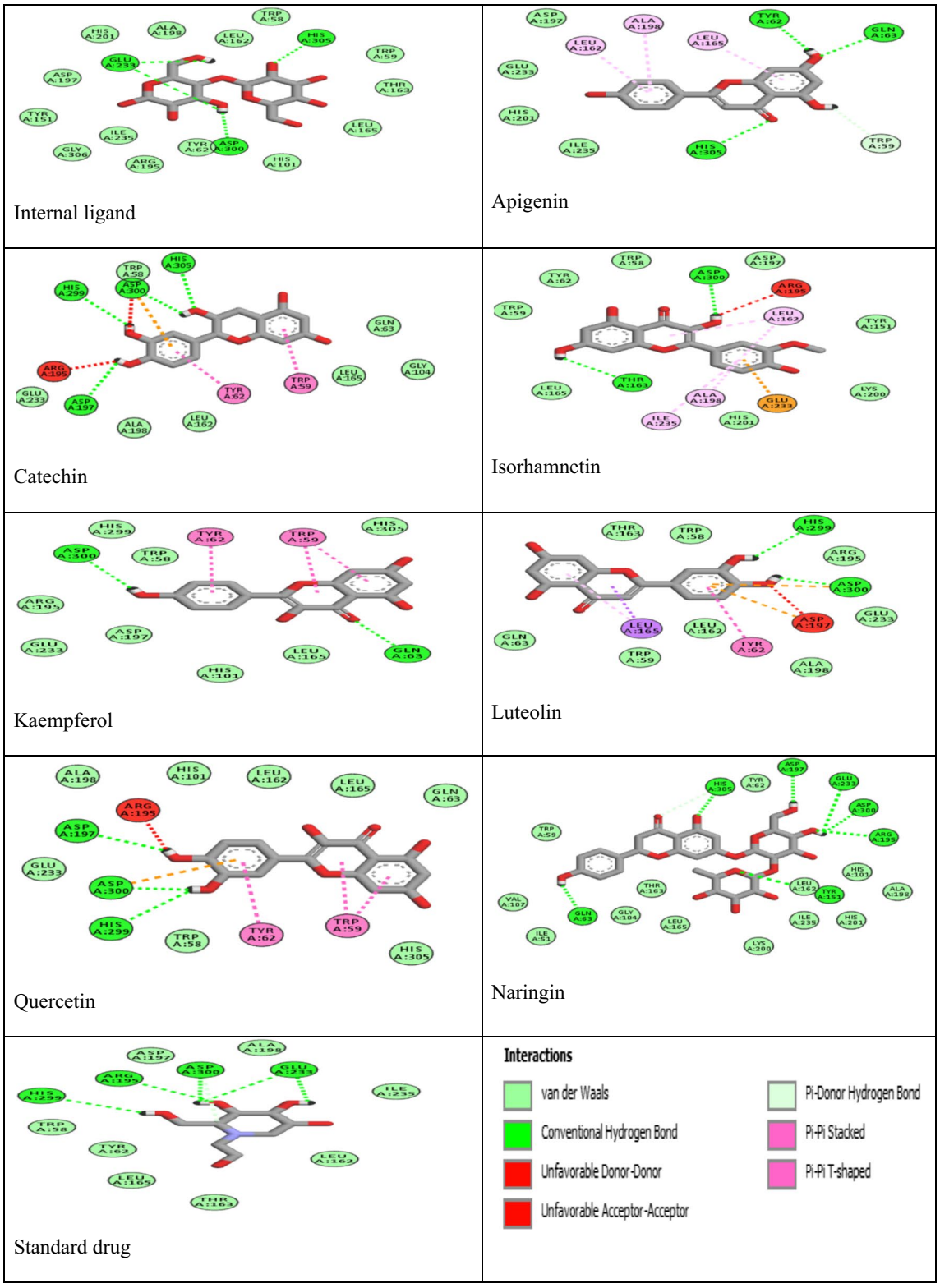


**Fig. 4** 2D views of interactions with amylase

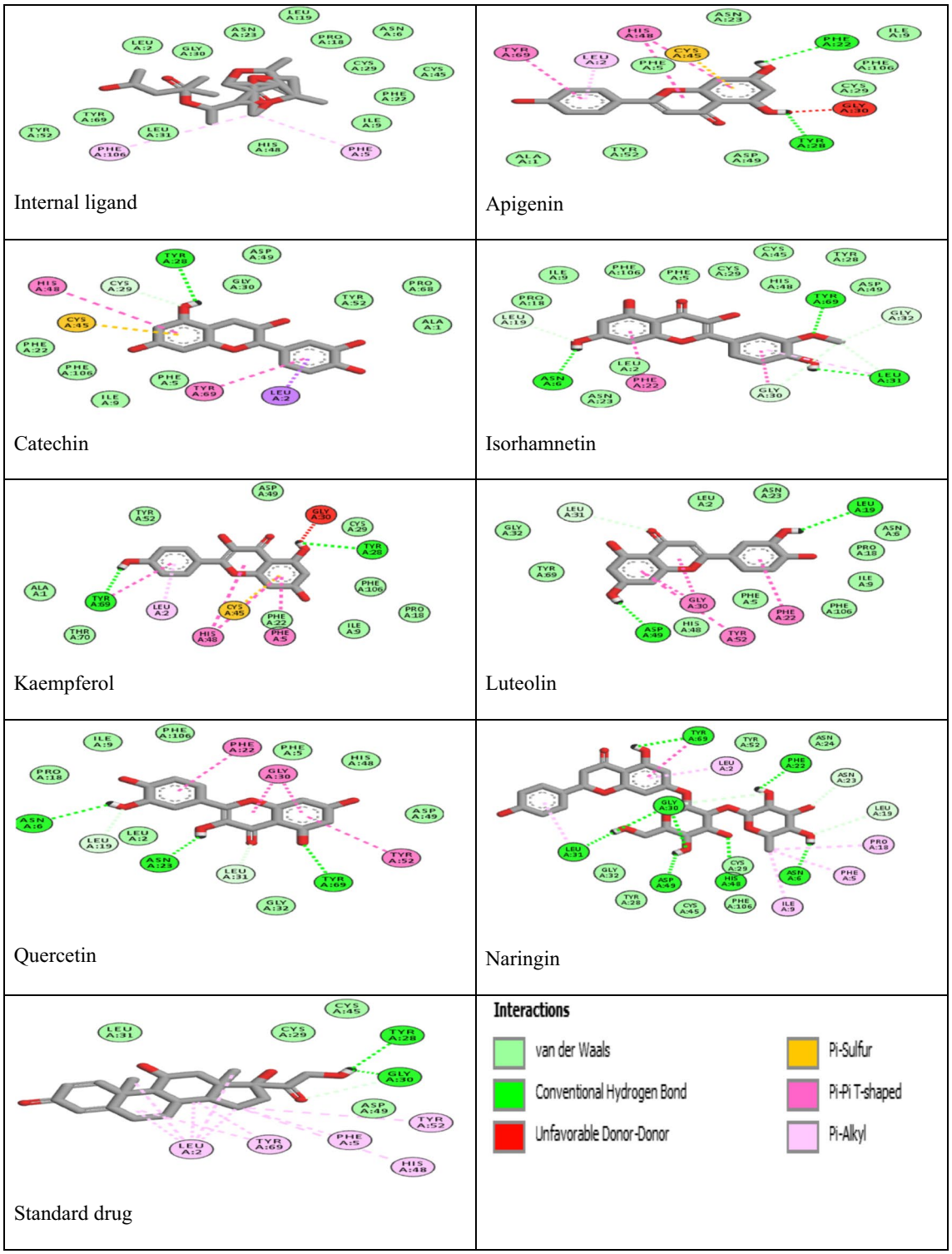


**Fig. 5** 2D views of interactions with phospholipase $A_2$

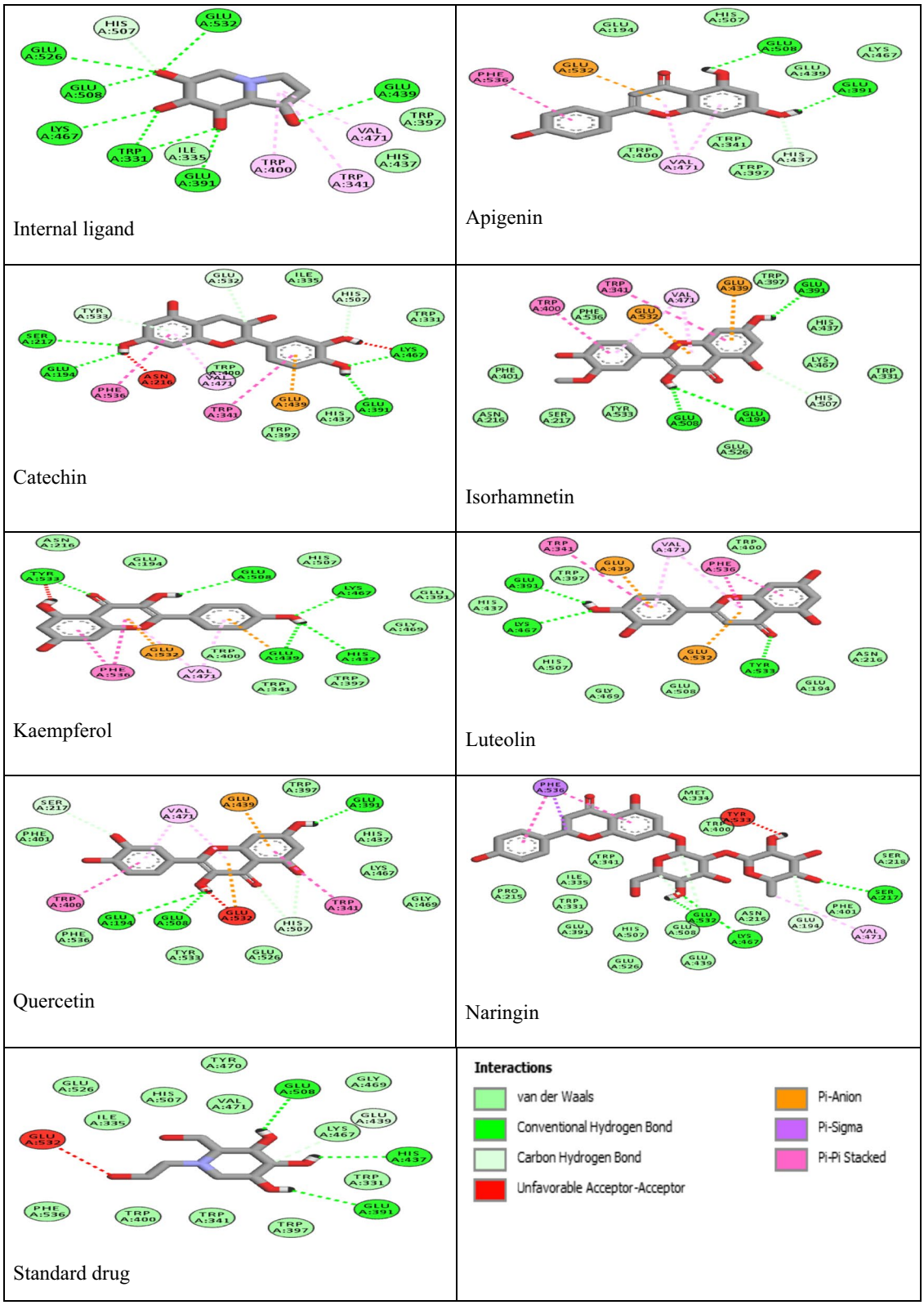


**Fig. 6** 2D views of interaction of butyrylcholinesterase

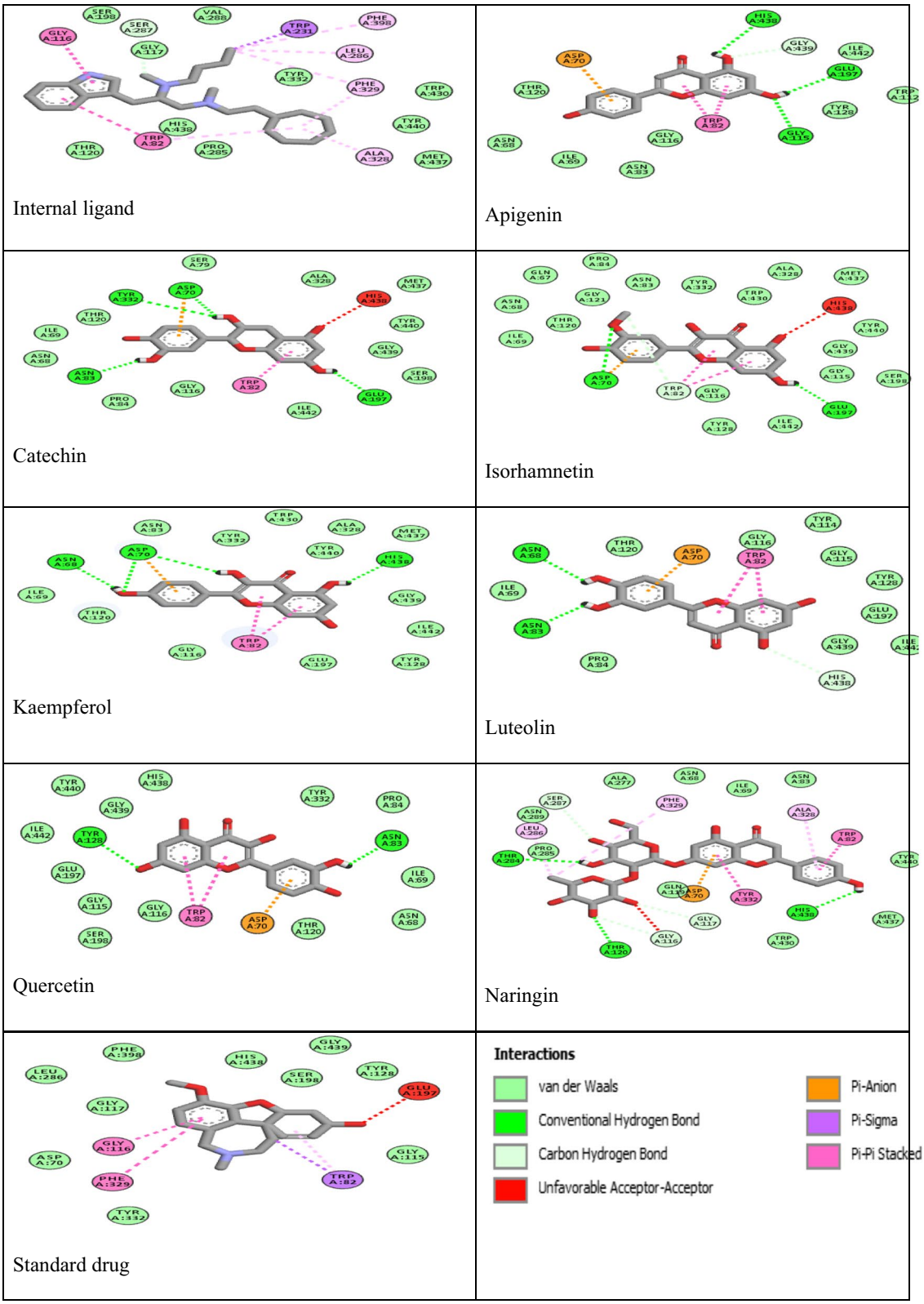


**Fig. 7** 2D views interaction of α-glucosidase

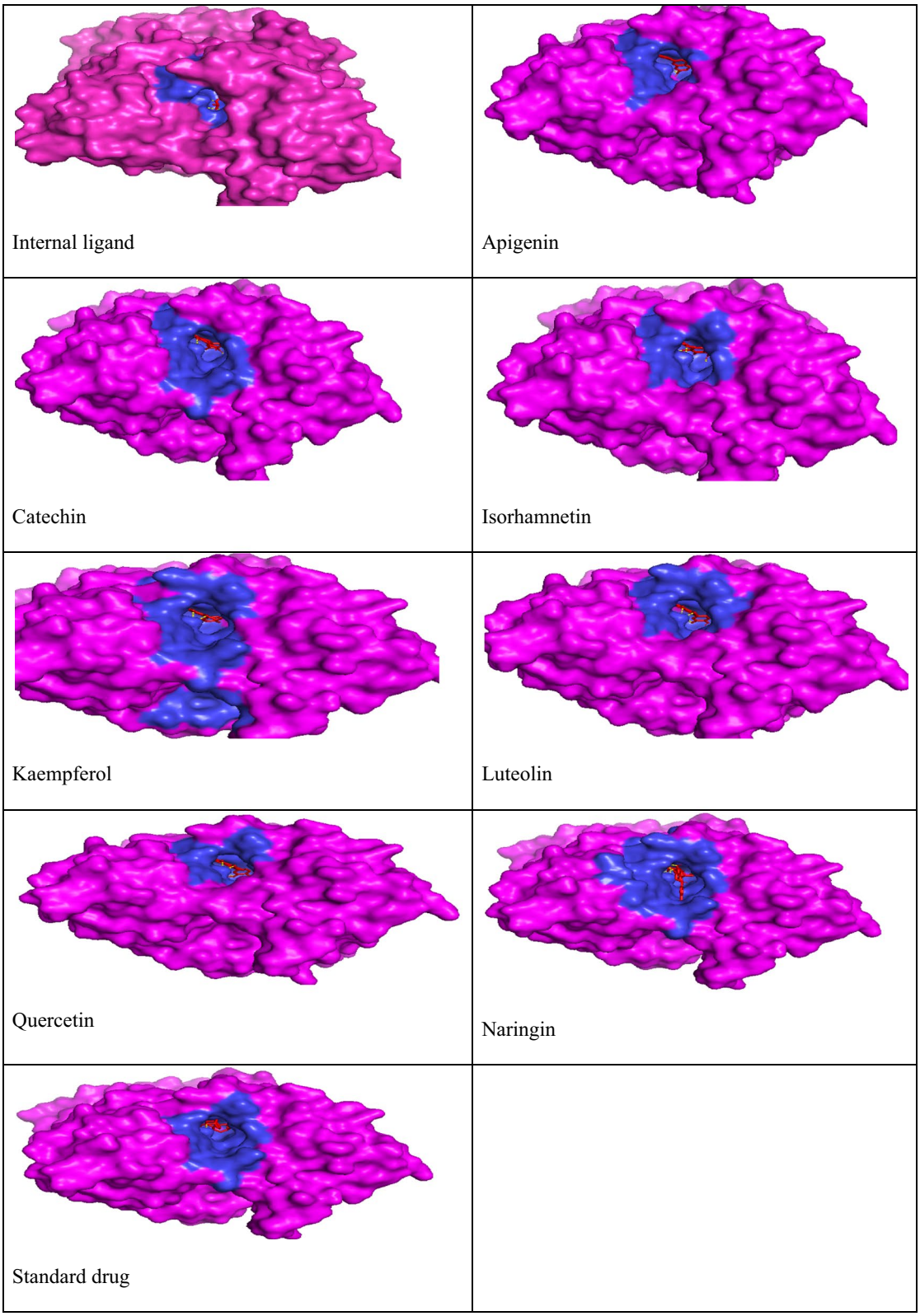


**Fig. 8** 3D views of interactions with acetylcholinesterase

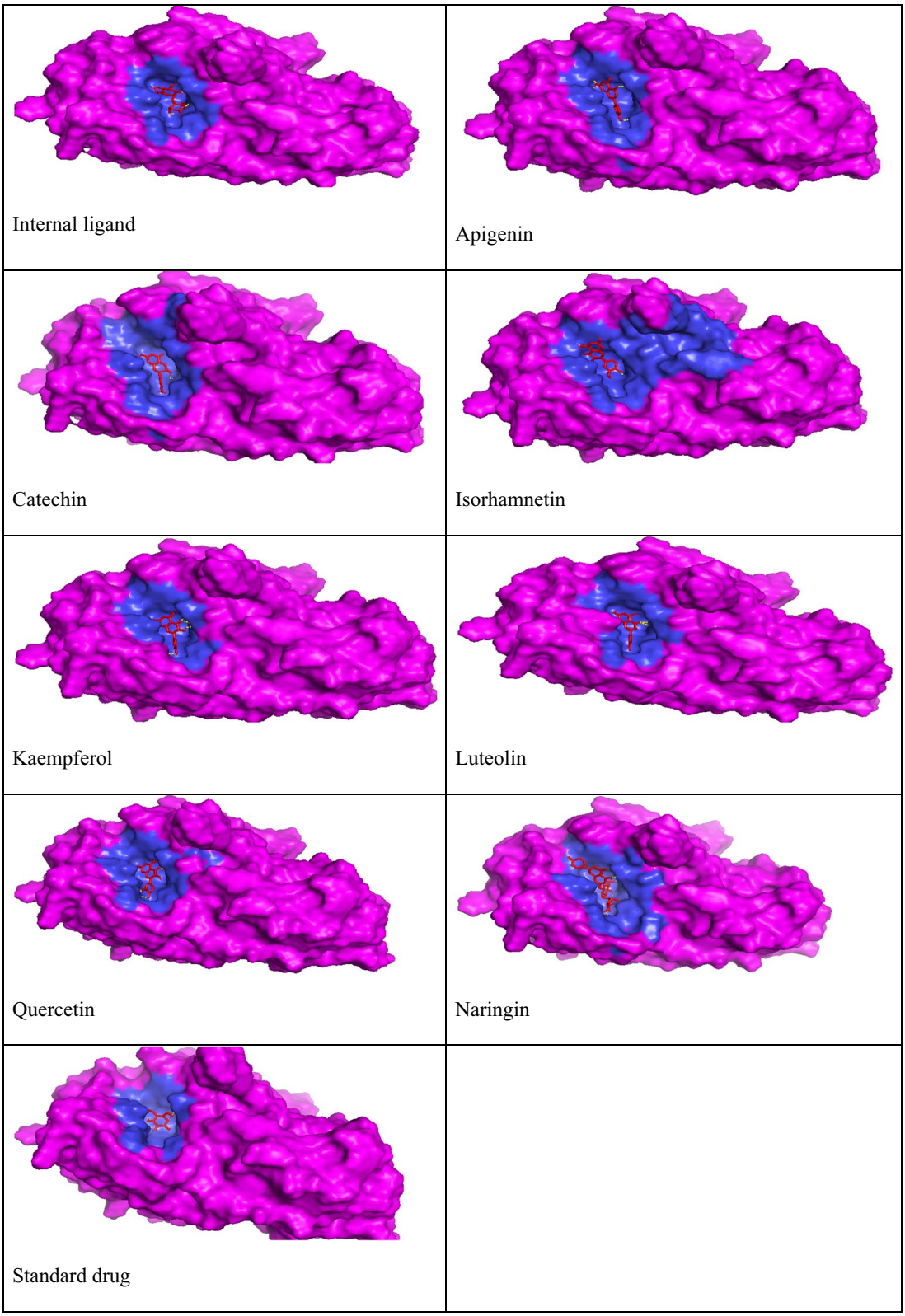


**Fig. 9** 3D views of interactions with α-amylase

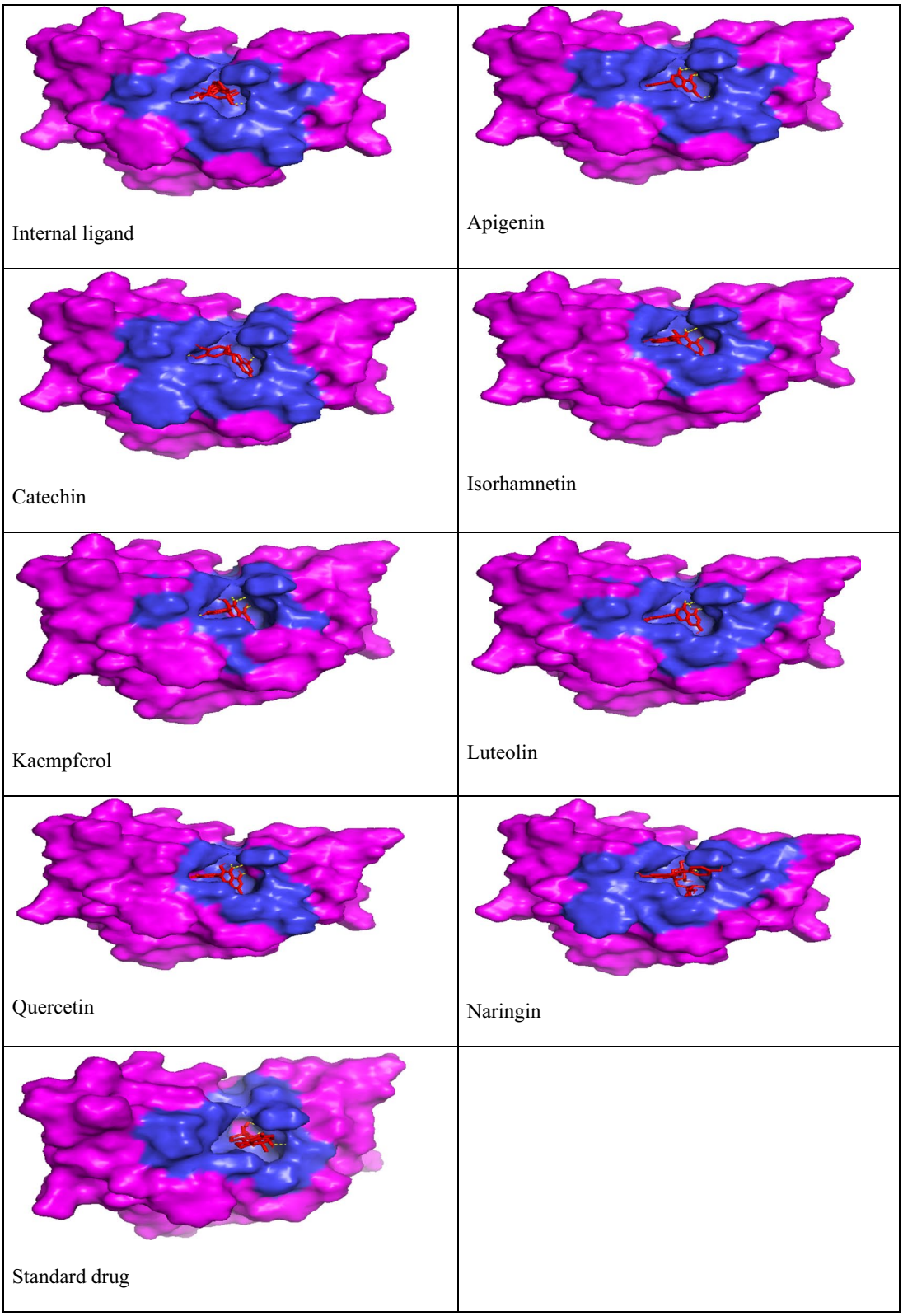


**Fig. 10** 3D views of interactions with phospholipase $A_2$

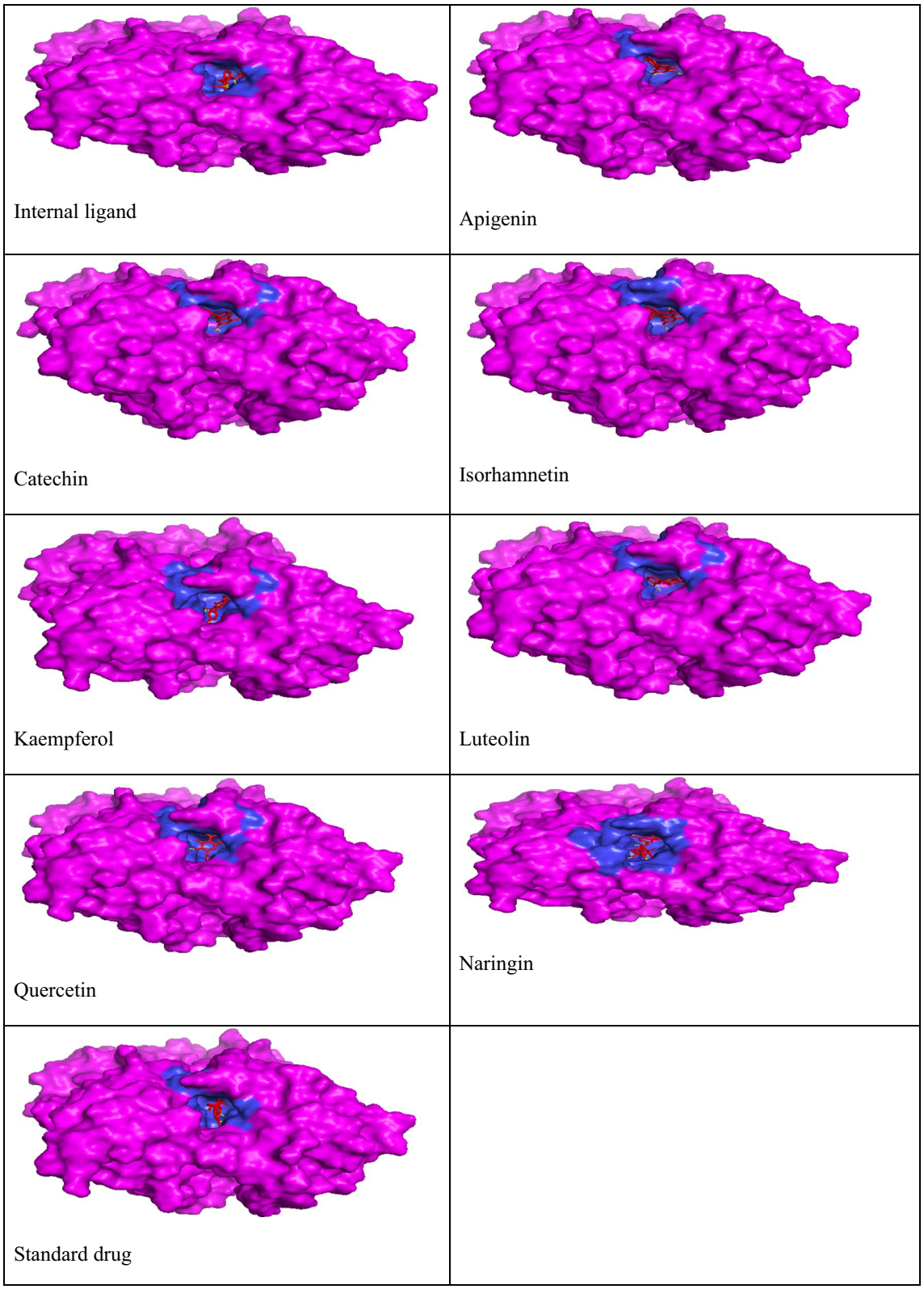


**Fig. 11** 3D views of interaction of butyrylcholinesterase

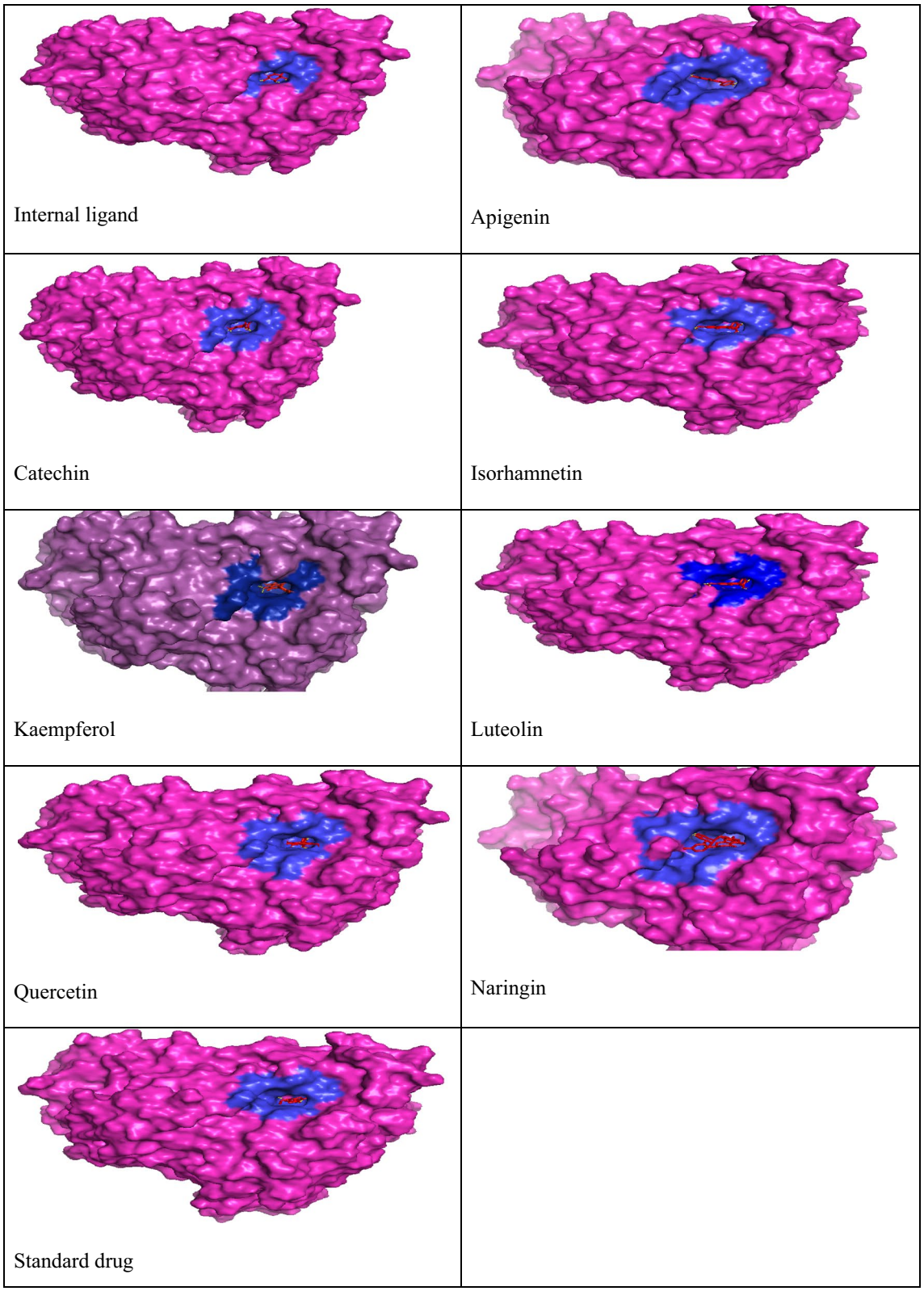


**Fig. 12** 3D views interaction of α-glucosidase

**Table 6** Predicted toxicity analysis

| Phyto-compounds | Predicted LD50 value (mg/kg) | Predicted toxicity class (1–6) | Prediction accuracy (%) | Hepatotoxicity | Immunotoxicity | Cytotoxicity | Carcinogenicity | Mutagenicity |
|---|---|---|---|---|---|---|---|---|
| Quercetin | 159 | 3 | 100 | Inactive | Inactive | Inactive | Active | Active |
| Kaempferol | 3919 | 5 | 70.97 | Inactive | Inactive | Inactive | Inactive | Inactive |
| Apigenin | 2500 | 5 | 70.97 | Inactive | Inactive | Inactive | Inactive | Inactive |
| Catechin | 10,000 | 6 | 100 | Inactive | Inactive | Inactive | Inactive | Inactive |
| Isorhamnetin | 5000 | 5 | 70.97 | Inactive | Active | Inactive | Inactive | Inactive |
| Naringin | 2300 | 5 | 70.97 | Inactive | Active | Inactive | Inactive | Inactive |
| Luteolin | 3919 | 5 | 80.26 | Inactive | Active | Inactive | Active | Inactive |

prediction analysis reported in Table 6. This in silico toxicity prediction is consistent with in vitro toxicity studies on the plant, reporting their non-toxicities in several animal models and cell lines.

In summary, our findings are similar to in silico studies, with several studies conducted singly against the identified phytochemicals and several targets [56, 58, 59]. In a recent study, naringenin and other interesting flavonoid derivatives were synthesized and shown to possess vitro AChE inhibitory activities ($IC_{50} < 100$ μM) [60]. Similarly, in in vitro and in silico studies, luteolin exhibited exciting inhibitory activities against α-glucosidase [61]. More so, apigenin isolated alongside other flavonoids from the extract and the flavonoid-rich fraction of *Merremia tridentata* (L.) showed remarkable antidiabetic properties in vitro [62]. Although these phytochemicals in our plant extract and their impressive binding properties to various targets promise tremendous therapeutic activities against diabetic complications, there is a need for in vivo and other in vitro studies on cell cultures to corroborate these findings.

## Conclusion

The research presented the flavonoids fingerprint of *N. laevis* stem-bark extract which showed high concentrations of catechin, apigenin, luteolin, kaempferol and quercetin, respectively, which are efficacious flavonoids with proven bioactivities. Also, the power to scavenge radicals generated in vitro suggests it could be a good antioxidant agent. The extract showed good attributes of an antidiabetic agent due to its ability to inhibit α-glucosidase and α-amylase with inhibition constants (*Ki*) of 2.95 and 2.11, respectively, and its potential to ameliorate other complications associated with diabetes by inhibiting AchE, BuchE and $PLA_2$. The power of the extract to mop up some radicals and inhibit some of these physiological enzymes in vitro suggests that the stem bark could be effective in ameliorating the complications associated with diabetes.

**Abbreviations**

| | |
|---|---|
| GC–FID | Gas chromatography/flame ionization detector |
| HPLC | High-performance liquid chromatography |
| DPPH | 2,2-Diphenyl-1-picrylhydrazyl |
| OH* | Hydroxyl radical |
| SO* | Sulfoxide radical |
| NO* | Nitric oxide radical |
| $PLA_2$ | Phospholipase $A_2$ |
| Ki | Inhibition constant |
| PDB | Protein data bank |
| AchE | Acetylcholinesterase |
| BuchE | Butyrylcholinesterase |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43094-023-00486-0.

**Additional file 1:** HPLC data for *N. laevis* flavonoids.

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**Author contributions**

KOO and SOOE contributed to conceptualizing the work, validation of the work and drafted the paper; BOE and CNO performed the experiment, revised the draft paper. SCO contributed to data analysis; TPCE interpreted data and methodology. All the authors read and approved the final version of the manuscript.

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**Availability of data and materials**

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

## Declarations

**Ethics approval and consent to participate**

Not applicable for this work. ***Newbouldia laevis*** stem bark was used in this study. The plant/plant part was provided and identified by Mr Felix Nwafor of the department of plant science and biotechnology, University of Nigeria, Nsukka and deposited as ***Newbouldia laevis G. Don***. (***Sapotaceae)*** with voucher number PCG/UNN/0359. The study was approved by the ethics committee of the Faculty of Biological Sciences, University of Nigeria, Nsukka, with reference Number FBS/2022/00183.

**Consent for publication**
The authors declare no conflict of interest.

**Competing interests**
The authors declare that they have no competing interests.

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