Validation of phytochemicals from Houttuynia cordata for their effect on dipeptidyl peptidase IV and sodium/glucose cotransporter 2: an in silico study

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Abstract

The study identified and screened phytochemicals present in *Houttuynia cordata* and characterized their ADME/Tox properties. The powdered plant extracted using water and methanol for about 24h was refluxed, filtered, and evaporated to dryness under reduced pressure and subjected to High-Performance Thin Layer Chromatography, and Gas Chromatography tandem mass spectrometry analysis using a standard protocol. The compounds identified from *Houttuynia cordata* were docked with antidiabetic targets- dipeptidyl peptidase-IV and sodium/glucose cotransporter 2. High-Performance Thin Layer Chromatography and Gas Chromatography tandem mass spectrometry analysis showed the presence of several compounds by matching their recorded spectra with the data bank mass spectra from the NIST library. ADME/Tox properties and docking of the identified compounds indicate the potentiality of the compounds as drug candidates. Selected compounds from *Houttuynia cordata* have good pharmacokinetic properties and binding affinity for DPP-IV and SGLT2. However, the isolation of these phytoconstituents and their *in vivo* activity will help give a better insight and will open a new area of investigation of individual components and their pharmacological potency.

Introduction

India is a land of immense biodiversity with over 6000 plants used in traditional, folk, and herbal medicine, representing about 75% of the medicinal needs of the third world countries (Dubey et al., 2004).Several substances derived from plants are currently used as drugs. Plants being non-narcotic, and cost-effective, as well as their association with fewer side effects, have acquired an important position in both developing and developed countries (Salmerón-Manzano et al., 2020). However, the pharmacological properties of many of these metabolites are still unknown. The metabolites present in these plants usually secondary, are responsible for their medicinal properties (Hussein & El-Anssary, 2019). But not all metabolites present in the plant are beneficial. Some of these may be toxic and not all plants display any form of pharmacological or therapeutic action (Haddad-Kashani et al., 2012) and as a consequence in the field of drug development, it is important to classify the phytoconstituents (Nakka & Devendra, 2012). Therefore, in-depth phytochemical screening and evaluation of these plants are valuable to understanding the complete action of the plant as a whole. This will contribute to and ease the discovery of leads for the treatment of various diseases.

One of the most promising metabolites– the flavonoids constitutes the largest group of secondary metabolites (de Rijke etal., 2006). They have been documented to have antidiabetic, anti-inflammatory, enzyme inhibition, antimicrobial, anticancer, antiallergy, and antioxidant properties (Harborne & Williams, 2008). Diabetes the fastest-growing health crisis is the third leading cause of death in the twenty-first century. According to the International Diabetes Federation (International Diabetes Federation, 2021), diabetes was responsible for 6.7 million fatalities. It is a serious, chronic illness that causes increased blood sugar levels because the body is unable to produce enough insulin or use it effectively. The need for novel treatment methods for type 2 diabetes mellitus (T2DM) is critical given how much of a burden it has been on the global community. Treatments of T2DM that raise the levels of insulin in the blood have

been shown to be effective. A promising novel treatment for T2DM, dipeptidyl peptidase-IV (DPP-IV) inhibitors act at least in part, as indirect stimulators of insulin production (Lankas et al., 2005). DPP-IV is expressed in a number of organs and cleaves oligopeptides after the second amino acid from the N-terminal end as one of its principal functions, acting preferentially on Glucagon-Like peptide-1 (GLP-1). GLP-1 is a complex hormone with a wide range of therapeutic applications and metabolic effects which include the glucose-dependent stimulation of insulin secretion, a reduction in stomach emptying, food intake, an increase in natriuresis and dieresis. It also influences learning and memory, reward behaviour, and palatability in addition to having cardio- and neuroprotective effects and reducing inflammation and apoptosis (Müller et al., 2019). The half-life of active GLP-1 is less than 2 minutes and only 60% of total GLP-1 in the circulation is active GLP-1. This half-life is markedly extended by DPP-IV inhibition, which also raises the percentage of active GLP-1 in the overall pool of circulating GLP-1 (Ahrén, 2007). Uncontrolled T2DM is characterized by high plasma glucose levels that are over the limit of glucose reabsorption, saturating the sodium-glucose cotransporter (SGLT) receptors, and increasing glucose excretion in the urine. By inhibiting SGLT, one might decrease urine glucose reabsorption and excretion, lower plasma glucose levels, and perhaps provide a novel treatment approach without the side effects associated with the currently available T2DM medications (Chao & Henry, 2010). SGLT1 and SGLT2 are key contributors to the transport of glucose through epithelial cells. While the majority of dietary glucose is absorbed in the gut by SGLT1, the majority (~ 90%) of glucose is reabsorbed in the kidney's tubular system by SGLT2 (Reig & Vallon, 2018).

Houttuynia cordata Thunb. (HC) is a traditional edible and medicinal herb belonging to the Saururaceae family. In Meghalaya, it is used to treat a variety of illnesses. Raw leaves of HC are consumed for blood cleansing, and they are also used topically to treat boils and ulcers. In Korea and China, it is used to treat dysentery, fever, dyspepsia, and hematochezia (de Rijke et al., 2006). The evidence for the numerous pharmaceutical effects of HC is mounting, including its anti-cancer, anaphylactic inhibitory, anti-mutagenic, anti-inflammatory, anti-allergic, anti-oxidative, anti-viral, anti-bacterial, anti-obesity, and anti-diabetic activities (Laldinsangi, 2022; Wu et al., 2021) Although the effects of plant extracts on several diabetic-related parameters have been documented in the past, there is no documentation of the effects of this plant on sodium/glucose cotransporter 2 (SGLT2) or dipeptidyl peptidase IV (DPP-IV). Hence, the present study was designed to identify potential compounds from HC to provide additional insights regarding their SGLT2 and DPP-IV inhibitory activity. The ADMET properties of the identified phytoconstituents were also analyzed for their compatibility as drug molecules and these molecules were also docked with DPP-IV and SGLT2 to determine their binding affinity to the antidiabetic targets.

Materials and methods

Chemicals

All chemicals used were of analytical grade. Catechin (purity > 98%), rutin (purity > 90%) and gallic acid (purity > 98%) was procured from Sigma Aldrich Co. (St. Louis, MO, USA). Epicatechin (purity > 99%) and kaempferol (purity > 99%) were procured from HWI Analytik GMBH pharma solutions, Germany. Quercetin

(purity > 99%) and methanol from Sisco Research Limited, India. All other chemicals used were purchased from S.D.fine–Chem. Ltd., Hi-Media and Fischer Scientific, India.

Plant Collection and Extraction

HC was collected from Mairang Village, West Khasi Hills District, Meghalaya, India and the plant specimen (voucher no. NEHU-11922) was submitted and identified by herbarium curator Dr. P.B. Gurung, Department of Botany, NEHU, Shillong.

The leaves of HC were washed, shredded, dried in the shade, and grounded. 50 gm of dried powdered mass was weighed and extracted with 10 volumes of aqueous-methanol solution (1:4) for 24h by maceration method with continuous stirring at room temperature. The mixture was filtered using a muslin cloth followed by whattmann no.1 and the filtrate was concentrated in a rotary evaporator (Yamato RE800) and then lyophilized (Scanvac Coolsafe) to obtain the crude powder (gm) (Thabah et al., 2021).

High-Performance Thin Layer Chromatography (HPTLC)

A powdered sample of aqueous extract of HC (AHC), and methanolic extract of HC (MHC) were reconstituted in methanol and applied onto a pre-coated HPTLC plate (silica gel F254) using a Camag Linomat 5 system applicator. Prior to usage, plates were activated at 50–60 C for 15-20min. The application was programmed through WinCATS planar chromatography manager (Version 1.4.8). The plates were resolved, dried, developed, and documented using a Camag TLC Visualizer under ultraviolet light at λ254nm and λ366nm.

Identification of plant metabolites using GC-MS/MS

GCMS analysis of the plant extracts was performed using Claurus 680 Gas Chromatograph/Claurus 600C Mass spectrometer, Perkin Elmer (USA). An HP Elite-35MS Capillary Column: Length: 60 m, I.D: 0.25 mm; Phase Reference: 35% diphenyl 65% dimethyl polysiloxane (low bleed) was used for GC/MS. The column temperature was programmed at a maximum of 350°C at a rate of 4°C/min with the lower and upper temperatures being held for 3 and 10min, respectively. Helium (99.99% purity) was used as carrier gas at a flow rate of 1.0 ml/min. Compounds were identified by their retention times and mass fragmentation patterns using Turbomass (Version5.4.2). Interpretation of Mass-Spectrum was carried out by using S/W Turbomass NIST 2008 Library having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components which was stored in the NIST library. The molecular weight, name, chemical structure, and molecular formula of the components of the test materials were ascertained.

ADME-Tox

Compounds identified in HC were evaluated for their Absorption, Distribution, Metabolism, and excretion including partition coefficient, solubility, and several other parameters using the SwissADME module provided in SIB (Swiss Institute of Bioinformatics) webserver (https://www.sib.swiss). Furthermore, the toxicity and drug-likeness of the compounds were predicted using Osiris DataWarrior (Version5.2.1)

Docking

The 3-D structure of the target and ligands with their respective PubChem CID were redeemed from the Protein Data Bank and Pubchem Database. Vildagliptin and dapagliflozin, known standard drugs for inhibition of DPP-IV and SGLT2 were used as the positive control. Using Pymol software metals were removed from the structure of the ligand and were saved in PDB format for docking. The binding energies of the proteins and ligands were calculated based on the Equation:

$$G=({}_{Bound}V^{L-L} - {}_{Unbound}V^{L-L}) + ({}_{Bound}V^{P-P} - {}_{Unbound}V^{P-P}) + ({}_{Bound}V^{P-L} - {}_{Unbound}V^{P-L} + S_{conf})$$

where P refers to the protein; L refers to the ligand; V represents the pair-wise evaluations and S denotes the loss of conformational entropy upon binding.

Results

HPTLC analysis

The qualitative analysis of AHC, and MHC indicated the presence of secondary metabolites like kaempferol, quercetin, and catechin (Fig. 1and **Table-1**). Both aqueous and methanolic extract of HC had well resolved bands at λ366 nm and λ254 nm. At λ254nm and white light, kaempferol (Rf = 0.67) and quercetion (Rf = 0.70) (Fig. 1a) were detected in HC. At λ366nm, in addition to kaempferol (Rf = 0.65), and quercetin (Rf = 0.57), catechin (Rf = 0.31) (Fig. 1b) was also found to be present. The Rf value of the resolved bands of the plant extracts and the reference standards are presented in **Table-1**.

GCMS/MS analysis

Identification of phytoconstituents of HC using GCMS/MS was based on the retention time, area%, and comparison of the mass spectra of known compounds listed in S/W Turbomass NIST 2008 Library. The GC-MS chromatogram showed the presence of several components (Fig. 2) with different retention times and area%. Based on their retention time (Rt) and area %, three peaks from AHC (11.08, 14.49, 47.31), and eight peaks from MHC (8.26, 9.36, 10.96, 12.08, 15.98, 16.65, 17.90, 21.16) were selected for analyzing the mass spectra. The mass spectra of the phytochemicals identified in AHC, and MHC are presented in Fig. 1S, **and** Fig. 2S. The various compounds identified are 3-carene, alpha-pinene, alpha-thujene, D-limonene, sylvestrene, bis(2-ethylhexyl)ester hexanedioc acid, isopulegol, 2,5-cyclohexadione, 4-methylcyclohexanone, 2-methylcyclohexanone, cycloheptanone, 2-methyl-n-2-(methylbutylidene)-1-butanamine (**Table-2**).

ADMET study

Compounds identified using HPTLC, GCMS/MS and previously reported by Anal (2014) and Choudhary (2015) were assessed for their ADME and toxicity properties. Based on the ADME values from SwissADME, the compounds listed (**Table-3** and **Table-4**) have a variable ADME properties. Out of the 15

compounds identified, structures of only 14 compounds were available and retrieved from PubChem. The ADME study of the 14 identified and 21 reported compounds showed that 87.5% of the compounds violated at least one of Lipinski's Rule of five (Ro5) (**Table-3a**, **Table-4b**, and **Table-S1**). Of the 35 compounds identified from HC, 18 compounds were found to be non-toxic (**Table-3b**and **Table 4c**) and 26 compounds had a bioavailability score ≥ 0.55 (**Table-3a** and **Table 4b**). 62.85% of the compounds were hydrophilic (Fig. 4, **Table-3a** and **Table 4b**). 48.57% of the identified compounds had a high gastrointestinal absorption (GIA) while 31.42% crossed the blood brain barrier (BBB) (Fig. 5).

Docking

Phytochemicals present in HC were investigated for identification of the binding domains by conducting molecular docking analysis of the selected protein. Compounds that had high GIA and bioavailability score, and non-toxic were selected for the docking studies. The first molecular docking investigations were carried out over the course of ten runs, and all created docking complexes were examined. The suitable docked complex with the lowest binding energy and maximum binding affinity was selected for its superior interaction with the proteins. The employed docking methods showed effective binding energies (**Table-6a** and **Table-6b**). The ligands' lowest binding energy values and maximum binding affinities served as the foundation for critical analysis.

Discussion

HPTLC and GCMS/MS are chromatographic tools widely used for the phytochemical profiling and estimation of biochemical markers of herbal plants (Misra et al., 2014; Nair & Clarke 2017; Yamunadevi et al., 2011). Such fingerprinting is helpful in the quality management of herbal products (Teo et al., 2013) and also for the assessment of various pharmaceutical preparations on the market (Patel et al., 2013). Secondary metabolites found in plants, such as phenols, flavonoids, alkaloids, terpenoids, and glycosides, are a useful source of potential novel medications and drug precursors. Through processes involving reactive oxygen species, phenols exhibit significant properties like inhibition of pathogens and decaying microorganisms, prevention of triglyceride deposition, reduction of the incidence of non-communicable diseases like cardiovascular disease, diabetes, cancer, stroke, and anti-inflammatory and anti-allergic effects (Ozcan et al., 2014). In nature, phenolic compounds can take many different forms, from mono-phenolic molecules to compounds containing multiple phenolic groups, like stilbenoids and flavonoids, to polymeric compounds like proanthocyanidins and tannins (Ho et al., 2020). Low molecular weight secondary metabolites flavonoids have anti-aging, anti-bacterial, antioxidant, and anti-cancer effects (Abraham et al., 2020). Terpenoids and terpenes play a significant role in the treatment of a variety of diseases through the activity of monoterpenes, sesquiterpenes, diterpenes, triterpenes, and tetraterpenes and glycoside compounds as anticancer agents, antimicrobial, anti-inflammatory, antioxidants, antiallergenic, neuroprotective, anti-aggregator, anti-coagulation, sedative and analgesic (Masyita, 2020). Phytochemical analysis revealed that HC contained secondary metabolites like flavonoids, phenols, terpenoids, and anthocyanidins, conferring cumulative pharmacological properties.

A powerful molecule has to be concentrated sufficiently to reach its target in the body and remain there in a bioactive state long enough for the anticipated biologic reactions to take place for it to be effective as a medication. Bioavailability, defined as the percentage of a dosage that enters the circulation unaltered is a composite metric depending on absorption and metabolism. Evaluation of the bioavailability of substances taken orally can be done using Lipinski's rule of five (RO5) (Lipinski et al., 2012). According to the RO5, the potential for oral bioavailability of a molecule is greatly increased if it satisfies the following criteria:(i) molecular weight < 500; (ii) hydrogen bond donors < 5 (OH and NH groups); (iii) hydrogen bond acceptors < 10 (N and O atoms);(iv) rotatable bonds < 5, and (v) logP < 5 (Lipinski et al., 2012).

Many drug development processes are considerably facilitated by the presence of soluble molecules, especially handling and formulation. Furthermore, solubility is a key factor affecting absorption for research initiatives aiming for oral delivery. One of the parameters affecting medication absorption and distribution is solubility, as evaluated by LogS, ESOL LogS, Ali LogS, and Silicos-IT LogSw. According to Daina (2017) compounds with a LogS value of <-6 are said to be poorly soluble. In accordance with this, all of the above compounds had a good solubility with exception to bis(2-ethylhexyl)ester hexanedioic acid. LogP, the partition coefficient between n-octanol and water (log Po/w) is the classical descriptor for lipophilicity. A molecule with an XLOGP3 between − 0.7 and + 5.0 is said to have a good bioavailability [39]. With exception to bis(2-ethylhexyl)ester hexanedioic acid all the compounds had a logP value between − 0.7 and + 5 (Daina et al., 2017).

The number of rotatable bonds (RB) also correlates with reduced oral bioavailability and has been widely used as a filter. Molecules with RB of more than 10 have lower bioavailability (Veber et al., 2002). Alpha pinene, alpha-thujene, 3-carene, d-limonene, 3-carene, sylvestrene, bis(2-ethylhexyl)ester hexanedioic acid, isopulegol, 2,5-cyclohexadiene-1,4-diol, 4-methyl-cyclohexanone, 2-methyl-cyclohexanone, cycloheptanone, 2-methyl-n-(2-methylbutylidene)-1-butanamine, quercetin, kaempferol, apigenin, afzelin, protocatechuic acid, phloridzin, quinic acid, caffeic acid, vanillic acid, isorhamnetin, chlorogenic acid methyl ester, cis-methylferulate, vanillin, and catechin have an RB lesser than 10 and hence have a high oral bioavailability (> 0.55). Although the oral bioavailability of phloridzin, quinic acid, chlorogenic acid methyl ester is high, they have a low GIA. Bioavailability is multifactorial but is primarily driven by gastrointestinal absorption (Newby et al., 2015) and absorption of an orally administered compound is more likely if the RO5 is fulfilled (Lipinski et al., 2012).

The BOILED-Egg model (Daina et al., 2017) helps in drug development by filtering the chemical libraries. The analysis predicts that bis(2-ethylhexyl)ester hexanedioic acid, isopulegol, 2,5-cyclohexadiene-1,4-diol, 4-methyl-cyclohexanone, 2-methyl-cyclohexanone, cycloheptanone, 2-methyl-n-(2-methylbutylidene)-1-butanamine, quercetin, kaempferol, apigenin, protocatechuic acid, caffeic acid, vanillic acid, isorhamnetin, cis-methylferulate, vanillin, catechin(Fig. 4) show high GIA.On the other hand, alpha pinene, alpha-thujene, 3-carene, d-limonene, 3-carene, sylvestrene, isopulegol, 4-methyl-cyclohexanone, 2-methyl-cyclohexanone, cycloheptanone, 2-methyl-n-(2-methylbutylidene)-1-butanamine, and vanillin can cross the blood brain-barrier (Fig. 4). Drug molecules may cross the BBB via lipid-mediated free diffusion providing it has a molecular weight < 500 Da and forms < 8 hydrogen bonds (Veber et al., 2002). BBB penetration is only

mandatory for substances that target the central nervous system (CNS) (Borra & Kuna, 2013). However, since the study focuses on antidiabetic drugs, the compounds of interest are those with a high GIA.

Based on the toxicological predictions including mutagenicity, carcinogenicity/tumorigenicity, reproductive effectiveness, and irritant shown in **Table-3b** and **Table-4c**, sylvestrene, isopulegol, 2,5-cyclohexadiene-1,4-diol, 2-methyl-n-(2-methylbutylidene)-1-butanamine, quercetin, kaempferol, rutin, apigenin, afzelin, chlorogenic acid, hyperin, quinic acid, quercitrin, avicularin, vanillic acid, chlorogenic acid methyl ester, methyl-cis-ferulate, isoquercitrin, neochlorogenic acid, and catechin were found to be safe as they did not exhibit any toxicity effects. Toxicity has been associated with lipophilicity. An increase in lipophilicity raises the risk of compounds binding to targets of hydrophobic proteins rather than those required, thus causing toxic effects in biological systems.

Methyl-cis-ferulate, vanillic acid, catechin, and 2,5-cyclohexadiene-1,4-diol had good ADME-Tox properties and since the study is focused on finding potential antidiabetic drug compounds from HC that target DPP-IV and SGLT2, these compounds were chosen for the docking study. Methyl-cis-ferulate is an active inhibitor of stem rust uredospores germination (Hess et al., 1975; Macko et al., 1972). Vanillic acid inhibits the related molecular pathways to exhibit a variety of bioactivity against illnesses such as cancer, diabetes, obesity, neurodegenerative, cardiovascular, and hepatic diseases. Along with the ability to heal bacterial and fungal infections, its derivatives have the therapeutic potential to treat autoimmune illnesses (Kaur et al., 2022). Catechins have anti-oxidant properties via scavenging and blocking free radicals, antibacterial and antiviral activity, as well as the ability to delay or lessen skin damage (Isemura, 2019; Musial et al., 2020; Reygaert, 2018). They can shield the body from UV rays and neurological diseases (Bae et al., 2020; Isemura, 2019). Due to these advantages, these molecules have a great deal of promise for use as nutraceuticals and offer room for therapeutic purposes outside of their usual application. The optimal interaction between the protein and the ligands in the current investigation is represented by the binding energy with the lowest value. These compounds had good binding affinities for DPP-IV and SGLT2. However, catechin had a higher binding affinity for DPP-IV and SGLT2, (**Table-6**) and thus may represent a strong therapeutic chemical entity for the treatment of T2DM.

Declarations

Statements and Declaration

Conflict of interests

The authors declare no conflicts of interest.

Supporting Information

Mass spectrum and Lipinski's violation of compounds are available as Supporting Information.

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Tables

Tables 1-6 are available in the Supplementary Files section.

Figures

Fig.1:

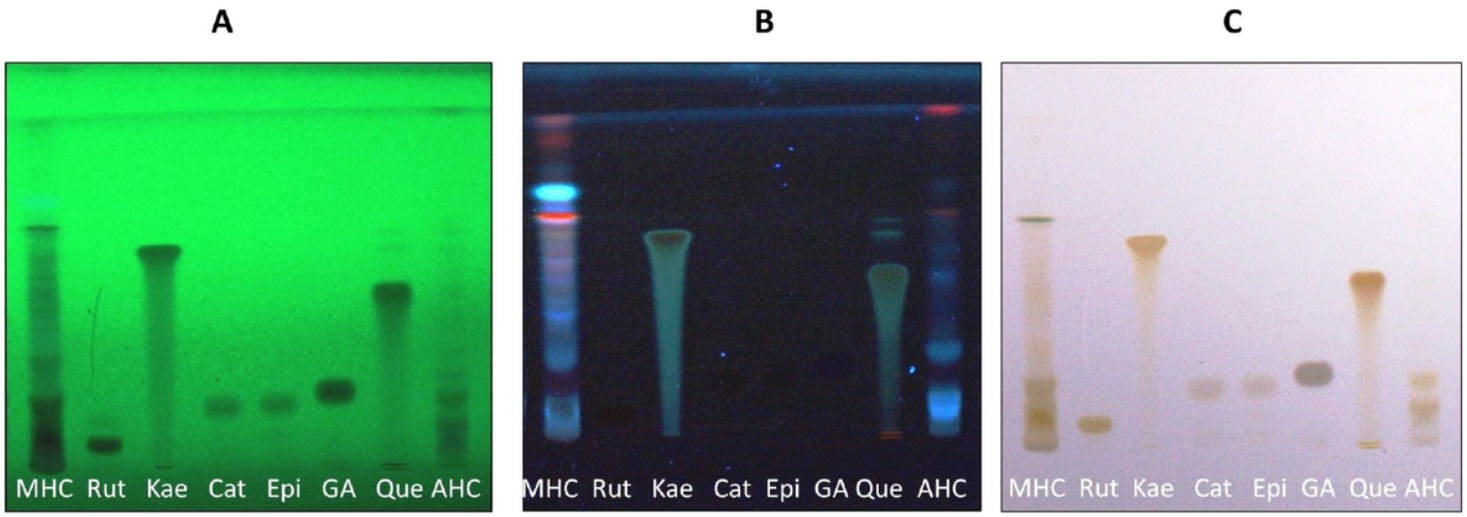


Figure 1

HPTLC Chromatogram of *H.cordata* at (A) λ254nm, (B) λ366 nm, and (C) White Light. Rut: rutin; Kae: kaempferol; Cat: catechin; Epi: epicatechin; GA: gallic acid; Que: quercetin; AHC: aqueous extract of *H.cordata*; MHC: methanolic extract of *H.cordata*.

Fig.2:

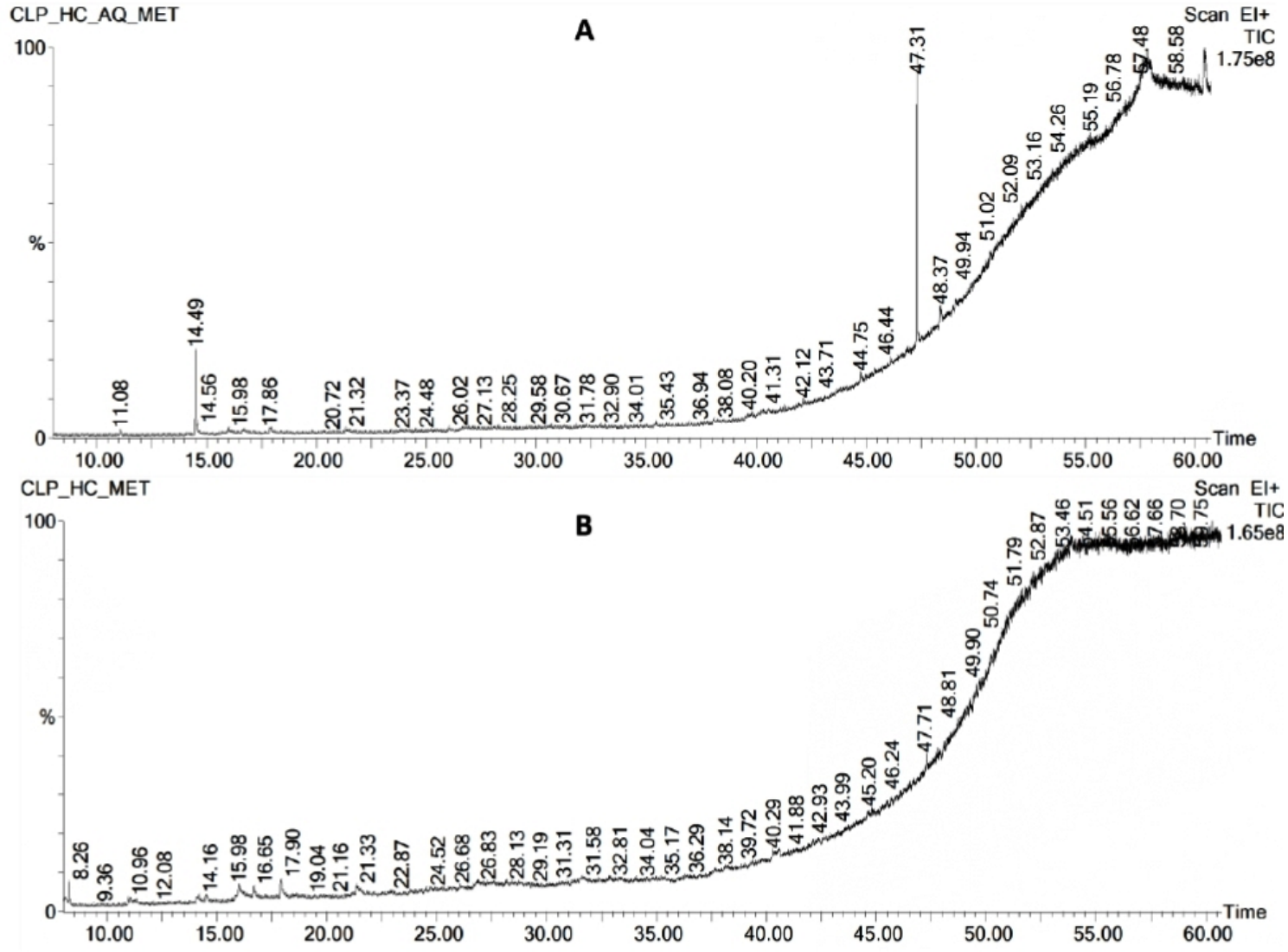
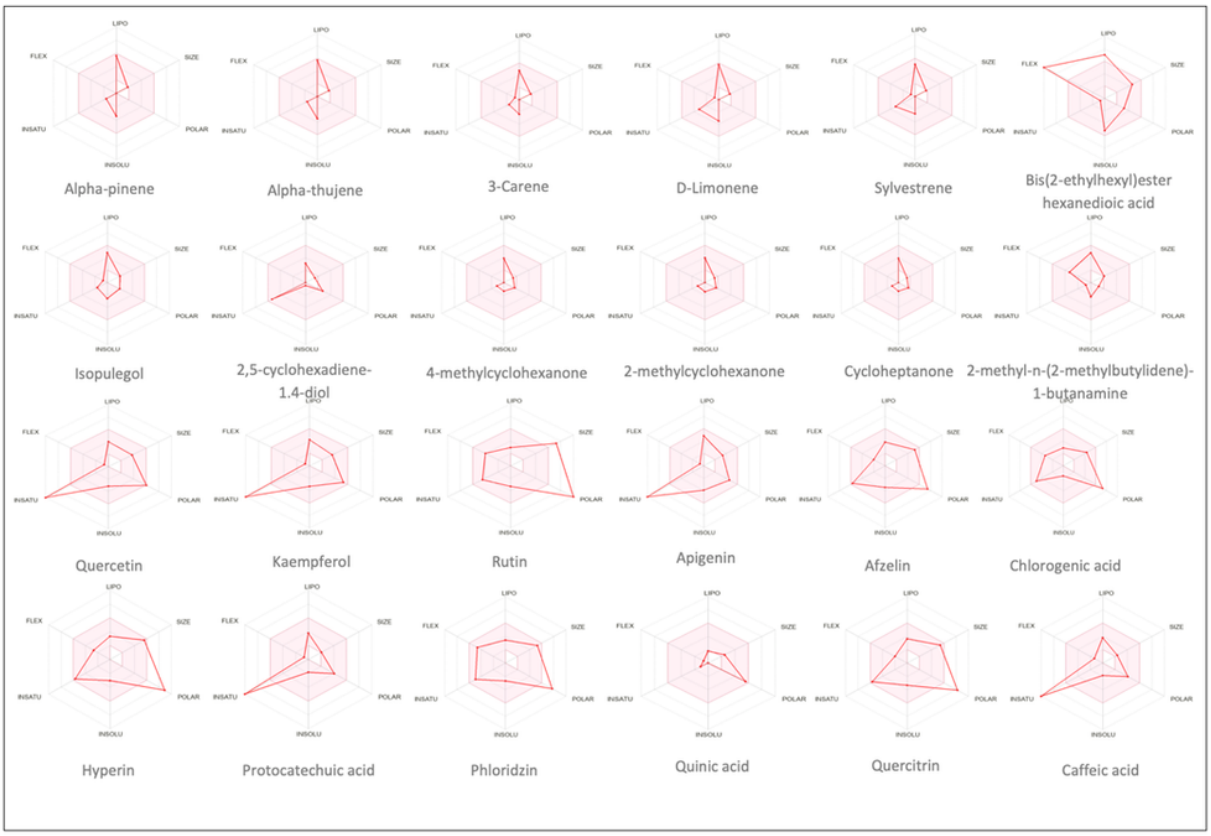


Figure 2

GCMS/MS Chromatogram of (A) Aqueous extract of *H.cordata,* and (B) Methanolic extract of *H.cordata*.



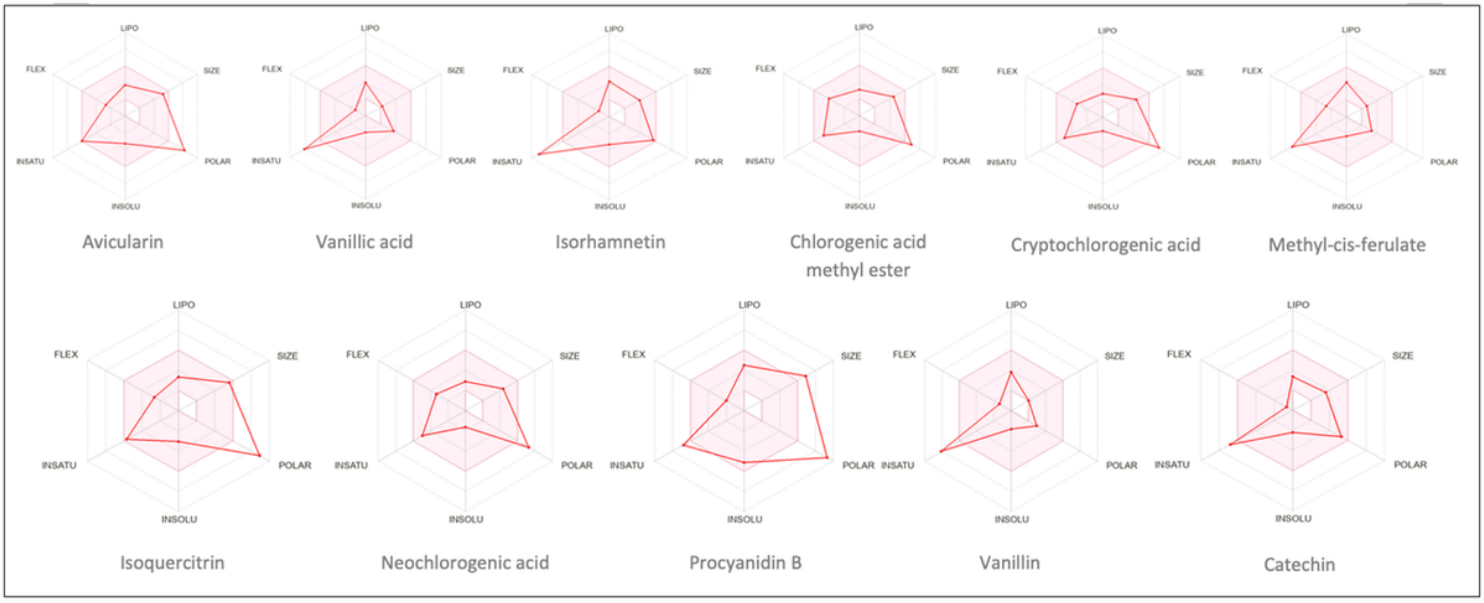


Figure 3

Bioavailability radar of compounds identified from *H.cordata*. The pink region represents the optimal range for lipophilicity, molecular size, polarity, solubility, saturation, and flexibility. Lipophilicity (XLogP3):-0.7-+5.0; molecular size: 150-5000g/mol; Polarity (TPSA): 20-130Å^2; solubility (LogS): ≤6; saturation: fractions of carbon in sp3 hybridisation ≥0.25; flexibility: ≤10 rotatable bonds.

Fig.4:

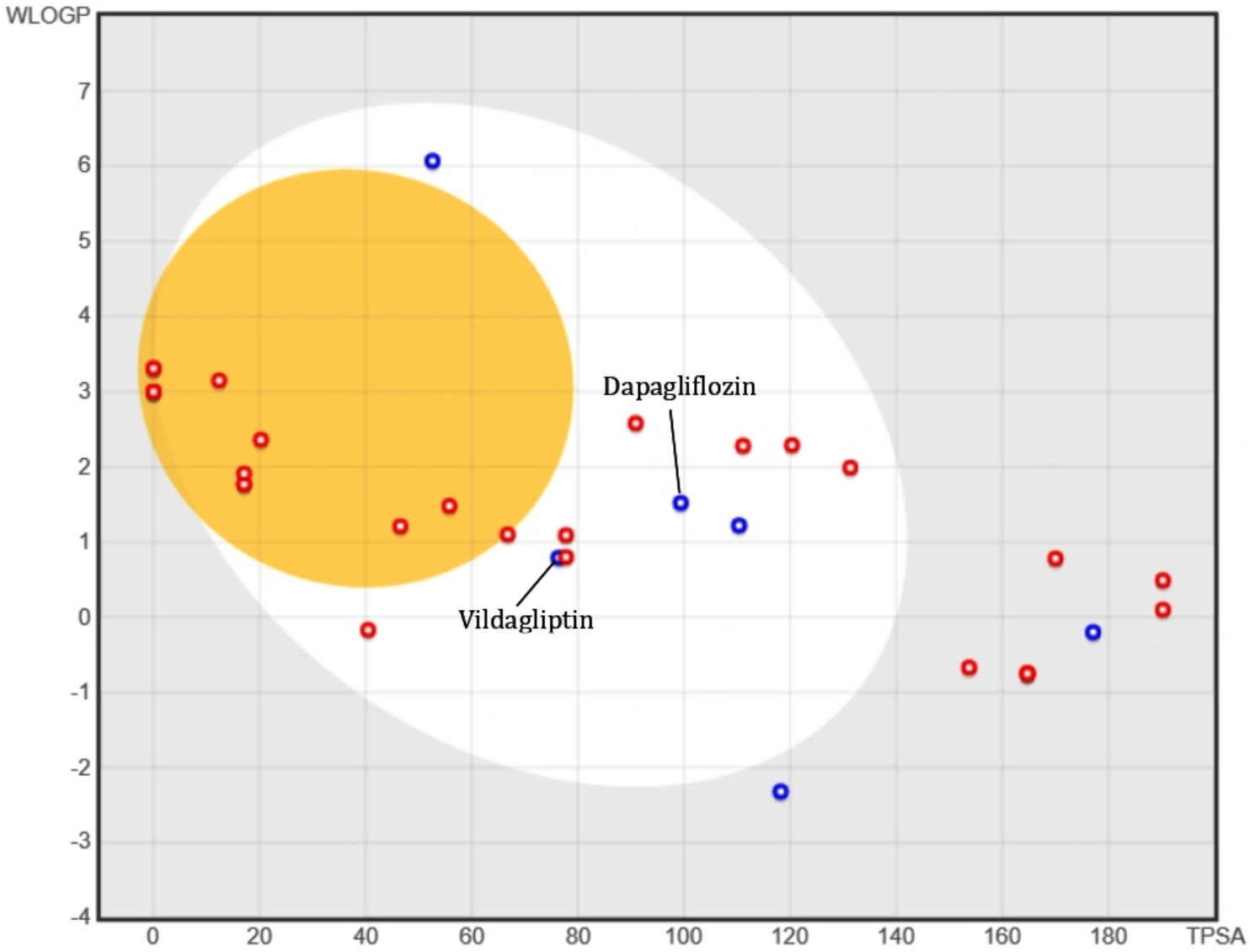


Figure 4

BOILED-EGG model representing the passive gastrointestinal absorption (GIA) and brain penetration of compounds identified from *H.cordata*. The white region represents probability for high GIA and the yellow region represents probability for penetration of the blood-brain barrier. Molecules that are not absorbed and not BBB permeant are depicted as out of range.The blue dot represent substrates of Pgp (Pgp+) while red dots represent non-substrates of Pgp (Pgp-).

Fig.5:

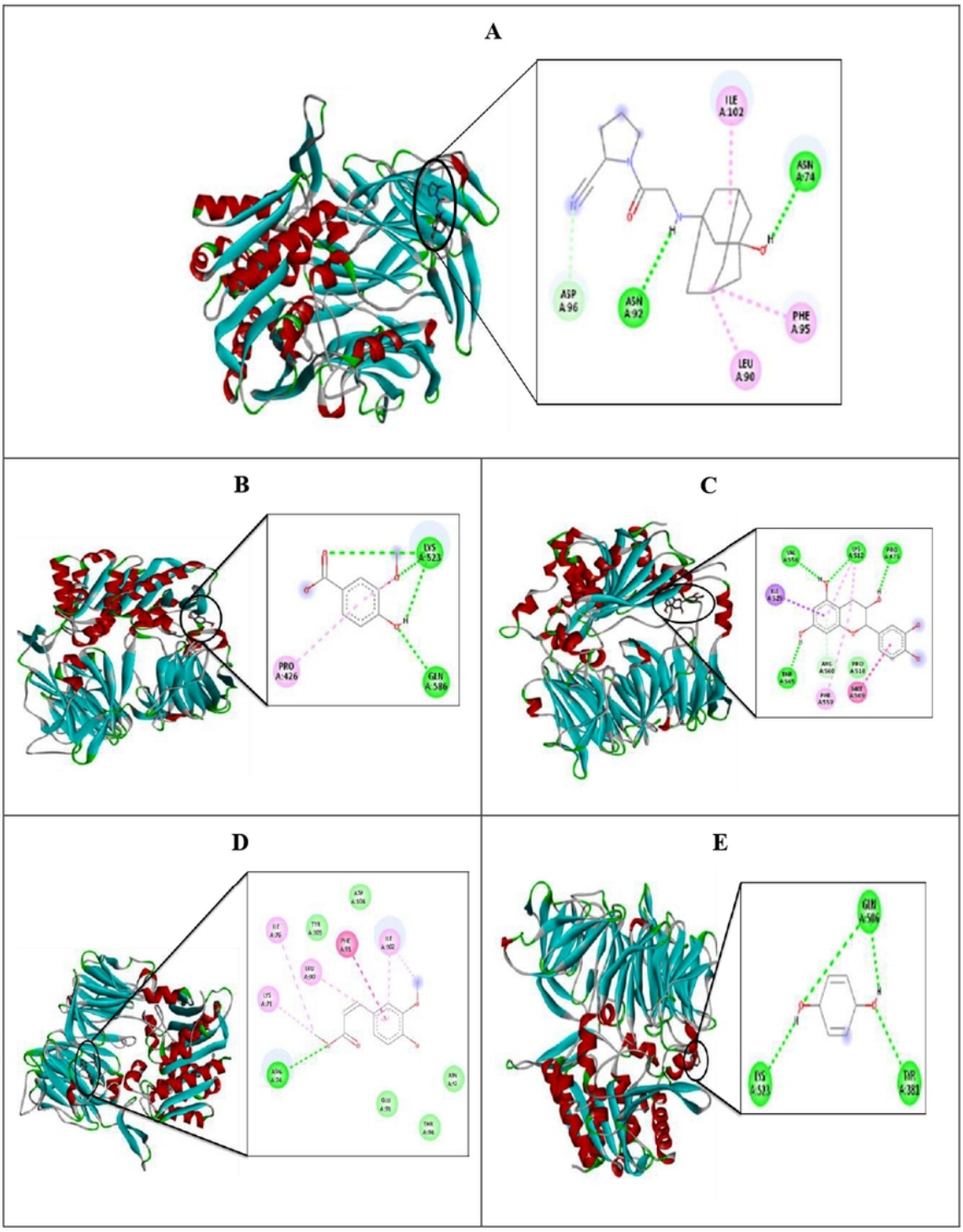


Figure 5

Interaction of (A) vildagliptin (6918537), (B) vanillic acid (8468), (C) catechin (9064), (D) methyl-cis-ferulate (10176654), and (E)2,5-cyclohexadiene (548773) with DPP-IV (1NU6). Green dotted lines represent hydrogen bonds.

Fig.6:

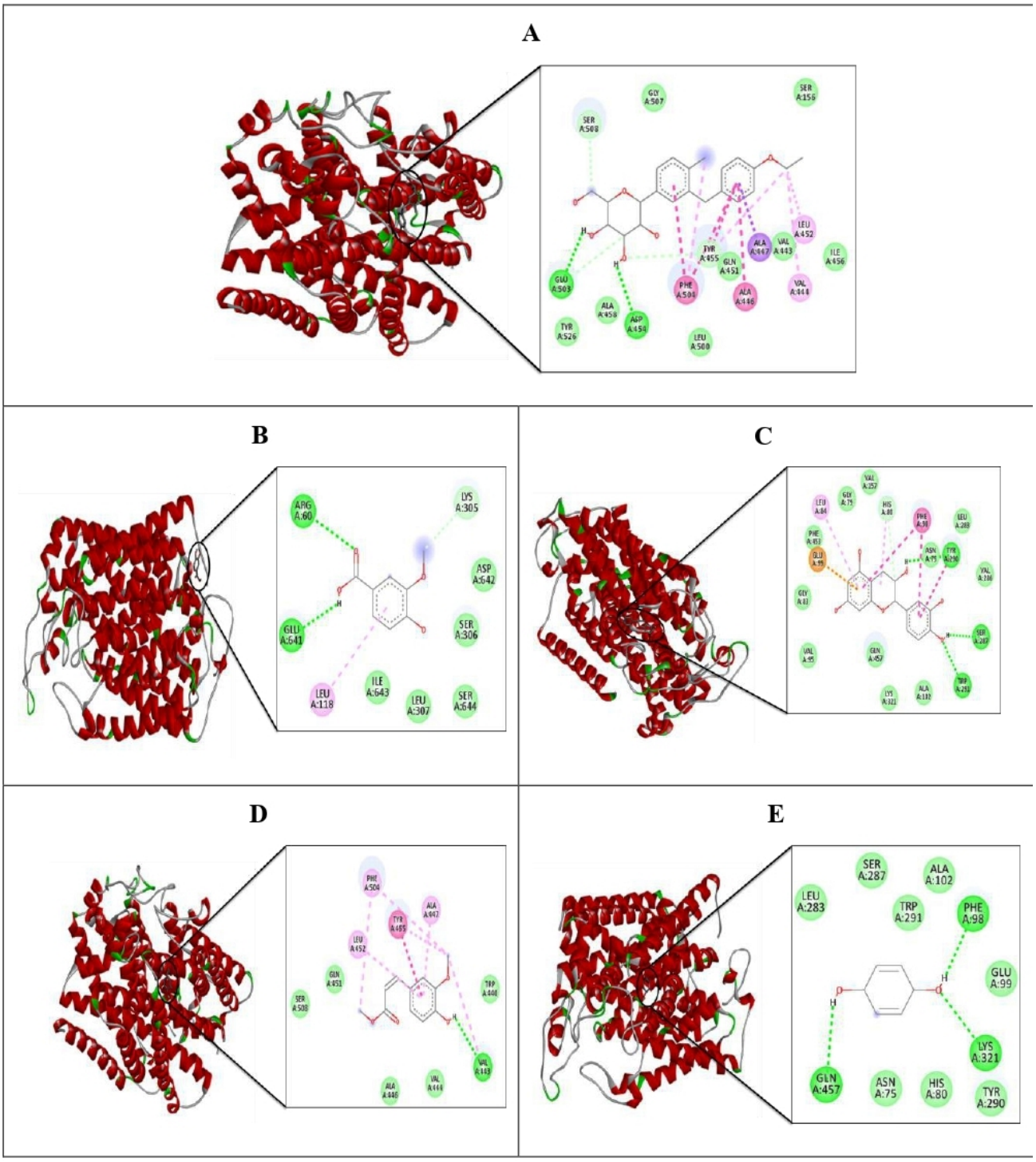


Figure 6

Interaction of (A) dapagliflozin (9887712), (B) vanillic acid (8468), (C) catechin (9064), (D) methyl-cis-ferulate (10176654), and (E)2,5-cyclohexadiene (548773) with SGLT2 (7VSI). Green dotted lines represent hydrogen bonds.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.docx
- Table2.docx
- Table3a.docx
- Table3b.docx
- Table4a.docx
- Table4b.docx
- Table4c.docx
- Table5.docx
- Table6.docx
- SupportingInformation1.docx
- SupportingInformation2.docx
- SupportingInformation3.docx