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Gene set enrichment analysis of alpha−glucosidase inhibitors from *Ficus benghalensis*

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ABSTRACT

Objective: To identify alpha-glucosidase inhibitors from *Ficus benghalensis* and analyze gene set enrichment of regulated protein molecules.

Methods: The phytoconstituents of *Ficus benghalensis* were queried for inhibitors of alpha-glucosidase, also identified as aldose reductase inhibitors. Druglikeness score, absorption, distribution, metabolism, excretion and toxicity profile, biological spectrum, and gene expression were predicated for each compound. Docking study was performed to predict the binding affinity with alpha-glucosidase and aldose reductase and compared with clinically proven molecules. Kyoto Encyclopedia of Genes and Genomes pathway analysis was performed for the regulated genes to identify the modulated pathways.

Results: Apigenin, 3,4',5,7-tetrahydroxy-3'-methoxyflavone, and kaempferol were identified as inhibitors of alpha-glucosidase and aldose reductase. Kaempferol was predicted to possess the highest binding affinity with both targets. The p53 signaling pathway was predicted to modulate the majority of protein molecules in diabetes mellitus. All the alpha-glucosidase inhibitors were also predicted as membrane integrity agonist and anti-mutagenic compounds.

Conclusions: The current study indicates alpha-glucosidase inhibitors from *Ficus benghalensis* can act as aldose reductase inhibitors after absorption from the intestinal tract. Furthermore, these phytoconstituents are involved in the regulation of numerous protein molecules and pathways. Hence, the anti-diabetic efficacies of these compounds are due to their action on multiple protein molecules and synergistic effects which should be confirmed by future investigations.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder, and occurs due to deficiency of insulin secretion/insulin resistance or combination of both[1]. Although the current pharmacotherapy of diabetes utilizes a large category of synthetic anti-diabetic molecules *i.e.* insulin secretagogues, dipeptidyl-peptidase 4 and alpha-glucosidase inhibitors, glucagon-like peptide-1 agonists, biguanides, thiazolidinediones, amylin analogs, and sodium-dependent glucose transporters 2 inhibitors[2], their utilization is limited due to various side effects such as gastrointestinal toxicities and weight gain[3]. To overcome these side effects, various traditional medicines can be utilized. However, these folk medicines lack evidence for detailed mechanism of action and its complete phytoconstituent composition.

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The current trend of drug discovery utilizes the concept of "lock and key" to design the ligand molecules that act on selective targets. Nevertheless, a drug can modulate a number of protein molecules as a "single master key can unlock multiple locks"[4]. Further, the disease is a condition of complex multi-gene network interlinked to each other[5] which suggests that single drug-protein interaction may not be sufficient enough to treat polygenic diseases like DM[6].

Inhibition of alpha-glucosidase at the brush rim of the small intestine mimics the absorption of glucose and is a well-accepted approach in the pharmacotherapy of DM[2]. Phytoconstituents from *Ficus benghalensis* (*F. benghalensis*) are reported to inhibit alpha-glucosidase[7]. However, the management of DM by folk medicine is not only due to single compound-protein interaction but is due to the synergistic effect of compounds over the multiple targets[8]. Further, it is also possible that alpha-glycosidase inhibitors in the intestine could get absorbed from the intestine and modulate a number of proteins involved in the pathogenesis of DM.

Hence, the present study aimed to identify alpha-glucosidase inhibitors from *F. benghalensis* and discern the pathways based on the drug-gene set enrichment analysis and network pharmacology approach. Interestingly, alpha-glucosidase inhibitors were also predicted as aldose reductase inhibitors, one of the major proteins targeted in the management of diabetic complications.

2. Materials and methods

2.1. Mining of phytoconstituents

The list of compounds available in the *F. benghalensis* was retrieved from the ChEBI database by using the keyword "*Ficus benghalensis*". The identified compounds were confirmed in the PubChem database. Canonical SMILES of all the compounds were retrieved from the PubChem Database along with their molecular weight and molecular formula.

2.2. Identification of alpha-glucosidase inhibitors

BindingDB[9], a public, web-accessible database was utilized to forecast the assorted targets of each compound by querying the SMILES of each phytoconstituent at the similarity of 0.7 (70%). After the prediction of possible targets, the phytoconstituents inhibiting alpha-glucosidase and aldose reductase were identified.

2.3. Druglikeness and absorption, distribution, metabolism, excretion and toxicity (ADMET) profile of phytoconstituents

Canonical SMILES was queried for the prediction of druglikeness and ADMET of individual phytoconstituents. MolSoft (http://molsoft.com/mprop/), admetSAR2.0[10], and ADVERPred[11] were used to calculate druglikeness score, ADMET profile and probable side effects, respectively.

2.4. Prediction of biological spectrum

The probable biological spectrum of phytoconstituents was predicted using Prediction of Activity Spectra for Substance[12]. The probable activities were expressed in probable activity (Pa) and probable inactivity (Pi). In the present study of biological spectrum evaluation, the activities with Pa>Pi were considered.

2.5. Prediction of gene expression profiles

DIGEP-Pred[13] was used to identify the phytoconstituent-induced change in gene expression profile. Canonical SMILES was queried for the identification of protein molecules which were predicted either to be upregulated or downregulated at the probable activity of 0.5.

2.6. Enrichment analysis

STRING[14] was used to understand the protein-protein interaction by submitting the set of proteins and the respective pathway involved in diabetes was identified by using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database.

2.7. Network construction

Cytoscape 3.7.1[15] was used to construct the network between compounds, target proteins, and pathways. The command "Network Analyzer" was used to analyze the network by treating the network as directed. The map node size was set from "low values to small sizes" and map node color from "low values to bright colors" based on edge count for both settings.

2.8. Docking studies

The .sdf 2D formats of the compounds were retrieved from PubChem database, converted into .pdb using Discovery Studio 2017. The energy of ligand molecules was minimized using MarvinSketch to obtain ten different confirmations. The confirmation scoring lowest energy was chosen as a ligand molecule. The 3D X-Ray crystallographic structure of aldose reductase (PDB ID:3RX2) was retrieved from the RCSB protein data bank. The alpha-glucosidase crystallographic moiety with a complete amino acid sequence was not found for *Homo sapiens*. Hence, BLASTp was used to identify

Table 1. Druglikeness of compounds.

Compounds	PubChem CID	Molecular formula	Molecular weight (g/mol)	NHBA	NHBD	MolLogP	DLS
Apigenin	5280443	$C_{15}H_{10}O_5$	270.05	5	3	3.06	0.77
3,4',5,7-tetrahydroxy-3'-methoxyflavone	5280681	$C_{16}H_{12}O_7$	316.06	7	4	2.46	0.93
Kaempferol	5280863	$C_{15}H_{10}O_6$	286.05	6	4	2.49	0.77
Acarbose	41774	$C_{25}H_{43}NO_{18}$	645.25	19	14	-7.96	0.51
Miglitol	441314	$C_8H_{17}NO_5$	207.11	6	5	-3.31	0.17
Epalrestat	1549120	$C_{15}H_{13}NO_3S_2$	319.03	5	1	3.22	-0.43

NHBA: Number of hydrogen bond acceptor, NHBD: Number of hydrogen bond donor, DLS: Druglikeness score.

the template and Modeller 9.10 was used to construct the homology model by adding the missing amino acid residues. Query sequence (Accession number: ABI53718.1) and PDB ID: 5KZW were used for the homology modeling of alpha-glucosidase. The energy of the target molecules was minimized by using YASARA. The docking study was performed using autodock4.0[16]. After the completion of docking, the pose having the lowest energy was chosen to visualize the ligand-protein interaction in Discovery Studio 2017.

3. Results

3.1. Druglikeness and ADMET profile of phytoconstituents

Among the twenty-one phytoconstituents, three flavonoids *i.e.* apigenin, 3,4',5,7-tetrahydroxy-3'-methoxyflavone, and kaempferol were predicted to inhibit alpha-glucosidase and aldose reductase at the similarity score of 70%. Further, 3,4',5,7-tetrahydroxy-3'-methoxyflavone was predicted for highest druglikeness score *i.e.* 0.93 (Table 1). The ADMET profile of the phytoconstituents is represented in Heat map (Figure 1a). Most phytoconstituents scored probable side effects Pa<0.7. However, acarbose, a well identified alpha-glucosidase inhibitor was predicted for its nephrotoxicity (Table 2).

3.2. Biological spectrum of phytoconstituents

The highest biological activity was predicted for membrane integrity agonist and antimutagenic activity for all the compounds. The heat map (Figure 1b) represents the probability of all three compounds for various biological activities.

Table 2. Probable side effects of the compounds.

Compounds	Side effect	Pa	Pi
Apigenin	Hepatotoxicity	0.525	0.182
3,4',5,7-tetrahydroxy-3'-methoxyflavone	Hepatotoxicity	0.422	0.243
Kaempferol	Hepatotoxicity	0.525	0.182
Acarbose	Nephrotoxicity	0.710	0.017
	Hepatotoxicity	0.350	0.299
Epalrestat	Myocardial infarction	0.569	0.033
	Hepatotoxicity	0.535	0.177

Pa: Probable activity, Pi: Probable inactivity. The probable side effect is detected experimentally if Pa>0.7. No side effects were predicted for Miglitol.

3.3. Docking study

Among the three flavonoids, kaempferol scored the highest binding affinity with alpha-glucosidase (-8.04 kcal/mol) and aldose reductase (-5.89 kcal/mol) and their interactions are represented in Figure 2. The binding affinity, inhibitory constant and hydrogen bond interaction of each compound with an individual target is compared with clinically accepted standard molecules (Table 3).

3.4. Prediction of gene expression profiles

Apigenin, kaempferol, and 3,4',5,7-tetrahydroxy-3'-methoxyflavone were predicted to regulate 32, 30 and 27 proteins respectively. Among them, 25 proteins were commonly regulated by three phytoconstituents (Figure 3).

3.5. Gene set enrichment and network analysis

The KEGG pathway analysis identified six different pathways, among them p53 signaling pathway was predicted to be majorly regulated by apigenin, kaempferol and 3,4',5,7-tetrahydroxy-3'-methoxyflavone (Table 4). Further, the peer interpretation of the constructed network also identifies the p53 signaling pathway as a major signaling pathway in the management of DM (Figure 4).

4. Discussion

Primarily, the study aimed to identify alpha-glucosidase inhibitors from *F. benghalensis* and assess the probable mechanisms for anti-diabetic activity based on the gene set enrichment analysis. Interestingly, only flavonoids were predicted as alpha-glucosidase inhibitors, also identified as aldose reductase inhibitors. This observation kindled us to assess the interaction between identified phytoconstituents and aldose reductase *via* the computational docking studies. Further, the reported alpha-glucosidase inhibitory activity of *F. benghalensis*[7] could be due to the presence of flavonoids as major phytoconstituents[17]. In one of our studies, which included the fractionation of a few parts of *F. benghalensis*, the maximum yield of flavonoids was obtained (Data not published).

The probability of false positive hits arises in docking studies due to negligence in the protein/target reorganization[18] which can be

Table 3. Binding affinity of ligand molecules with respective targets.

Compounds	Alpha-glucosidase (Homology modelled protein)			Aldose reductase [PDB ID: 3RX2]		
	BE (kcal/mol)	IC_{50} (μM)	Amino acid…..ligand interaction	BE (kcal/mol)	IC_{50} (μM)	Amino acid…..ligand interaction
3,4',5,7-tetrahydroxy-3'-methoxyflavone	-7.32	4.29	ILE98(=O)…..HO ILE98(=O)…..HO PRO94(=O)…..HO ARG275(-H)…..O- ARG331(-H)…..O-	-5.85	51.77	GLY 151(=O)…..HO LEU 96 (=O)…..HO LEU 101(=O)…..HO GLN93(=O)…..HO
Apigenin	-7.50	3.16	ASP91(=O)…..HO GLN115(=O)…..HO CYS127(=O)…..HO	-5.54	86.19	LEU101(=O)…..HO LEU96(=O)…..HO GLN93(-H)…..O=
Kaempferol	-8.04	1.27	ASP91(=O)…..HO ILE98(=O)…..HO GLY116(=O)…..HO GLY123(=O)…..HO GLY123(=NH)…..O-	-5.89	48.10	GLU150(=O)…..HO ASP120(=O)…..HO GLN93(=O)…..HO
Acarbose	-8.17	1.03	ASP91(HO)…..HO ALA93(=O)…..HO PRO94(=O)…..HN ARG275(NH)…..O- VAL544(=O)…..HO VAL544(=O)…..HO PRO541(=O)…..HO PRO541(=O)…..HO	-	-	-
Miglitol	-4.19	855.39	ALA105(=O)…..HO ARG106(=O)…..HO ARG106(=O)…..HO THR158(=O)…..HO THR158(=O)…..HO	-	-	-
Epalrestat	-	-	-	-7.25	4.83	LYS154(-H)…..O= GLY151(=O)…..HO

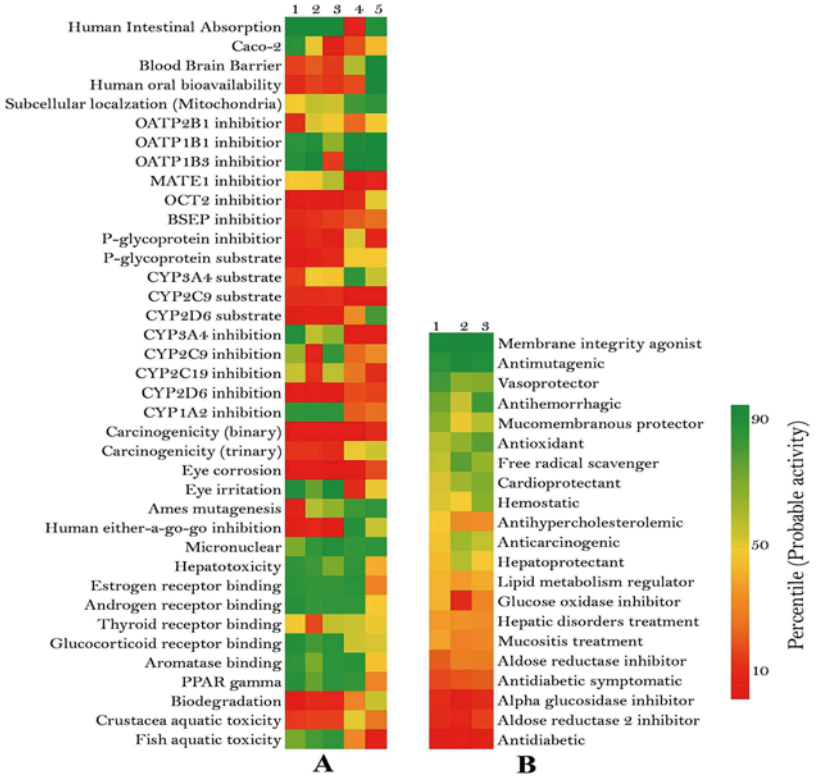

Figure 1. Absorption, distribution, metabolism, excretion and toxicity profile of phytoconstituents compared to standard moiety (A) and *in silico* biological spectrum of phytoconstituents (B). In Figure 1A, line 1: apigenin; line 2: 3,4',5,7-tetrahydroxy-3'-methoxyflavone; line 3: kaempferol; line 4: acarbose; line 5: miglitol. In Figure 1B, line 1: apigenin; line 2: 3,4',5,7-tetrahydroxy-3'-methoxyflavone; line 3: kaempferol.

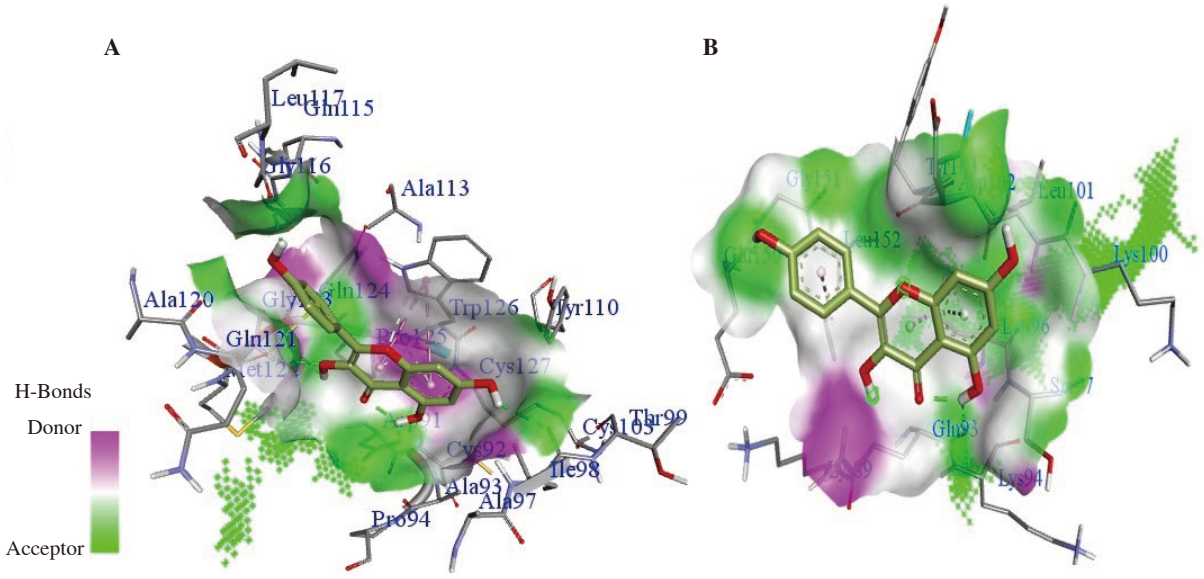

Figure 2. Interaction of kaempferol with (A) alpha-glucosidase and (B) aldose reductase.

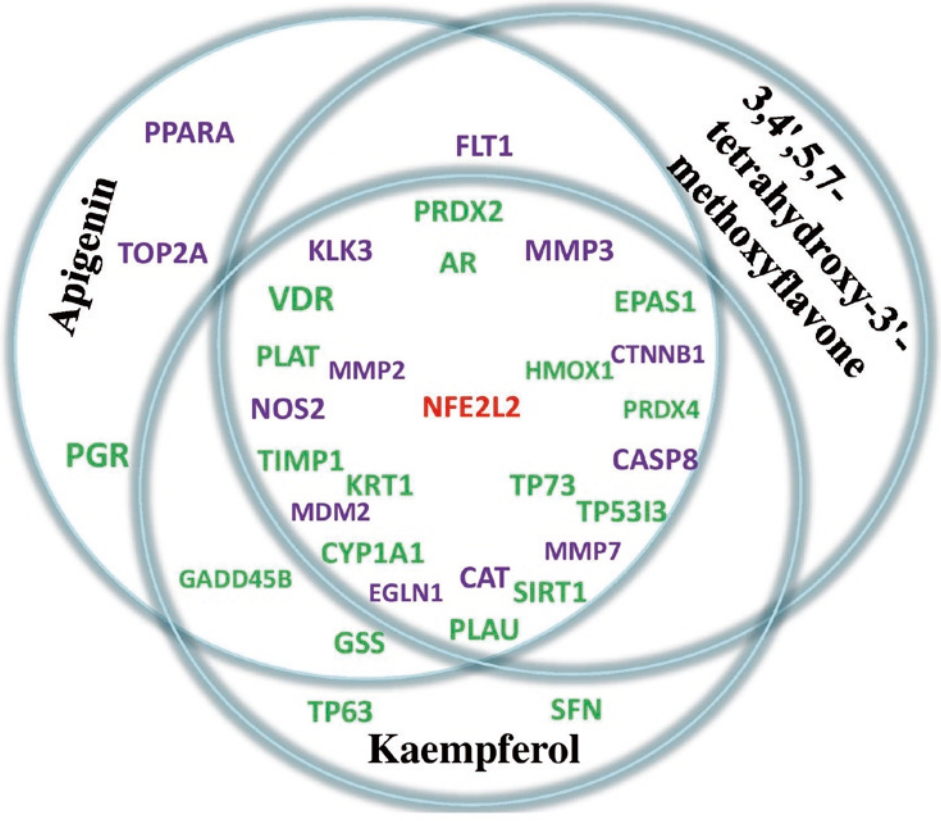

Figure 3. Proteins regulated by phytoconstituents. Red: upregulated, downregulated proteins, green: upregulated proteins and purple: downregulated proteins. Apigenin∩3,4',5,7-tetrahydroxy-3'-methoxyflavone∩Kaempferol=25; Apigenin∩3,4',5,7-tetrahydroxy-3'-methoxyflavone=1; Apigenin∪3,4',5,7-tetrahydroxy-3'-methoxyflavone∪Kaempferol=33.

minimized by utilizing the cheminformatic information. BindingDB predicts probable targets to molecules based on the principle "similar compounds target similar proteins". Hence, the current study utilizes the docking study only after the prediction of aldose reductase as a target for alpha-glucosidase inhibitors. Kaempferol scored maximum binding affinity with both targets, and interacted with ASP91, ILE98, GLY116, GLY123 and GLY123 amino acid residues of alpha-glucosidase and GLU150, ASP120 and GLN93 of aldose reductase.

Alpha-glucosidase digests dietary carbohydrates *via* the cleavage of 1,4-glycosidic linkage and releases glucose in the intestine which gets absorbed from the gastrointestinal tract[19]. Inhibitors of this enzyme are well recognized in the pharmacotherapy of DM. However, these molecules may not limit within the intestine but may get absorbed into the systemic circulation to modulate non-pathogenic protein molecules. This could be one of the reasons why well established alpha-glucosidase inhibitors are not free from side effects[20]. The current prediction of side effects also identifies acarbose as nephrotoxic and hepatotoxic; however, the predicted side

Table 4. Enrichment analysis of phytoconstituents.

Phytoconstituents	KEGG pathway	Description	Count in gene set	Gene codes
Apigenin	map04151	PI3K-Akt signaling pathway	2	FLT1, MDM2
	map04310	Wnt signaling pathway	2	MMP7, CTNNB1
	map04114	Oocyte meiosis	2	AR, PGR
	map04110	Cell cycle	2	GADD45B, MDM2
	map04115	p53 signaling pathway	5	GADD45B, CASP8, TP73, TP53I3, MDM2
	map01100	Metabolic pathways	4	GSS, NOS2, HMOX1, CYP1A1
Kaempferol	map04115	p53 signaling pathway	6	GADD45B, CASP8, MDM2, TP73, TP53I3, SFN
	map04110	Cell cycle	3	GADD45B, MDM2, SFN
	map01100	Metabolic pathways	4	GSS, NOS2, HMOX1, CYP1A1
	map04310	Wnt signaling pathway	2	MMP7, CTNNB1
3,4',5,7-tetrahydroxy-3'-methoxyflavone	map04115	p53 signaling pathway	4	TP53I3, MDM2, TP73, CASP8
	map01100	Metabolic pathways	3	HMOX1, NOS2, CYP1A1
	map04151	PI3K-Akt signaling pathway	2	FLT1, MDM2
	map04310	Wnt signaling pathway	2	MMP7, CTNNB1

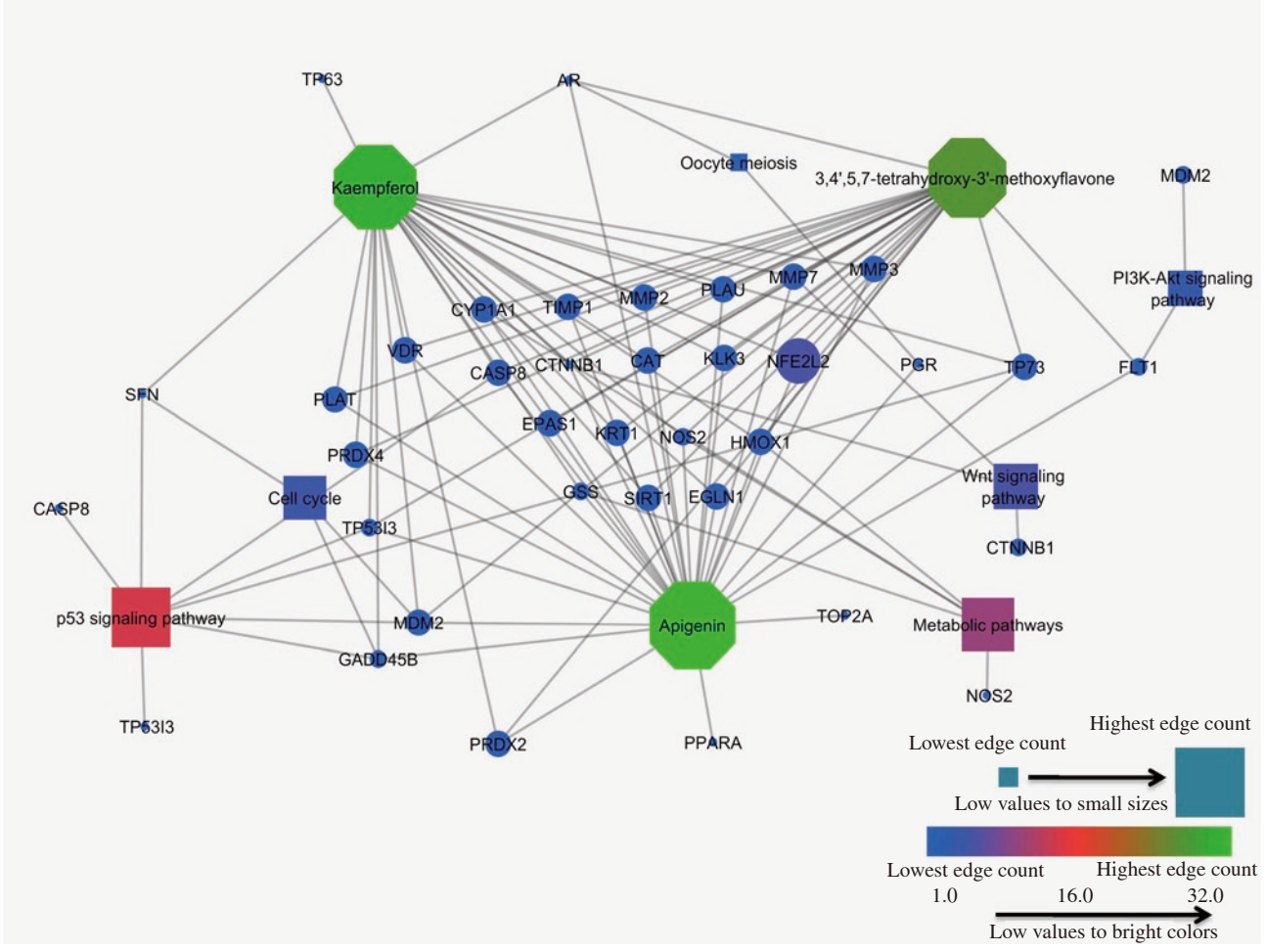

Figure 4. Network interaction between phytoconstituents, targets and pathways.

effects of phytoconstituents may not be expressed clinically since the side effects are detected experimentally provided Pa>0.7.

It is well known that a single drug molecule can regulate (upregulate or downregulate) the number of proteins[5] reflecting the necessity for the identification of probable biological activities (may be related to side effects or synergistic activity). Hence, the identified compounds were predicted for the regulation of protein molecules at the minimum probable activity of 0.5. Interestingly, the phytoconstituents regulated the number of protein molecules, and 25 were commonly regulated.

The network constructed in the current study identifies the p53 signaling pathway to be majorly modulated, and is also supported by the KEGG pathway analysis based on the count in gene sets. Four common protein molecules *i.e.* TP53I3, MDM2, TP73, CASP8 were identified under p53 signaling pathways and were modulated by the phytoconstituents. TP53I3 gene encodes putative quinone oxidoreductase enzyme involved in the redox reaction and modulates the proliferation of liver cells and insulin-degrading enzyme activity[21]. MDM2 is E3 ubiquitin ligase involved in the ubiquitination, stimulation of nuclear exportation, and proteasomal degradation of p53[22–24]. Further, it also regulates the proliferation of glomerular mesangial cell and deposition of excessive extracellular matrix in diabetic kidney disease[25]. Hence, apart from the management of blood glucose level in diabetes, the identified alpha-glucosidase inhibitors could contribute to the minimization of kidney injury. Tumor protein P73 (TP73) regulates autophagy, glycolysis, gluconeogenesis, and mitochondrial metabolism of pyruvate[26,27] and was predicted to be upregulated. CASPASE-8 (CASP8) is reported to play the prime role in diabetic embryopathy by regulating Bid-stimulated mitochondrion/caspase-9 pathway and its inhibition is reported to reduce the rate of embryonic malformation[28] which was predicted to be downregulated by alpha-glucosidase inhibitors.

The PI3K-AKT signaling pathway regulates glucose homeostasis, improves insulin secretion, and activates GLUT4 for glucose uptake[29]. Apigenin and 3,4',5,7-tetrahydroxy-3'-methoxyflavone were identified to regulate two protein molecules *i.e.* vascular endothelial growth factor receptor 1 (FLT1) and murine double minute-2 (MDM2) for the glucose homeostasis modulating above pathway. Wnt signaling pathway is identified to have a role in glucose homeostasis and lipid metabolism[30] which was modulated by all the three phytoconstituents.

In conclusion, the current study confirms that though flavonoids were purposed as alpha-glucosidase inhibitors, they may not be restricted within the gastrointestinal tract but also get absorbed to the systemic circulation and modulate multiple proteins which are involved in the homeostatic/pathogenic condition. Further, we utilized the concept of network pharmacology approach to purpose the additional mechanism of recognized compounds in the biological system. The current study adds the knowledge in scientific literature by explaining the additional mechanism of flavonoids from *F. benghalensis* in the management of DM. Although the current purpose is the reflection of computer simulation, futuristic well designed wet-lab protocols will prove the findings accordingly.

Conflict of interest statement

Authors declare that there are no competing interests.

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