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Research Article

In silico exploration of phytoconstituents of *Enicostemma littorale* as potential DPP-4 inhibitors for the treatment of type 2 diabetes mellitus

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Abstract

The goal of the current investigation was to use a computational technique to examine the DPP-4 inhibitory potential of phytoconstituents of *E. littorale*. The Lipinski rule of five, the Veber's rule, and the ADMET characteristics have all been used to filter all of the phytoconstituents. Only a few compounds were chosen from this first screening to conduct molecular docking experiments on the DPP-4 enzyme: Apigenin, Ferulic acid, Genkwanin, p-coumaric acid, Protocatechuic acid, Syringic acid, and Vanillic acid. The native ligand had a binding free energy of -9.1 kcal/mol and a beneficial way of binding into the enzyme's allosteric site, while it also created four hydrogen bonds with Tyr662, Arg125, Arg358 and Phe357. In comparison to the native ligand, apigenin, ferulic acid, p-coumaric acid, protocatechuic acid, syringic acid, and vanillic acid formed more hydrogen bonds and had binding free energies that were, respectively, -8.7, -6.4, -6.1, -5.7, and -6.1 kcal/mol, indicating stronger DPP-4 inhibition. The formation of hydrogen bonds with allosteric amino acid residues by apigenin, ferulic acid, p-coumaric acid, protocatechuic acid, syringic acid, and vanillic acid with these phytoconstituents indicates their effective enzyme inhibition. *E. littorale* extract, which is previously recognised to have therapeutic benefits in diabetic patients, is a common ingredient in anti-diabetic Ayurvedic preparations. We discovered and described the primary phytochemical with the antidiabetic potential.

Keywords: *Enicostemma littorale*; DPP-IV inhibitors, Apigenin; Swertiamarin; Verticilliside; Betulin

Introduction

Diabetic people with type 2 diabetes may benefit from contemporary therapies such dipeptidyl peptidase-4 (DPP-4) inhibitors, which have improved glucose control and long-term efficacy. DPP-IV inhibitors, which have also been demonstrated to be well tolerated and to lessen hypoglycemia and unfavourable cardiovascular consequences, help in the regeneration and differentiation of pancreatic beta-cells[1–3]. DPP-4 may be found in the human body in a variety of locations, including the biliary system, kidney, gastrointestinal tract, uterine, and liver. This enzyme functions as a signalling protein for the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) (GIP). The ability of these hormones to boost insulin production and lessen beta-cell degeneration is one of their most crucial functions. The half-life is just 1-2 minutes for GLP-1

and 7 minutes for GIP. This is because DPP-4 quickly degrades. Some of the structurally different DPP-IV inhibitors that are now available on the market are Sitagliptin, Alogliptin, Linagliptin, Anagliptin, and Teneligliptin[4–7].

There have been several phytoconstituents identified from the plant *E. littorale*. According to records, the plant's aerial parts produced 15.7 percent of the ash and 34% of the dry alcoholic extract[8–10]. It has been stated in the literature that this plant produces five alkaloids, two sterols, and volatile oils[11,12]. Another sapogenin, betulin, was also isolated from this plant[13]. For the first time in this species, the occurrence of catechins, saponins, steroids, sapogenin, triterpenoids, flavonoids, and xanthones and a new flavonous C-glucoside called verticilliside was isolated[14]. The compound of swertiamarin was isolated from *E. littoral* by the alcoholic extract[15–20]. There have also been six phenolic acids identified: vanillic acid, syringic acid, p-hydroxybenzoic acid, protocatechuic acid, p-coumaric acid and ferulic acid[21–23]. The methanol extract contained numerous amino acids such as L-glutamic acid, tryptophan, alanine, serine, aspartic acid, L-proline, L-tyrosine, threonine, phenylalanine, L-histidine mono-hydrochloride, methionine.[24,25]. Regular 2 grams intake of *E. littorale* fresh leaves is a widely recommended for diabetic patients (Upadhyay and Goyal 2004). E. littorale has therefore been chosen as the subject of the investigation into its antidiabetic potential. We made an effort to determine the probable natural lead compounds from *E. littorale* that inhibit DPP-4 by looking at how they bind to the enzyme's allosteric region.

Material and Methods

Calculation of Lipinski's Rule of Five

All phytoconstituents were examined for violations of the Lipinski's rule of five, the Veber's rule, and the pharmacokinetic (ADMET) features in order to further optimise the molecules. SwissADME is an online application that was used to determine the characteristics of each phytoconstituent(http://www.swissadme.ch/index.php).

Molecular Docking

We conducted molecular docking on Lenovo ThinkPad T440p using PyRx-Virtual Screening Tool[26]. The structures of all the phytoconstituents and native ligand (.sdf File format) were downloaded from the National Center for Biotechnology Information PubChem

(https://pubchem.ncbi.nlm.nih.gov/). The energy minimization (optimization) was performed by Universal Force Field (UFF)[27]. A crystal structure of human DPP-4 enzyme was obtained as input 2P8S from the Protein Data Bank (PDB) of RCSB (https://www.rcsb.org/structure/1V4S). The native ligand was used as a reference molecule for molecular docking. In PyRx 0.8, Autodock vina 1.1.2 was used to conduct molecular docking analyses of both the phytoconstituents and native ligands against the crystal structure of DPP-4 [26]. With the aid of Discovery Studio Visualizer 2019, the composition of the enzyme was refined, purified, and prepared for molecular docking [28]. The molecular docking was executed by using Vina Wizard Tool in PyRx 0.8. Molecules (PDBQT Files), both ligands and target (DPP-4 enzyme), were selected for MD. For the purpose of MD simulation, the three-dimensional grid box (size_x = 43.35 A^0; Size_y = 59.36 A^0; Size_z = 43.92 A^0) was built using Autodock tool 1.5.6 with exhaustiveness value of 8 [26]. The active amino acids in the protein were analyzed and illuminated using Visualizer in BIOVIA Discovery Studio (version-19.1.0.18287) [28]. The full MD process, the identification of cavity and active amino acid residues, were performed as defined by S. L. Khan *et al.* [29–34]. The enzyme cavity is depicted in fig. 1 with the co-crystallized ligand molecule.

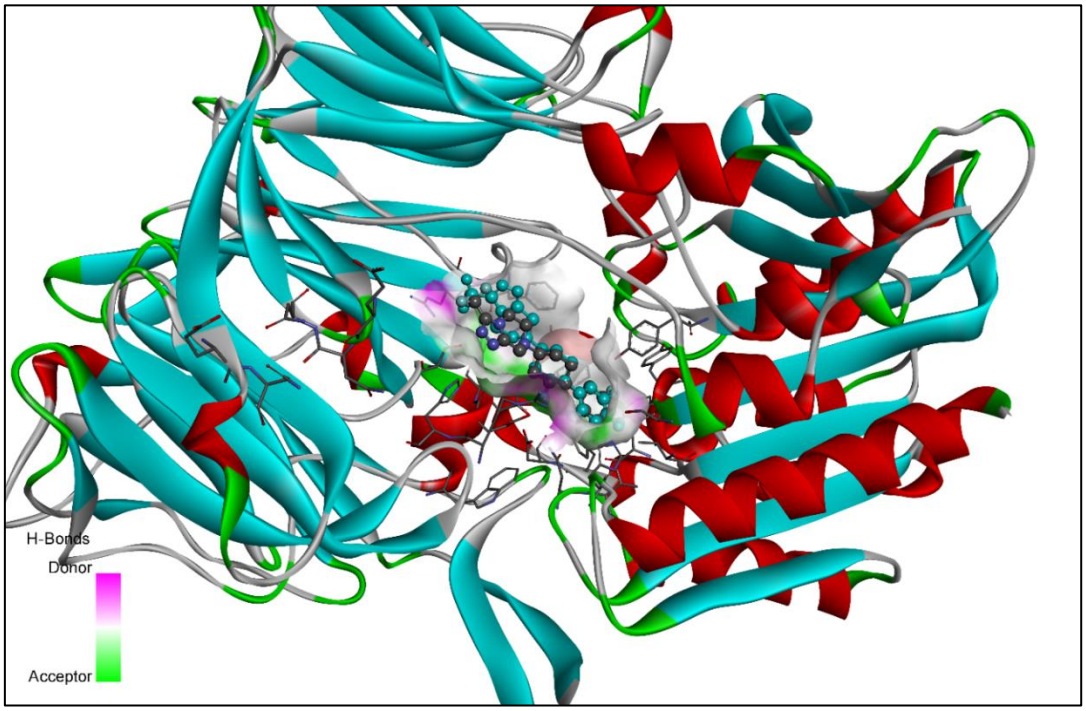


Fig. 1. The cavity of the enzyme is depicted with the co-crystallize ligand molecule (PDB ID: 2P8S)

Results

Because they allow researchers to evaluate the biological features of drug candidates, pharmacokinetic characteristics are a crucial part of drug development. Lipinski's rule of five and Veber's criteria were used to determine if the compound was ideal for oral bioavailability (Table 1). To better understand the pharmacokinetic profiles and drug-like properties of all the phytoconstituents, their ADMET features were investigated (Table 2). The ligand energies (kcal/mol), binding free energy (kcal/mol), root mean square deviation/upper bound (rmsd/ub), and root mean square deviation/lower bound (rmsd/lb) of the conformers generated of all the docked phytoconstituents are tabulated in Table 3. The active amino residues, reactive atom of ligands, bond length (A^0), and type of interactions of phytoconstituents with glucokinase enzyme are depicted in Table 3. The 2D- and 3D-docking poses of all the docked molecules are represented in Table 4.

Table 1. The molecular formula, Lipinski rule of five and Vebers's rule

Molecule Name	Molecular Formula	Lipinski rule of 5					Veber's rule	
		Mol. Wt.*	HBA*	HBD*	LogP	Violation	Total polar surface area ($Å^2$)	No. of rotatable bonds
Native Ligand	$C_{14}H_{12}FN_5OS_2$	3.29	419.37	10	01	00	59.97	03
Apigenin	$C1_5H_{10}O_5$	270.24	05	03	3.02	00	90.90	1
Betulin	$C_{30}H_{50}O_2$	442.72	02	02	8.28	01	40.46	2
Ferulic acid	$C_{10}H_{10}O_4$	194.18	04	02	1.51	00	66.76	3
Genkwanin	$C_{16}H_{12}O_5$	284.26	05	02	3.35	00	79.90	2
Isovitexin	$C_{21}H_{20}O_{10}$	432.38	10	07	0.21	01	181.05	3
p-coumaric acid	$C_9H_8O_3$	164.16	03	02	1.46	00	57.53	2
Protocatechuic acid	$C_7H_6O_4$	154.12	04	03	1.15	00	77.76	1
Saponarin	$C_{27}H_{30}O_{15}$	594.52	15	10	-1.60	03	260.20	6
Swertiamarin	$C_{16}H_{22}O_{10}$	374.34	10	05	-2.00	00	155.14	4
Syringic acid	$C_9H_{10}O_5$	198.17	05	02	1.04	00	75.99	3
Vanillic acid	$C_8H_8O_4$	168.15	04	02	1.43	00	66.76	2
Verticilliside	$C_{23}H_{24}O_{13}$	508.43	13	08	0.00	00	219.74	5

*Mol. Wt.=Molecular Weight; HBA=Hydrogen bond acceptor; HBD=Hydrogen bond donor.

Table 2 The pharmacokinetic and drug-likeness properties of selected phytoconstituents

Parameters		Compound names												
		Native Ligand	Apigenin	Betulin	Ferulic acid	Genkwanin	Isovitexin	p-coumaric acid	Protocatechuic acid	Saponarin	Swertiamarin	Syringic acid	Vanillic acid	Verticilliside
Pharmacokinetics	GI absorption	High	High	Low	High	High	Low	High	High	Low	Low	High	High	Low
	BBB permeation	High	No	No	Yes	No	No	Yes	No	No	No	No	No	No
	P-gp substrate	High	No	No	No	No	No	No	No	Yes	No	No	No	No
	CYP1A2 inhibitor	High	Yes	No	No	Yes	No	No	No	No	No	No	No	No
	CYP2C19 inhibitor	High	No	No	No	No	No	No	No	No	No	No	No	No
	CYP2C9 inhibitor	High	No	No	No	Yes	No	No	No	No	No	No	No	No
	CYP2D6 inhibitor	High	Yes	No	No	Yes	No	No	No	No	No	No	No	No
	CYP3A4 inhibitor	High	Yes	No	No	Yes	No	No	Yes	No	No	No	No	No
	Log K_p (skin permeation, cm/s)	High	-5.80	-3.12	-6.41	-5.66	-8.79	-6.26	-6.42	-11.06	-10.00	-6.77	-6.31	-9.40
Drug-likeness	Ghose	High	Yes	No	Yes	Yes	Yes	Yes	No	No	No	Yes	Yes	No
	Egan	High	Yes	No	Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	No
	Muegge	High	Yes	No	No	Yes	No	No	No	No	No	No	Yes	No
	Bioavailability Score	High	0.55	0.55	0.85	0.55	0.55	0.85	0.56	0.17	0.11	0.56	0.85	0.17

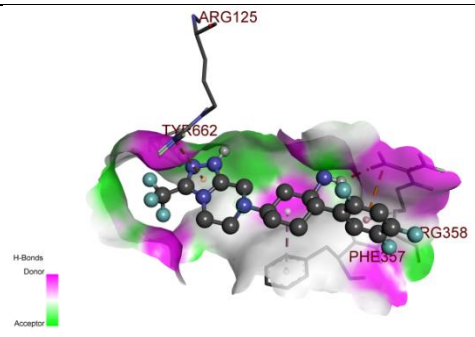
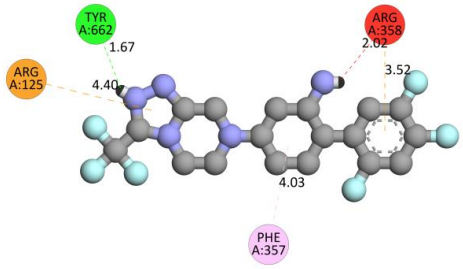
Where: GI, gastrointestinal; BBB, blood brain barrier; P-gp, p-glycoprotein

Table 3. The ligand energies (kcal/mol), binding free energy (kcal/mol), rmsd/ub, and rmsd/lb of the conformers generated of all the docked phytoconstituents

Active amino acids	Bond length	Bond Type	Bond Category	Docking Score	Binding Energy
Apigenin_5280443					
LYS512	2.28299	Hydrogen Bond	Conventional Hydrogen Bond	189.84	-8.7
GLN527	2.61104				
THR565	1.93326				
THR565	2.29423				
ILE529	3.96569	Hydrophobic	Pi-Sigma		
ILE529	3.23148				
PHE559	5.49623		Pi-Pi T-shaped		
PHE559	5.1623				
PRO510	5.44892		Pi-Alkyl		
LYS512	4.70281				
LYS512	4.86276				
ARG560	4.17593				
Ferulic acid_445858					
PRO475	2.32144	Hydrogen Bond	Conventional Hydrogen Bond	470.59	-6.4
LYS512	2.47829				
ILE529	3.46661	Hydrophobic	Pi-Sigma		
LYS512	5.24245		Alkyl		
ILE529	5.05345				
ARG560	3.70582				
PRO510	5.47846		Pi-Alkyl		
LYS512	5.01682				
ARG560	5.43288				
Genkwanin 5281617					
ARG560	3.69997	Hydrogen Bond	Carbon Hydrogen Bond	211.23	-8.3
ILE529	3.3219	Hydrophobic	Pi-Sigma		
ARG560	3.66069				
PHE559	4.8671		Pi-Pi T-shaped		
PHE559	5.10339				
PRO475	4.72341		Alkyl		
LYS512	4.76662		Pi-Alkyl		
LYS512	5.04651				
LYS512	5.16934				
p-coumaric acid_637542					
LYS512	2.16734	Hydrogen Bond	Conventional Hydrogen Bond	86.34	-6.4
GLN527	2.09956				
ARG560	1.8805				
PHE559	5.27592	Hydrophobic	Pi-Pi T-shaped		

LYS512	3.99596		Pi-Alkyl		
ILE529	5.42866				
Protocatechuic acid 72					
GLN153	2.66033	Hydrogen Bond	Conventional Hydrogen Bond	69.01	-6.1
ASN170	1.99527				
TRP201	2.74729				
TYR195	4.94502	Hydrophobic	Pi-Pi T-shaped		
Syringic acid_10742					
ASP588	2.8558	Hydrogen Bond	Conventional Hydrogen Bond	837.82	-5.7
THR350	1.89087				
THR350	2.42989				
THR351	2.15137				
THR351	1.95459				
VAL354	3.93585	Hydrophobic	Alkyl		
Vanillic acid_8468					
TYR211	2.30204	Hydrogen Bond	Conventional Hydrogen Bond	89.94	-6.1
GLN153	2.16023				
GLN153	2.70681				
ASN170	2.02857				
PRO255	3.66914		Carbon Hydrogen Bond		
TYR195	4.96787	Hydrophobic	Pi-Pi T-shaped		
VAL254	3.68724		Alkyl		
PRO255	5.16976				
TRP124	5.359		Pi-Alkyl		
TRP124	5.18784				

Table 4. The binding poses and 2D interactions of native ligand, and most potent derivatives

3D-docking poses	2D-docking poses
	
Native ligand	

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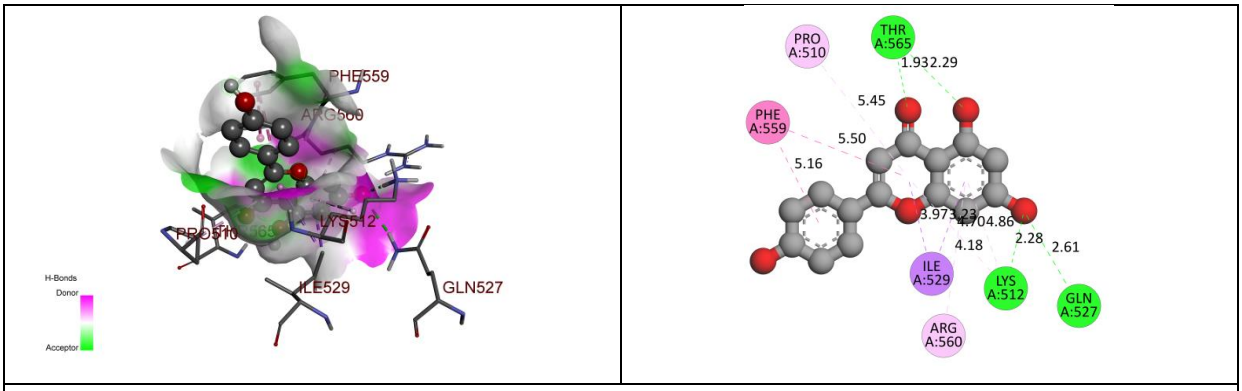
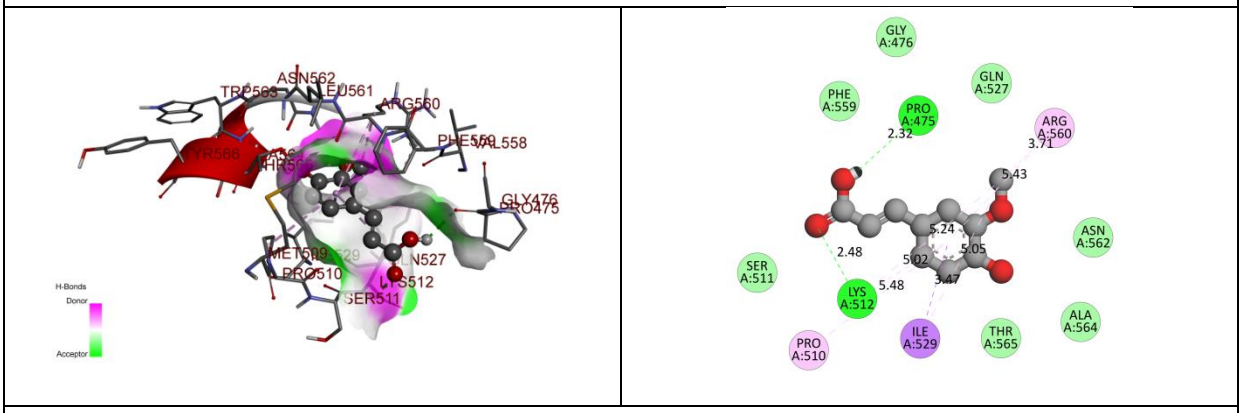
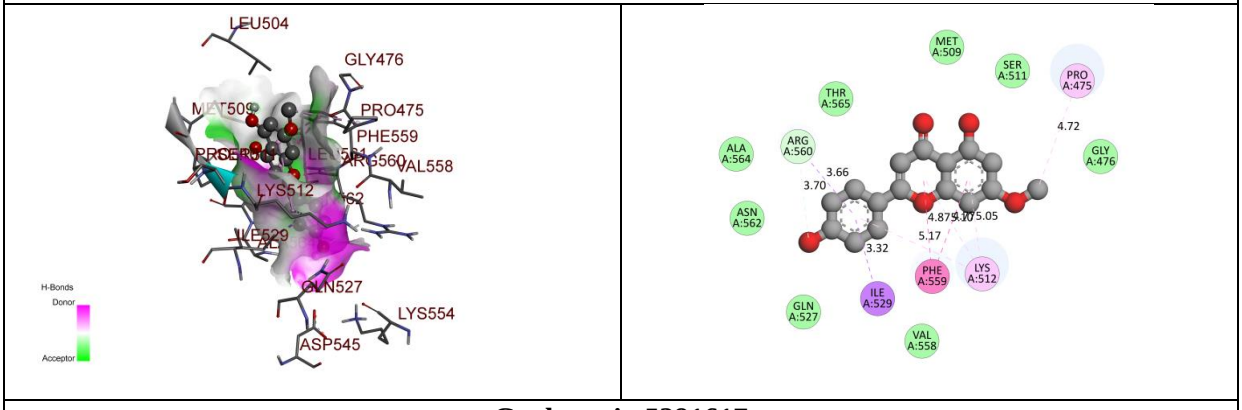


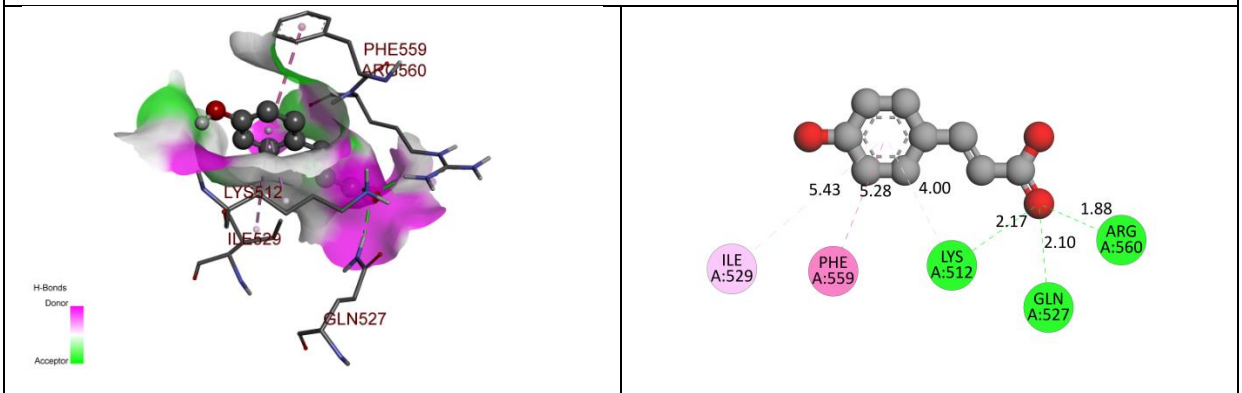
Apigenin_5280443



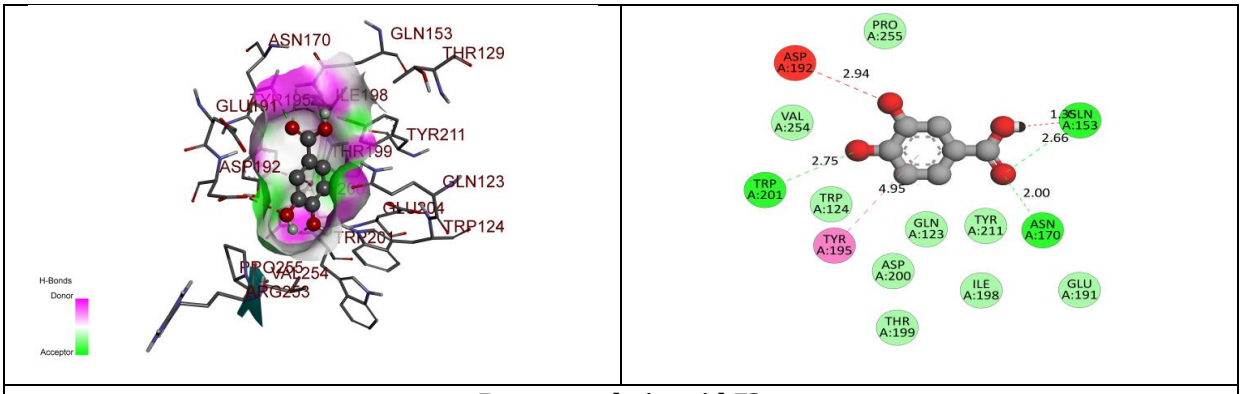
Ferulic acid_445858



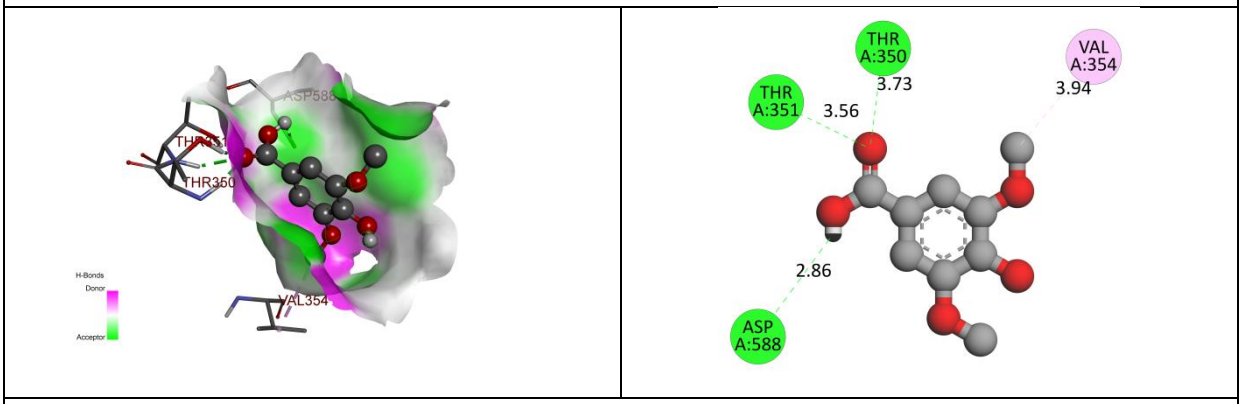
Genkwanin 5281617



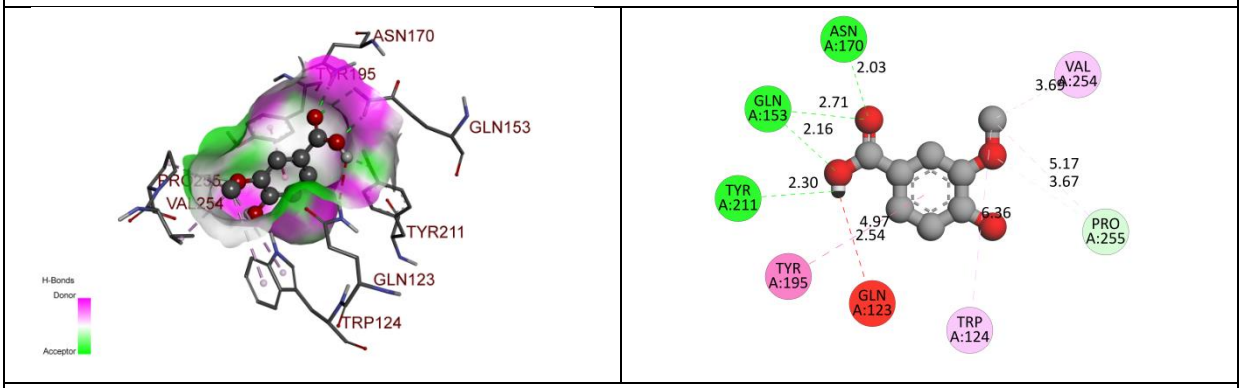
p-coumaric acid_637542



Protocatechuic acid 72



Syringic acid_10742



Vanillic acid_8468

Discussion

By looking at binding mode at the enzyme's allosteric site and binding free energies, we attempted to discover the possible natural lead compounds from *E. littorale* as DPP-4 inhibitors. Numerous phytoconstituents breached both Lipinski's and Veber's standards (Table 1) by failing to exhibit the traits that make a compound similar to a medicine. Amongst all the molecules, betulin has a log P value of 8.28, which has violated the Lipinski rule of 5 and indicates poor lipophilicity. An essential aspect of the compound that influences its function in the human body is lipophilicity. The compound's Log P value shows the

permeability of the drugs in the body to enter the target tissue[35,36]. Isovitexin was found to have 7 hydrogen bond donors, which has violated the Lipinski rule of 5. Saponarin has a molecular weight of 594.52 Da, 15 hydrogen bond acceptors, and 10 hydrogen bond donors with 3 violations of the Lipinski rule of 5. It is preferable to look for substances that exceed the Lipinski limit of 500 Da, since this will only boost absorption. Still, there are several reports of relatively more significant compounds that are transported effectively through the cells. The remaining phytoconstituents, including native ligand, had fortunately not violated the Lipinski rule of 5, indicating better absorption and/or lipophilicity of the molecules. Many phytoconstituents violated the Veber's rule with total polar surface area (TPSA, should be less than 140) values and the number of rotatable bonds (which should be less than 10) which is not falling within the acceptable range for oral availability. Isovitexin, Saponarin, Swertiamarin, and Verticilliside violated the Veber's rule.

All compounds have been put through pharmacokinetics and drug-likeness calculations for further optimization. All of the compounds lacked BBB penetration potential, which is a bad quality for medications that are meant to target the central nervous system. Unfortunately, many molecules did exhibit optimum log *Kp* (skin permeation, cm/s) and bioavailability scores. Many molecules violated the Ghose, Egan, and Muegge filters (Table 2). The molecules which displayed low GI absorption and violation of Lipinski and Veber's rule have been eliminated from the further optimization. Also native ligand displayed low GI absorption. Therefore only Apigenin, Ferulic acid, Genkwanin, p-coumaric acid, Protocatechuic acid, Syringic acid, and Vanillic acid have been selected to perform molecular docking studies on GK enzyme.

A total 9 conformers were generated through molecular docking for each molecule (Table 3). The conformer with zero rmsd/ub and rsmd/lb values has been treated as the best fit model for the DPP-4 enzyme activation. The binding free energy and binding mode of the native ligand in the allosteric site of the enzyme have been considered a reference for validating the other molecules. **Native ligand** only made one conventional hydrogen bond with TYR662 through the N-H of the amide group and had a binding free energy of -9.1 kcal/mol. It has developed electrostatic interactions with ARG125, ARG358 and hydrophobic interactions with ARG358 and PHE357.

Apigenin (4′,5-trihydroxyflavone), a flavonoid, falls under the flavone class that is the aglycone of many naturally-occurring glycosides[37–40]. Apigenin exhibited -8.7 kcal/mol of binding energy and formed four conventional hydrogen bonds with Lys512, Gln527 and Thr565. It displayed Hydrophobic (Pi-sigma, Pi-pi T shaped, Pi-alkyl) Interactions with Ile29, Phe559, Pro510, Lys512 and Arg560. **Ferulic acid** is an organic compound; chemically, it is 3-methoxy-4-hydroxycinnamic acid. In plant cell walls a rich phenolic phytochemical is present covalently attached to arabinoxyls as side chains[41,42]. Ferulic acid displayed docking score of -6.4 kcal/mol and formed two conventional hydrogen bonds with Pro475 and Lys512.It also formed hydrophobic (Pi-sigma, alkyl and Pi-alkyl) Interactions with Ile529, Lys512, Arg560 and Pro510. **Genkwanin** is a monomethoxyflavone, which is a derivative of apigenin. It has been biosynthesized by apigenin in plants by methylation of the hydroxyl group at 7th position [43,44]. Genkwanin has shown -7.5 kcal/mol of binding free energy with glucokinase enzyme and possesses stable ligand energy of 206.69 kcal/mol.

p-Coumaric acid is a hydroxyl derivative of cinnamic acid and widely distributed in many plant species[45]. p-coumaric acid exhibited three conventional hydrogen bonds with Lys512, Gln527 and Arg560 with the docking score of -6.4 kcal/mol. It also formed Hydophobic (Pi-Pi T-shaped and Pi-Alkyl) Interactions with Phe559, Lys512 and Ile529. **Protocatechuic acid** is a type of phenolic acid that is naturally present and over 500 plants have it or its derivatives (active constituents), and these substances have different therapeutic potential. It has structural similarities with gallic acid, caffeic acid, vanillic acid, and syringic acid, which are well-known antioxidants found in foods and other items [46]. Protocatechuic acid exhibited three conventional hydrogen bonds with Gln153, Asn170 and Trp201 with the docking score of -6.1 kcal/mol. It also formed Hydophobic (Pi-Pi T-shaped) Interactions with Tyr195. **Syringic acid** is a phenolic substance that is mostly present in fruits and vegetables. This compound is made by the shikimic acid process and is found in plants. It shows a wide variety of clinical applications in preventing diabetes, coronary disorders, cancer, ischemic stroke, etc. It can shield brain tissue from free radical injury, delay the development of diabetes, and is hepatoprotective medicine [47]. Syringic acid showed -5.7 kcal/mol docking score and formed five conventional hydrogen bonds with Asp588, Thr350 and Thr351. It also showed hydrophobic (alkyl) Interactions with Val354. **Vanillic acid** formed four conventional hydrogen bonds with Tyr211, Gln153, Asn170 and one carbon hydrogen bond

with Pro255. It also showed hydrophobic Interactions (Pi-Pi T-shaped, Alkyl and Pi-Alkyl) with Tyr195, Val254, Pro255 and Trp124.

Conclusion

The goal of the current investigation was to use a computational technique to examine the DPP-4 inhibitory potential of phytoconstituents of *E. littorale*. The Lipinski rule of five, the Veber's rule, and the ADMET characteristics have all been used to filter all of the phytoconstituents. Only a few compounds were chosen from this first screening to conduct molecular docking experiments on the DPP-4 enzyme: Apigenin, Ferulic acid, Genkwanin, p-coumaric acid, Protocatechuic acid, Syringic acid, and Vanillic acid. The natural ligand had a binding free energy of -9.1 kcal/mol and a beneficial way of binding into the enzyme's allosteric site, while it also created four hydrogen bonds with Tyr662, Arg125, Arg358 and Phe357. These results provide support for the interactions of phytoconstituents. In comparison to the native ligand, apigenin, ferulic acid, p-coumaric acid, protocatechuic acid, syringic acid, and vanillic acid formed more hydrogen bonds and had binding free energies that were, respectively, -8.7, -6.4, -6.1, -5.7, and -6.1 kcal/mol, indicating stronger DPP-4 inhibition. The formation of hydrogen bonds with allosteric amino acid residues by apigenin, ferulic acid, p-coumaric acid, protocatechuic acid, syringic acid, and vanillic acid with these phytoconstituents indicates their effective enzyme inhibition. *E. littorale* extract, which is previously recognised to have therapeutic benefits in diabetic patients, is a common ingredient in anti-diabetic Ayurvedic preparations. We discovered and described the primary phytochemical with the antidiabetic potential.

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Conflict of interest

Declared none.

Authors' contributions

All the authors have contributed equally.

Ethics approval

Not applicable.

Supplementary material

Not applicable.

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References:

(1) Okechukwu, P.; Sharma, M.; Tan, W. H.; Chan, H. K.; Chirara, K.; Gaurav, A.; Al-Nema, M. In-Vitro Anti-Diabetic Activity and in-Silico Studies of Binding Energies of Palmatine with Alpha-Amylase, Alpha-Glucosidase and DPP-IV Enzymes. *Pharmacia* **2020**, *67* (4), 363–371. https://doi.org/10.3897/pharmacia.67.e58392.

(2) Dowarah, J.; Singh, V. P. Anti-Diabetic Drugs Recent Approaches and Advancements. *Bioorganic Med. Chem.* **2020**, *28* (5). https://doi.org/10.1016/j.bmc.2019.115263.

(3) Huang, J.; Deng, X.; Zhou, S.; Wang, N.; Qin, Y.; Meng, L.; Li, G.; Xiong, Y.; Fan, Y.; Guo, L.; Lan, D.; Xing, J.; Jiang, W.; Li, Q. Identification of Novel Uracil Derivatives Incorporating Benzoic Acid Moieties as Highly Potent Dipeptidyl Peptidase-IV Inhibitors. *Bioorganic Med. Chem.* **2019**, *27* (4), 644–654. https://doi.org/10.1016/j.bmc.2019.01.001.

(4) Stoimenis, D.; Karagiannis, T.; Katsoula, A.; Athanasiadou, E.; Kazakos, K.; Bekiari, E.; Matthews, D. R.; Tsapas, A. Once-Weekly Dipeptidyl Peptidase-4 Inhibitors for Type 2 Diabetes: A Systematic Review and Meta-Analysis. *Expert Opin. Pharmacother.* **2017**, *18* (9), 843–851. https://doi.org/10.1080/14656566.2017.1324848.

(5) Salvatore, T.; Carbonara, O.; Cozzolino, D.; Torella, R.; Carlo Sasso, F. Adapting the GLP-1-Signaling System to the Treatment of Type 2 Diabetes. *Curr. Diabetes Rev.* **2007**, *3* (1), 15–23. https://doi.org/10.2174/157339907779802076.

(6) Safavi, M.; Foroumadi, A.; Abdollahi, M. The Importance of Synthetic Drugs for Type 2 Diabetes Drug Discovery. *Expert Opin. Drug Discov.* **2013**, *8* (11), 1339–1363. https://doi.org/10.1517/17460441.2013.837883.

(7) Arulmozhi, D. K.; Portha, B. GLP-1 Based Therapy for Type 2 Diabetes. *Eur. J. Pharm. Sci.* **2006**, *28* (1–2), 96–108. https://doi.org/10.1016/j.ejps.2006.01.003.

(8) Patel, S. S.; Shah, R. S.; Goyal, R. K. Antihyperglycemic, Antihyperlipidemic and Antioxidant Effects of Dihar, a Polyherbal Ayurvedic Formulation in Streptozotocin

Induced Diabetic Rats. *Indian J. Exp. Biol.* **2009**, *47* (7), 564–570.

(9) Sadique, J.; Chandra, T.; Thenmozhi, V.; Elango, V. The Anti-Inflammatory Activity of Enicostemma Littorale and Mollugo Cerviana. *Biochem. Med. Metab. Biol.* **1987**, *37* (2), 167–176. https://doi.org/10.1016/0885-4505(87)90023-5.

(10) Sanmugarajah, V. Phyto, Physicochemical Standardization of Medicinal Plant Enicostemma Littorale, Blume. *IOSR J. Pharm.* **2013**, *3* (2), 52–58. https://doi.org/10.9790/3013-32205258.

(11) Vishwakarma, S. L.; Sonawane, R. D.; Rajani, M.; Goyal, R. K. Evaluation of Effect of Aqueous Extract of Enicostemma Littorale Blume in Streptozotocin-Induced Type 1 Diabetic Rats. *Indian J. Exp. Biol.* **2010**, *48* (1), 26–30.

(12) Selvaraj, S.; Selvaraj, S.; Chittibabu, C. V.; Janarthanam, B. Studies on Phytochemical Screening, Antioxidant Activity and Extraction of Active Compound (Swertiamarin) from Leaf Extract of Enicostemma Littorale. *Asian J. Pharm. Clin. Res.* **2014**, *7* (4), 240–244.

(13) Indumathi, C.; Durgadevi, G.; Nithyavani, S.; Gayathri, P. K. Estimation of Terpenoid Content and Its Antimicrobial Property in Enicostemma Litorrale. *Int. J. ChemTech Res.* **2014**, *6* (9), 4264–4267.

(14) Jahan, E.; Perveen, S.; Malik, A. Verticilliside, a New Flavone C-Glucoside from Enicostemma Verticillatum. *J. Asian Nat. Prod. Res.* **2009**, *11* (3), 257–260. https://doi.org/10.1080/10286020802675019.

(15) Sonawane, R. D.; Vishwakarma, S. L.; Lakshmi, S.; Rajani, M.; Padh, H.; Goyal, R. K. Amelioration of STZ-Induced Type 1 Diabetic Nephropathy by Aqueous Extract of Enicostemma Littorale Blume and Swertiamarin in Rats. *Mol. Cell. Biochem.* **2010**, *340* (1–2), 1–6. https://doi.org/10.1007/s11010-010-0393-x.

(16) Alam, P.; Ali, M.; Singh, R.; Shakeel, F. A New HPTLC Densitometric Method for Analysis of Swertiamarin in Enicostemma Littorale and Commercial Formulations. *Nat. Prod. Res.* **2011**, *25* (1), 17–25. https://doi.org/10.1080/14786411003754348.

(17) Vaidya, H.; Rajani, M.; Sudarsanam, V.; Padh, H.; Goyal, R. Swertiamarin: A Lead from Enicostemma Littorale Blume. for Anti-Hyperlipidaemic Effect. *Eur. J. Pharmacol.* **2009**, *617* (1–3), 108–112. https://doi.org/10.1016/j.ejphar.2009.06.053.

(18) Leong, X. Y.; Thanikachalam, P. V.; Pandey, M.; Ramamurthy, S. A Systematic Review of the Protective Role of Swertiamarin in Cardiac and Metabolic Diseases. *Biomedicine and Pharmacotherapy*. 2016, pp 1051–1060.

https://doi.org/10.1016/j.biopha.2016.10.044.

(19) Vishwakarma, S. L.; Rajani, M.; Bagul, M. S.; Goyal, R. K. A Rapid Method for the Isolation of Swertiamarin from Enicostemma Littorale. *Pharm. Biol.* **2004**, *42* (6), 400–403. https://doi.org/10.1080/13880200490885095.

(20) Patel, T. P.; Soni, S.; Parikh, P.; Gosai, J.; Chruvattil, R.; Gupta, S. Swertiamarin: An Active Lead from Enicostemma Littorale Regulates Hepatic and Adipose Tissue Gene Expression by Targeting PPAR-γ and Improves Insulin Sensitivity in Experimental Niddm Rat Model. *Evidence-based Complement. Altern. Med.* **2013**, *2013*. https://doi.org/10.1155/2013/358673.

(21) Rathod, M. C.; Dhale, D. A. Pharmacognostic Characterization of Enicostemma Littorale Blume. *Int. J. Res. Ayurveda Pharm.* **2013**, *4* (6), 893–898. https://doi.org/10.7897/2277-4343.04624.

(22) Abirami P and Gomathinayagam M. A Review on Enicostemma Littorale. *Pharmacologyonline* **2011**, *1*, 75–83.

(23) Srinivasan, M.; Padmanabhan, M.; Prince, P. S. M. Effect of Aqueous Enicostemma Littorale Blume Extract on Key Carbohydrate Metabolic Enzymes, Lipid Peroxides and Antioxidants in Alloxan-Induced Diabetic Rats. *J. Pharm. Pharmacol.* **2005**, *57* (4), 497–503. https://doi.org/10.1211/0022357055722.

(24) Nagarathnamma, M.; Sudarshana, M. S.; Niranjan, M. H.; Pandurangamurthy. Rapid Regeneration of Enicostemma Littorale Blume from Leaf and Stem Cultures. *J. Plant Interact.* **2010**, *5* (1), 69–73. https://doi.org/10.1080/17429140903353549.

(25) Sawant, L. P.; Prabhakar, B. R.; Pandita, N. S. A Validated Quantitative HPTLC Method for Analysis of Biomarkers in Enicostemma Littorale Blume. *J. Planar Chromatogr. - Mod. TLC* **2011**, *24* (6), 497–502. https://doi.org/10.1556/JPC.24.2011.6.8.

(26) Dallakyan, S.; Olson, A. J. Small-Molecule Library Screening by Docking with PyRx. *Methods Mol. Biol.* **2015**, *1263* (1263), 243–250. https://doi.org/10.1007/978-1-4939-2269-7_19.

(27) Rappé, A. K.; Casewit, C. J.; Colwell, K. S.; Goddard, W. A.; Skiff, W. M. UFF, a Full Periodic Table Force Field for Molecular Mechanics and Molecular Dynamics Simulations. *J. Am. Chem. Soc.* **1992**, *114* (25), 10024–10035. https://doi.org/10.1021/ja00051a040.

(28) San Diego: Accelrys Software Inc. Discovery Studio Modeling Environment, Release

3.5. *Accelrys Softw. Inc.* **2012**.

(29) Khan, S. L.; Siddiqui, F. A.; Jain, S. P.; Sonwane, G. M. Discovery of Potential Inhibitors of SARS-CoV-2 (COVID-19) Main Protease (Mpro) from Nigella Sativa (Black Seed) by Molecular Docking Study. *Coronaviruses* **2020**, *2* (3), 384–402. https://doi.org/10.2174/2666796701999200921094103.

(30) Khan, S. L.; Siddiqui, F. A.; Shaikh, M. S.; Nema, N. V.; Shaikh, A. A. Discovery of Potential Inhibitors of the Receptor-Binding Domain (RBD) of Pandemic Disease-Causing SARS-CoV-2 Spike Glycoprotein from Triphala through Molecular Docking. *Curr. Chinese Chem.* **2021**, *01*. https://doi.org/10.2174/2666001601666210322121802.

(31) Khan, S. L.; Sonwane, G. M.; Siddiqui, F. A.; Jain, S. P.; Kale, M. A.; Borkar, V. S. Discovery of Naturally Occurring Flavonoids as Human Cytochrome P450 (CYP3A4) Inhibitors with the Aid of Computational Chemistry. *Indo Glob. J. Pharm. Sci.* **2020**, *10* (04), 58–69. https://doi.org/10.35652/igjps.2020.10409.

(32) Chaudhari, R. N.; Khan, S. L.; Chaudhary, R. S.; Jain, S. P.; Siddiqui, F. A. B-Sitosterol: Isolation from Muntingia Calabura Linn Bark Extract, Structural Elucidation And Molecular Docking Studies As Potential Inhibitor of SARS-CoV-2 Mpro (COVID-19). *Asian J. Pharm. Clin. Res.* **2020**, *13* (5), 204–209. https://doi.org/10.22159/ajpcr.2020.v13i5.37909.

(33) Siddiqui, F. A.; Khan, S. L.; Marathe, R. P.; Nema, N. V. Design, Synthesis, and In Silico Studies of Novel N-(2-Aminophenyl)-2,3- Diphenylquinoxaline-6-Sulfonamide Derivatives Targeting Receptor- Binding Domain (RBD) of SARS-CoV-2 Spike Glycoprotein and Their Evaluation as Antimicrobial and Antimalarial Agents. *Lett. Drug Des. Discov.* **2021**, *18* (9), 915–931. https://doi.org/10.2174/1570180818666210427095203.

(34) Khan, Sharuk L; Siddiui, F. A. Beta-Sitosterol: As Immunostimulant, Antioxidant and Inhibitor of SARS-CoV-2 Spike Glycoprotein. *Arch. Pharmacol. Ther.* **2020**, *2* (1). https://doi.org/10.33696/pharmacol.2.014.

(35) Krzywinski, M.; Altman, N. Points of Significance: Significance, P Values and t-Tests. *Nat. Methods* **2013**, *10* (11), 1041–1042. https://doi.org/10.1038/nmeth.2698.

(36) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. *Advanced Drug Delivery Reviews*. 2012, pp 4–17.

https://doi.org/10.1016/j.addr.2012.09.019.

(37) Salehi, B.; Venditti, A.; Sharifi-Rad, M.; Kręgiel, D.; Sharifi-Rad, J.; Durazzo, A.; Lucarini, M.; Santini, A.; Souto, E. B.; Novellino, E.; Antolak, H.; Azzini, E.; Setzer, W. N.; Martins, N. The Therapeutic Potential of Apigenin. *International Journal of Molecular Sciences*. 2019. https://doi.org/10.3390/ijms20061305.

(38) Shukla, S.; Gupta, S. Apigenin: A Promising Molecule for Cancer Prevention. *Pharmaceutical Research*. 2010, pp 962–978. https://doi.org/10.1007/s11095-010-0089-7.

(39) BAUMANN, L. S. Apigenin. *Ski. Allergy News* **2008**, *39* (3), 32. https://doi.org/10.1016/s0037-6337(08)70149-9.

(40) Ali, F.; Rahul; Naz, F.; Jyoti, S.; Siddique, Y. H. Health Functionality of Apigenin: A Review. *International Journal of Food Properties*. 2017, pp 1197–1238. https://doi.org/10.1080/10942912.2016.1207188.

(41) Wu, P.; Li, L.; Du, G. H. Ferulic Acid. In *Natural Small Molecule Drugs from Plants*; 2018; pp 75–80. https://doi.org/10.1007/978-981-10-8022-7_12.

(42) Mathew, S.; Abraham, T. E. Bioconversions of Ferulic Acid, an Hydroxycinnamic Acid. *Critical Reviews in Microbiology*. 2006, pp 115–125. https://doi.org/10.1080/10408410600709628.

(43) Nasr Bouzaiene, N.; Chaabane, F.; Sassi, A.; Chekir-Ghedira, L.; Ghedira, K. Effect of Apigenin-7-Glucoside, Genkwanin and Naringenin on Tyrosinase Activity and Melanin Synthesis in B16F10 Melanoma Cells. *Life Sci.* **2016**, *144*, 80–85. https://doi.org/10.1016/j.lfs.2015.11.030.

(44) Lee, H.; Kim, B. G.; Kim, M.; Ahn, J. H. Biosynthesis of Two Flavones, Apigenin and Genkwanin, in Escherichia Coli. *J. Microbiol. Biotechnol.* **2015**, *25* (9), 1442–1448. https://doi.org/10.4014/jmb.1503.03011.

(45) Pei, K.; Ou, J.; Huang, J.; Ou, S. P-Coumaric Acid and Its Conjugates: Dietary Sources, Pharmacokinetic Properties and Biological Activities. *J. Sci. Food Agric.* **2016**, *96* (9), 2952–2962. https://doi.org/10.1002/jsfa.7578.

(46) Kakkar, S.; Bais, S. A Review on Protocatechuic Acid and Its Pharmacological Potential. *ISRN Pharmacol.* **2014**, *2014*, 1–9. https://doi.org/10.1155/2014/952943.

(47) Srinivasulu, C.; Ramgopal, M.; Ramanjaneyulu, G.; Anuradha, C. M.; Suresh Kumar, C. Syringic Acid (SA) – A Review of Its Occurrence, Biosynthesis, Pharmacological and Industrial Importance. *Biomedicine and Pharmacotherapy*. 2018, pp 547–557.

https://doi.org/10.1016/j.biopha.2018.09.069.