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Esculetin and its similar compounds: Implications in binding Sirt1 and treating diabetic nephropathy

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Abstract:

Diabetic nephropathy(DN)occurs as a result of prolonged high levels of glucose in diabetic patients. It is diagnosed primarily upon the presence of microalbuminuria (MA). Factors like dyslipidemia, high blood sugar levels, free radicals, smoking, advanced glycation end products, environmental, and genetic factors play an important role in the progression of diabetic nephropathy. Many reports have highlighted the role of genetics and epigenetics including histone modifications in the progression of DN. Histone acetyl transferases (HATs) and Histone deacetylases (HDACs)which regulates histone acetylation levels are known to play an important role in chromatin remodelling, affecting various gene transcription that is related to metabolism, apoptosis, immunity, and angiogenesis. HDAC inhibitors are now emerging as new therapeutic target. Treatment methods still are not so prevalent in ameliorating DN; some of them include controlling hypertension, controlling high blood sugar level, cessation of smoking and drinking, and renal replacement. The number of side effects associated with synthetic drugs makes researchers look for alternatives that are less toxic. Phytochemicals can be a good alternative to these synthetic drugs as they are relatively safe and have multiple interactive targets. In order to fish out potent phytochemicals,a thorough review was done on current existing literature, and Esculetin was chosen. Structurally similar compounds were found by doing structural similarity searches with filter 80%. Top 3 similar compounds were chosen and further docked with human SIRT1 protein. Docking was done to identify potent compound for treating DN and similar binding energies proved similar compounds may have the potential to ameliorate DN.

Keywords: *Nephropathy, Microalbuminuria, Phytochemicals, Epigenetics, Histone modifications, HDAC Inhibitors, Docking.*

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Introduction:

In diabetic nephropathy, there are certain morphological changes along with abnormal kidney function[1].Anomalies in morphology of kidney include nodular and diffuse glomerularsclerosis, kidney hypertrophy, tubular atrophy, thickness in the glomerular basement membrane and interstitial fibrosis [2,3]. Kidney dysfunction includes persistent proteinuria, hypertension, increase in glomerular filtration rate (GFR) and loss of renal function[4]. There are five stages of DN as suggested by Mongessen: (a) increase in GFR and renal flow; (b) tubulointerstitial and mesangial expansion, thickening of the glomerular basement membrane, glomerular hypertrophy; (c) microalbuminuria due to hypertension; (d) dipstick-positive proteinuria; and (e) ESRD with uremia[6] (Figure 1)

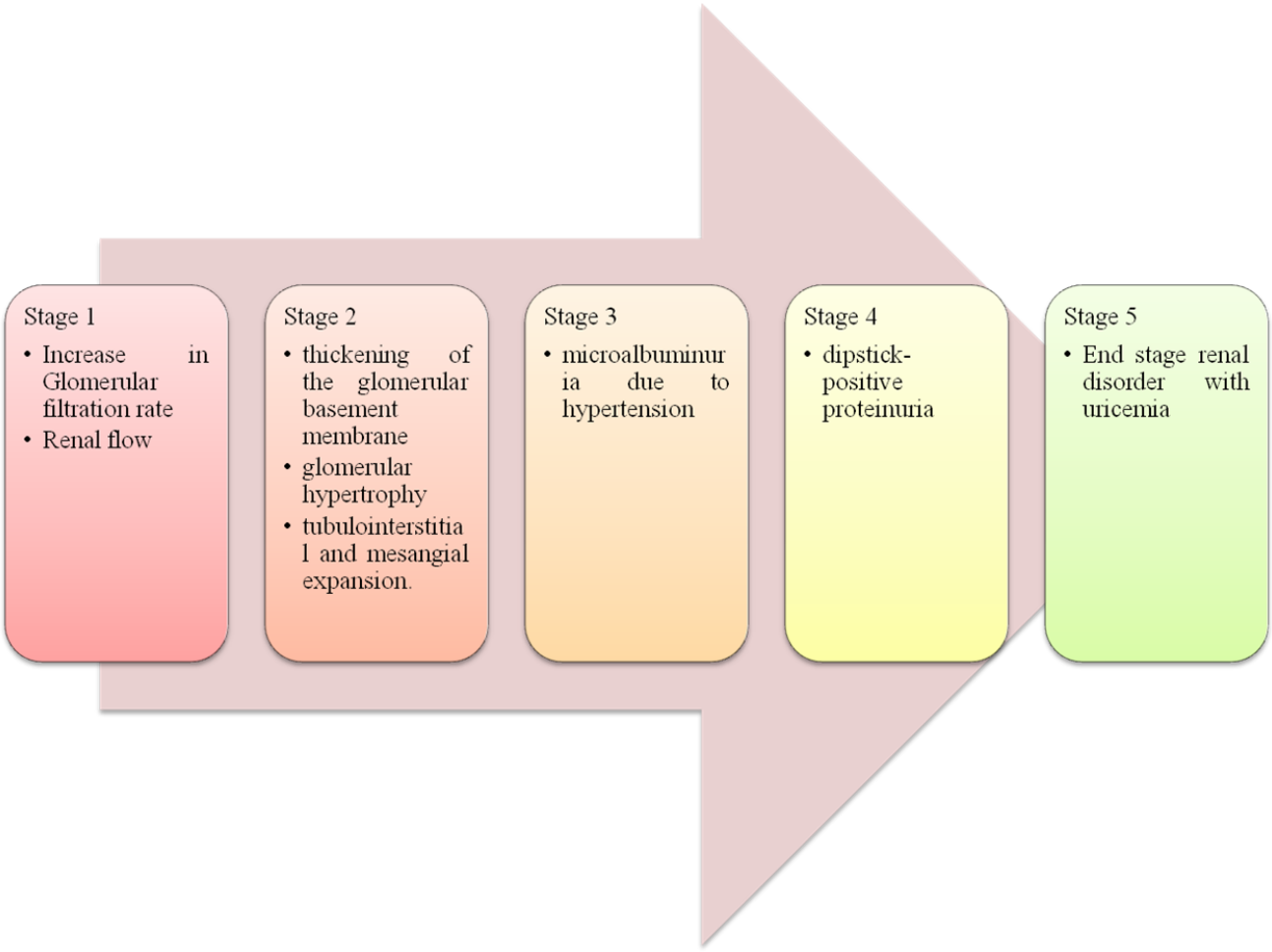


Figure 1: Various stages of Diabetic nephropathy

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Other features of DN include the accumulation of extracellular matrix(ECM)in tubulointerstitial compartments, glomerular mesangial, and epithelial-to-mesenchymal transition (EMT) of renal tubular epithelial cells[7]. In DN, renin-angiotensin-aldosterone systems (RAAS) which are vaso-active systems, are activated that lead to the secretion of molecules like AGE, transforming growth factor b1 (TGF-b1), activation protein kinase C (PKC). There is an increase in intra-glomerular and systemic pressure due to the acceleration of aldose reductase pathway, hence hyperfiltration leads to the leakage of protein from the capillaries of glomerulus[8]. Podocytes are highly specialized epithelial cells, known to maintain and hold the integrity of the glomerular filtration barrier. Any damage to podocytes leads to DN[9].First clinical evidence of DN is microalbuminuria. Microalbuminuria is defined as excretion of urinary albumin 30-299 mg/24 hours in a 24-hour urine collection. 30-299 µg/mg creatinine in urine is also evident in DN[10]. Blacks, Pima Indians, and Hispanics are at higher risk of DN compared to Caucasians[11,12].Several mechanisms are known for the pathogenesis of DN: inflammation, autophagy, oxidative stress, genetic and epigenetic alterations[13-15].

Recent reports have highlighted that epigenetic modifications like DNA methylation, micro RNA and histone modificationsplays an important role in the progression of DN[16].Histone acetyl transferases (HATs) are enzymes that add acetyl group on histone and Histone deacetylases (HDACs) remove acetyl group on the histones, their interplay is known to regulate gene expression[17-19]. HDACs being involved in several biochemical pathways play a crucial role in DN progression[20-22]. HDAC1 is known to trigger proliferation and chemokine production in tubular epithelial cells and interstitial renal fibroblastsactivation[17]. In a very recent report, HDAC2/4/5 has been shown to be up-regulated in clinical as well as in experimental DN[23].

HDAC inhibitors have an attribute of being renoprotective and anti-fibrotic against many diabetic-related complications, confirming crucial role of HDAC in DN progression[24,25]. The kidneys of STZ- induced rats showed elevated levels of HDAC-2 activity that eventually increased the expression of fibronectin, while on the other hand, decreased E-cadherin expression[26].Moreover, HDAC inhibitors also have beneficial effects in different pathological conditions associated with nephrotic and non-nephrotic conditions by chromatin dependent and independent mechanisms[27-30].

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In general HDAC inhibitors works by regulating gene expression and led to hyperacetylation of histone proteins[31,32]. Histone acetylation causes increase transcriptional activity and decreased acetylation is related to transcriptional repression[33]. So by the theory, it is very much obvious that HDAC inhibitors would cause an increase in gene transcription.TrichostatinA(TSA) is a known HDAC inhibitor that was shown to prevent downregulation of e-cadherins induced by TGF-b1 and upregulation of collagen II in tubular epithelial cells[34].

Some of the plant-based natural molecules were also found to be effective against diabetic nephropathy and some are potent HDAC inhibitors. In a study, it was also reported that curcumin analog provides protection against diabetic nephropathy by suppressing the diabetes-induced increase in histone acetylation [35]. While doing a literature survey a phytochemical named esculetin was found to be a very good bioactive compound in treating DNand has anti-diabetic properties.Esculetin is a naturally occurring coumarin derivative having good potential against metabolic diseases. The crucial role of esculetin has been attributed to its ability to act as an anti-proliferative, anti-inflammatory, anti-cancer, and anti-diabetic agent. Esculetin as a class of coumarin is categorized as chichorigenin (a simple coumarin). It is a 6,7-dihydroxycoumarin found in, many plants [36-38]. It is found in *Viola yedoensis*[39], the leaves of *Citrus limonia*[40], *Artemisia capillaries*[36], *Cortex fraxini*[41,42], *Artemisia scoparia*[43], *Ceratostigma willmottianum*[43], *Cichorium intybus*[44], and *Aesculus hippocastanum*[45].

Esculetin is reported to be anti-diabetic because it exerts a protective effect by ameliorating oxidative stress and antioxidant competence[46]. It also has an anti-hyperglycemic effect on STZ-induced rats [47]. In another report, they have highlighted the fact that esculetin supplementation is beneficial for insulin resistance inHFD-induced obese mice by activating AdiopR2-AMPK pathway[48].In a report,it is reported that esculetin was able to reverse histone acetylation, and methylation by reducing hypertension, metabolic alterations, hypertrophy and fibrosis in the heart of diabetic patients. Esculetin was also able to attenuate any anomaly in the rennin-angiotensin system, cell proliferation and oxidative stress[49].In other experiments, when esculetin treatment was given to Sprague Dawley (SD) rats it was able to overcome the downregulation of PPAR-Y in the diabetic kidney, decrease in monomethylation, and acetylation of H3 simultaneously. The protection provided by esculetin is due to the alterations in the mRNA

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expression of two genes:Mmp13 and Bmp6[50]. In another report, it was shown that esculetin was able to ameliorate DN by giving reno-protection because of its renin-angiotensin system modulating properties[51]. Esculetin was able to overcome any changes in ANG-II due to vascular reactivity[52].Gout is known to be associated with renal injuries which are a notable cause of hyperuricemia [53]. Esculetin showed upregulation of mOAT1 (renal organic anion transporter 1), confirming its role in ameliorating hyperuricemia [53].

Moreover, there may be a plethora of phytochemicals present that are similar to esculetin, in structure and in bioactivity as well. Based on the perusal of the above literature it would be quite apt to say that esculetin has pleiotropic effects in ameliorating diabetes and diabetes-related complications.Therefore, in the present study, following the hypothesis that "similar structures have similar biological activities", we aim to identify esculetin's structurally similar phytochemicals and have elucidated their binding modes with HDAC (SirtI) similar to esculetin. These studies will help to identify potent new molecules and in understanding their protective effects against DN.

Methods

Data mining and similarity searching of Esculetin

Escueltin was used as a query molecule in several open databases like ChEMBL, PubChem, ZINC, ChemSpider. It's smile notation, 2-D, 3-D structures were used to look into databases for detailed studies and to fish out something important. Similarity searching was done on the filter 80% and above.

Molecular docking:

Molecular docking was done using AutoDockVina 4.2 [54]. The protein was downloaded from PDB and processed before docking. Several parameters were used like: polar hydrogen was added, water was deleted to clear out the binding pocket, charges were added. Ligand was also processed before docking. Ligand was drawn in ChemBio Office and energy was minimized. Torsions were fixed for ligand and the grid box was set for docking with an area of 40x40x40 (center on ligand).The box was made using AutoGrid. Lamarckian Genetic Algorithm is used in AutoDock Vina to get the best binding energies and pose. Docking program was validated first

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before docking. Validation of the program was done by docking protein with already bound ligand from the PDB file. This will make sure that the ligand binds at the same place without changing parameters. This study deduces the fact that how similar ligands bind to the active site of the same protein.

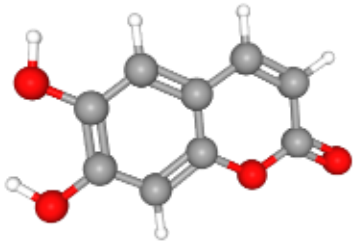
Results:

There were 51 compounds similar to esculetin when 80% filter was used during structural similarity searching. Top three similar compounds were taken for further study and docking process which are Isoscopoletin havingthe highest similarity of 94.12% with esculetin, Scoparone had similarity 90.18% and Fraxetin had 85.14% similarity (Table 1).

When we did docking with the Crystal structure of the SIRT1 catalytic domain bound to NAD and an EX527 analog (PDB ID: 4I5I), we found that esculetin had binding energy of -8.9 kcal/mol (Figure 2), Isoscopoletin had binding energy of -8.6 kcal/mol (Figure 3), Scoparone (Figure 4) also had the same binding energy as that of isoscopoletin and Fraxetin had a binding energy of -8.5 kcal/mol (Figure 5) (Table 2).

This shows that similar compounds had nearly similar binding energy to that of esculetin, highlighting the fact that these can be a potent drug in treating DN.

Table 1: Compounds similar to Esculetin

Similar Compound	Similarity %	Chembl ID	Structure
Esculetin		**CHEMBL244743**	

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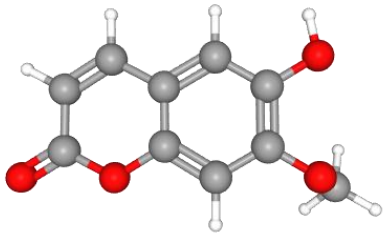
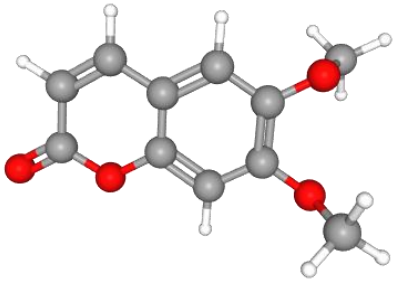
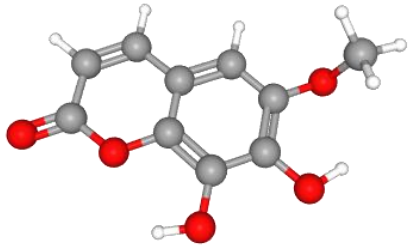
Isoscopoletin	**94.12**	**CHEMBL390320**	
Scoparone	**90.18**	**CHEMBL325864**	
Fraxetin	**85.16**	**CHEMBL54909**	

Table 2: Binding energies when protein docked with phytochemicals

Ligands	Binding energies (kcal/mol)	Interacting Amino Acids	Hydrogen Bonds
Esculetin	-8.9	Phe297, Ile347Phe273,	1 hydrogen bond with ULN-1
Isoscopoletin	-8.6	Ile411,Ile 347, Ile316, Phe297, Phe 273	None
Scoparone	-8.6	Phe273, Phe297, Ile411, Ile347, Ile316	None
Fraxetin	-8.5	Phe273, Phe297, Ile316, Ile347	None

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Figure 2:

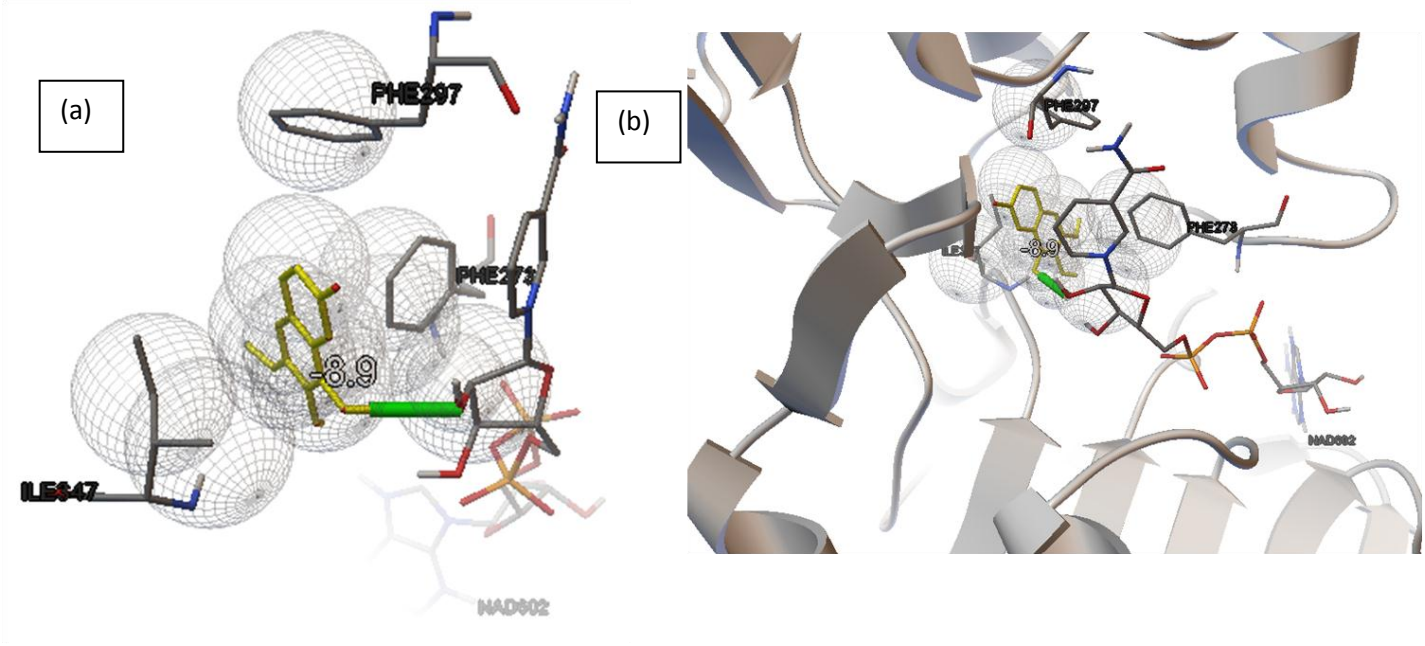
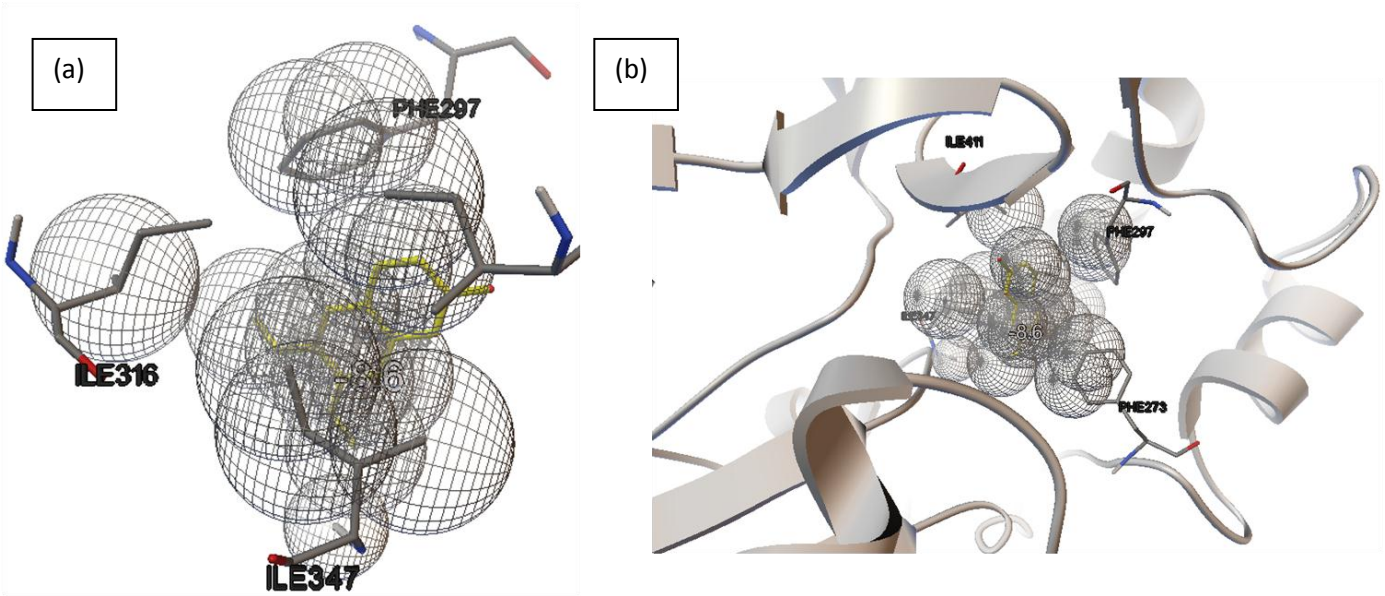


Figure 2: Molecular docking of Esculetin with Sirt1 active site (PDB ID: 4I5I): (a) Interaction of ligand with protein where hydrogen bond is represented in green color (b) Pictorial representation of ligand interacting with ribbon structure of the protein.

Figure 3:



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Figure 3: Molecular docking of Isoscopoletin with Sirt1 active site (PDB ID: 4I5I): (a) Interaction of ligand with protein (b) Pictorial representation of ligand interacting with ribbon structure of the protein.

Figure 4:

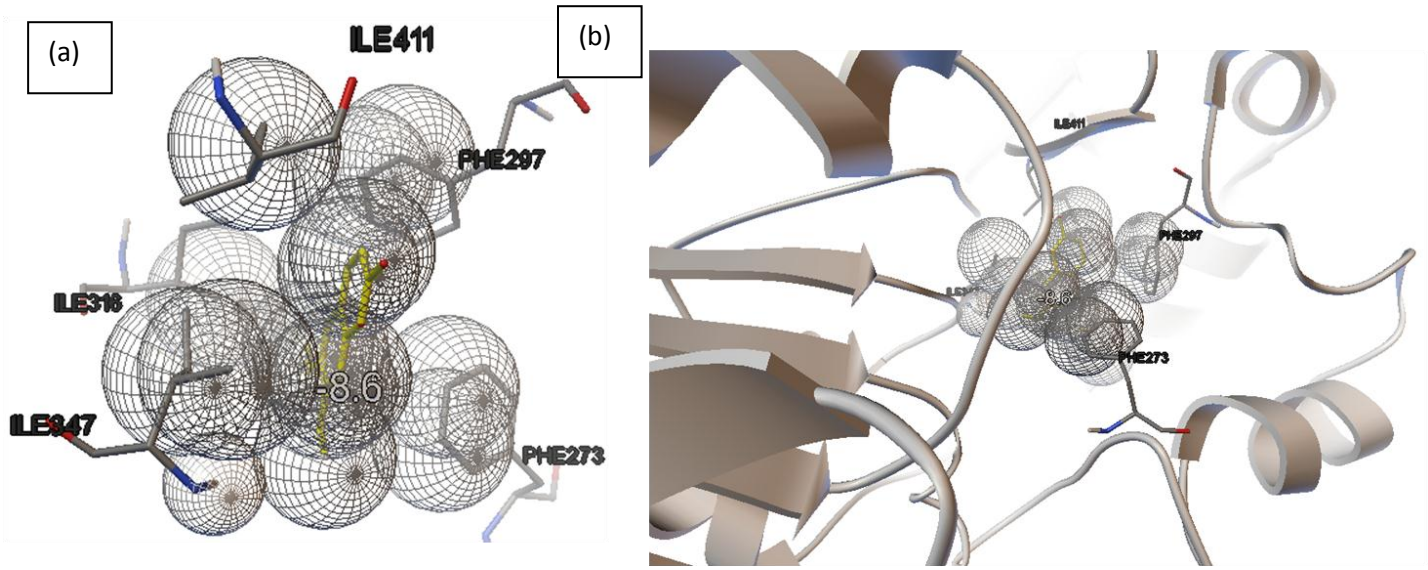
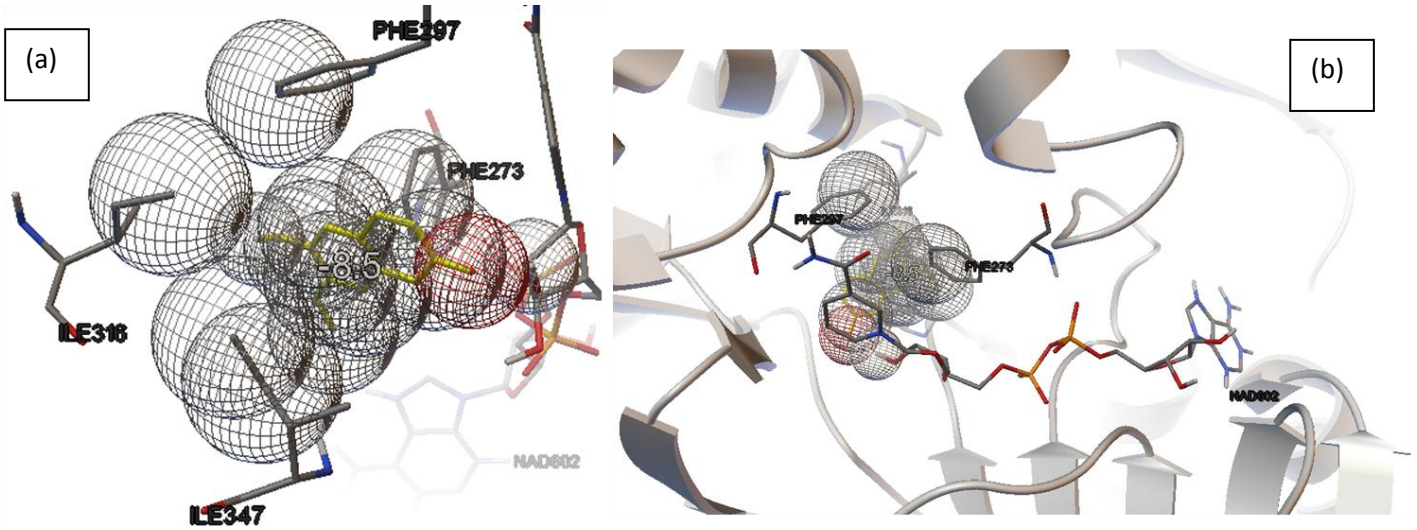


Figure 4: Molecular docking of Scoparone with Sirt1 active site (PDB ID: 4I5I): (a) Interaction of ligand with protein (b) Pictorial representation of ligand interacting with ribbon structure of the protein.

Figure 5:



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Figure 5: Molecular docking of Fraxetin with Sirt1 active site (PDB ID: 4I5I): (a) Interaction of ligand with protein (b) Pictorial representation of ligand interacting with ribbon structure of the protein

Discussion:

Plants have been used in treatment since Vedic ages. They are relatively safe without any side effects or atleast with lesser side effects. Structural similarity searching is a powerful tool to investigate potent drug targets. It also helps to identify biological activities associated with esculetin. Escueltin is a coumarin derivative and possesses many biological activities like anti-inflammatory, anti-proliferative, anti-diabetic and anti-cancerous[36-38].

Ligand-based similarity searching was done to elucidate compounds similar to it. For the purpose, open-source databases like PDB,CHEMBL,BindingDB, PubChem, and ChemSpider were searched. Top 3 similar compounds were chosen on the basis of the highest similarity percentage, similarity index was 0.8. Similar compounds are isoscopoletin, scoparone and fraxetin. These were further docked with human Sirt 1 protein. Similar binding energies showed that these similar compound may prove to be beneficial in treating DN.

Isoscopoletin is found in the crude extract of *Arracacia tolucensis* that showed antimycobacterial action against *Mycobacterium tuberculosis*[55]. From *Artemisia argyi* plants isoscopoletin was identified and it was discovered that it was effective enough to postpone cancer progression[56]. Its ethyl acetate extract showed inhibition in cell proliferation assay using human leukemia cells that have isocopoletin and scopoletin fractions [57]. Esculetin, fraxetin, and scoparone were found to be potent against edema and inflammation[58]. The mixture of 5 medicinal plants was tested for hypoglycaemic effect and the mixture was able to ameliorate this metabolic abnormality. Isoscopoletin was found in a significant amount in these mixtures. Thus can be said that isoscopoletin also exerts hypoglycaemic effects[59]. In another study, isoscopoletin have shown anti-cancer activity along with other coumarin derivatives[60].

Scoparone in a study has shown to protect mesangial cells against damage due to high blood glucose by inactivating ERK pathway [61].The inhibitory effect of scoparone was studied in 3T3 cell lines, where it was revealed that scoparone has PPARγ antagonistic activity. It suppressed the differentiation and proliferation of adipogenic genes by PPARγ inhibition[62]. Scoparone is

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also known to be a very good antioxidant as it inhibits UV induced lipid peroxidation[63]. It also showed antioxidant activity in DPPH assay[64]. Scoparone also exhibits antitumor activity against prostate cancer by inhibiting STAT3 activity.

Fraxetin has been reported to be antihyperglycemic when administered to diabetic rats improved body weight and hepatic glycogen[65], levels of plasma glucose were also decreased and plasma insulin level was increased [65]. Fraxetin is known to provide protection against rotenone-induced neurotoxicity[66] and prevents oxidative damage by increasing glutathione reductase[67]. Fraxetin can reduce apoptosis by decreasing free radical generation in SH-SY5Y cells.Fraxetin alongwith esculetin, when administered to osteosarcoma LM8 cells, showed anti-metastatic and antitumor effects [68].

This highlights the fact that many biological activities have been reported previously on esculetin and its similar compound. They have been reported to be anticancer, antidiabetic, antitumor, antioxidant, antibacterial, and anti-inflammatory. But there is always a scope for more. We have realized that not much focus is laid on their capability as being a potent drug for DN. For the first time here, we are reporting their interaction with SIRT1, an HDAC protein. Their ability to regulate SIRT1 activity might be responsible for regulating gene expression and providing these beneficial health effects.

Conclusion:

Scientific works highlighted here supports the fact that plant can be used as therapeutic agents either as an extract or as pure compounds. Due to the complex mechanisms of DN, use of synthetic drugs as nephroprotective agents is questionable because of their notable higher side effects. So for the protection of kidney alternatives are phytochemicals. Many reports have already proved the potential of these phytochemicals but many of them are still to be evaluated. Our study shows that esculetin and its similar compound are abundantly present in plants and have numerous health benefits as they possess many bioactivities. They have also shown to be a potent HDAC inhibitor-based on docking studies, compelling us to look further for their potency in vitro and in vivo. This is the study of its own kind that uses the tool molecular docking and structural similarity searching to identify their potential as anti-nephrotic drug. Once validated in

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vitro and in vivo can be helpful for pharmaceutical companies to develop a drug with no or least side effects and can increase the life of diabetic patients.

Conflict of interest

The authors state that no conflict of interest exists with the publication of this work.

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