

# Novel *In Silico* and *In Vivo* Insights of Flavonoids as Anti-Diabetic and Anti-Oxidant in Rodent Models

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## Mudunuri *et al.*: Flavonoids as Antidiabetic and Antioxidant

Insulin sensitivity is a symptom of diabetes, which is caused by insulin resistance, decreasing insulin production and ultimately pancreatic cancer. The current study is focused on the anti-diabetic and anti-oxidant properties of *Chrysanthemum indicum* flower extract and to scientifically validate the claim by *in silico* docking studies of natural compounds present in the extract for their respective sites against selected proteins. Pharmacological evaluations were done using 200 and 400 mg/kg body weight of the extract. Upon treatment with methanolic extract of *Chrysanthemum indicum* at 200 mg/kg body weight, 400 mg/kg body weight and glibenclamide 0.5 mg/kg body weight, significant reduction in blood glucose levels was observed. The extract was screened against reducing power assay and hydrogen peroxide scavenging assay for its anti-oxidant potential and the results were compared with the standard ascorbic acid. In both assays, the findings clearly demonstrated the extract's ability to scavenge free radicals. Docking studies were performed for natural compounds present in the extract such as epigallocatechin, quercetin, kaempferol, apigenin, myricetin, rutin, luteolin and standard drug glibenclamide against protein data bank ID: 1VA5, protein data bank ID: 1Z2C, protein data bank ID: 5WUA using Mcule software. The statistical verification of the model was evaluated with PROCHEK; a structure verification program relies on Ramachandran plot which determines the quality of the predicted structures. The results revealed that epigallocatechin and rutin had shown highest glide scores which indicates a stronger receptor-ligand binding affinity among the various phytochemical constituents present in the extract and this might be the possible mechanism by which the extract showed antidiabetic and antioxidant activities. From the above, it is concluded that the extract possess anti-diabetic and anti-oxidant activity.

**Key words:** Antidiabetic, Antioxidant, *Chrysanthemum indicum*, docking, glibenclamide

Insulin sensitivity as a result of insulin resistance, decreasing insulin production and subsequent pancreatic beta ( $\beta$ ) cell failure characterize Diabetes Mellitus (DM)<sup>[1]</sup>. Majority of people with type 2 diabetes are obese, with central visceral adiposity. It is a potentially morbid condition with high prevalence worldwide and currently global prevalence of the disease is around 200 million and would increase to 300 million by 2025. In spite of important developments in the management of DM with prescription medicines, the quest for natural antidiabetic plants as an alternate continues. Free radicals can play a part in the pathogenesis of DM and its complications, according to experimental evidence. As a result, plants that can neutralize free radicals can help to avoid diabetes and reduce the incidence of diabetic complications<sup>[2]</sup>. Phenolics and flavonoids can exert antioxidant activity through a variety of mechanisms, including quenching free radicals, chelating metal ions, and inhibiting enzymatic systems that generate free radicals<sup>[3]</sup>.

Glibenclamide is a sulphonylurea of the second generation that is used as a standard medication. It primarily works by inducing insulin release from pancreatic  $\beta$  cells in the islets of Langerhans and by increasing the sensitivity of peripheral tissues to insulin<sup>[4]</sup>. The flowers of *Chrysanthemum indicum* of the family Compositae is native to India, commonly called as chaamanthi in telugu. The whole plant is used as anti-inflammatory action, in oriental medicine, anti-diabetic, antihyperlipidemic, anti-oxidant, diabetic nephropathy<sup>[5-10]</sup>. The leaves are used for migraine as they are deparative and essential oil used for antimicrobial activity<sup>[11]</sup>. The various phytochemicals present in the flowers of *Chrysanthemum indicum* are flavonoids,

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phenolic compounds, steroids, triterpenoids, glycosides, carbohydrates, volatile oils and saponins.

## MATERIALS AND METHODS

### Plant collection and drying:

The florae of *Chrysanthemum indicum* were collected from Bachannapet village Jangaon district, Telangana in the month of January was identified and authenticated. The flowers were cleaned, reduced to small fragments, dried under shade for about 6 d and coarsely powdered in a mixer grinder. The powdered material was stored or taken up for extraction process.

### Preparations of methanolic flower extract *Chrysanthemum indicum*:

Soxhlet extraction is a continuous extraction method in which the same solvent is pumped repeatedly through the extractor. This procedure entails solvent extraction accompanied by evaporation. The solvent vapours are collected in a condenser and the dissolved liquid is returned to the substance for further extraction<sup>[12]</sup>. Large amounts of drug can be extracted with a much smaller quantity of solvent.

### Preliminary phytochemical analysis of the extract:

The extract was subjected to preliminary phytochemical screening to identify various phytoconstituents present in *Chrysanthemum indicum*.

### Acute toxicity testing:

The acute toxicity studies were carried out using Organization for Economic Co-operation and Development (OECD) 425 guidelines. Present study was carried out in Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) approved animal house of Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, India. (Registered No. 1175/PO/ERe/S/08/CPCSEA).

### Animal housing:

The Albino mice and Wistar Albino rats were held in poly acrylic cages with a maximum of six animals per cage and a 12 h light/12 h dark period. Animals have unrestricted access to a standard diet and unlimited water. The animals were given a week to acclimate to the laboratory environment before the experiment began. The care and maintenance of the animals were carried

out as per the approved guidelines of the CPCSEA.

## ANTI-DIABETIC ACTIVITY

### Dexamethasone induced diabetic rat model:

30 Wistar albino rats weighing 250–300 g of either sex were selected for the present study. These rats were divided into 5 groups of 6 animals in each group. Dexamethasone (10 mg/kg, body weight (bd.wt.) p.o) was used to induce diabetes. The Methanolic Extract of *Chrysanthemum indicum* (MECI) (200 mg/kg, p.o and 400 mg/kg, p.o) is used for anti-diabetic effect and compared with standard glibenclamide (0.5 mg/kg, p.o). Group-I as normal vehicle control received (saline). Group II as disease control group received dexamethasone (10 mg/kg bd.wt. p.o) for 10 d, Group-III and IV as test groups received MECI (200 mg/kg, p.o) and MECI (400 mg/kg, p.o) for 10 d along with dexamethasone (10 mg/kg bd.wt. p.o) and Group-V as standard group received dexamethasone (10 mg/kg bd.wt. p.o) for 10 d along with glibenclamide (0.5 mg/kg, p.o). The blood glucose levels in the treated groups were compared with that of diabetic control group<sup>[13]</sup>.

## ANTIOXIDANT ACTIVITY

### Reducing power assay:

The theory behind this approach is that a decrease in the absorbance of reaction mixtures implies an increase in antioxidant activity. Substances with reduction potential react with potassium Ferricyanide ( $\text{Fe}^{3+}$ ) to form potassium Ferrocyanide ( $\text{Fe}^{2+}$ ), which then interacts with ferric chloride to form ferric ferrous complex with a 700 nm absorption limit. 2.5 ml potassium  $\text{Fe}^{3+}$  (1 % w/v) and 2.5 ml phosphate buffer pH 6.6 were applied to 1 ml of test and normal compounds and incubated at 50° for 30 min. 2.5 ml distilled water and 0.5 ml ferric chloride solution (0.1 % w/v) were applied to 2.5 ml of the above supernatant solvent. The absorbance of ferric ferrous complex was measured at 700 nm using a ultraviolet-visible spectrophotometer with phosphate buffer pH 6.6 as a control and the increase in absorbance was calculated. The following equation was used to measure the percent (%) increase in reducing power.

Percentage increase in reducing power (%) =  $\frac{(\text{Abs control}) - (\text{Abs sample})}{(\text{Abs control})} \times 100$

Where, Abs (control): Absorbance of the control and Abs (sample): Absorbance of the extracts/standard<sup>[14]</sup>.

### Hydrogen peroxide radical scavenging assay:

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential Thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, hydrogen peroxide can probably react with Fe<sup>2+</sup>, and possibly Copper (Cu<sup>2+</sup>) ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. A solution of hydrogen peroxide (2 mmol/l) was prepared in phosphate buffer (pH 7.4) and test compounds (10-100 µg/M) were added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide and compared with ascorbic acid, the reference compound.

Percent Hydrogen peroxide activity=(Abs control)-(Abs sample)×100

(Abs control)

Where, Abs (control): Absorbance of the control; Abs (sample): Absorbance of the extract/standard<sup>[14]</sup>.

### Molecular docking by Mcule:

Molecular docking is the study of how two or more molecular structures (e.g., drug and enzyme or protein) fit together. In a simple definition, docking is a molecular modeling technique that is used to predict how a protein (enzyme) interacts with small molecules (ligands). The ability of a protein (enzyme) and nucleic acid to interact with small molecules to form a supra molecular complex plays a major role in the dynamics of the protein, which may enhance or inhibit its biological function<sup>[15]</sup>. To understand the interactions between the ligands and proteins and to explore their binding mode, docking studies were performed using Mcule software. In the present study the docking studies were performed for natural compounds such as epigallocatechin, quercetin, kampherol, apigenin, myrcetin, rutin, luteolin present in the extract and standard drug glibenclamide against selected proteins. The statistical verification of the model was evaluated with PROCHEK; a structure verification program relies on Ramachandran plot which determines the quality of the predicted structures.

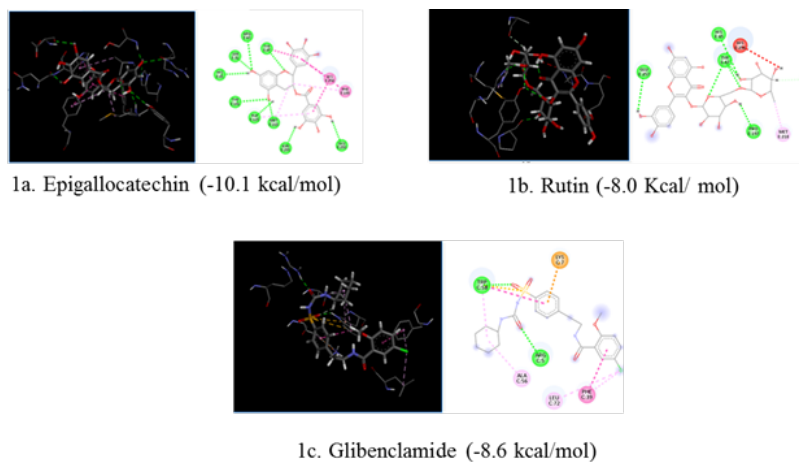
### Selection of proteins:

**Selection of Protein Data Bank (PDB) ID-1VA5:** Glucokinase (GK) is an enzyme that regulates glucose

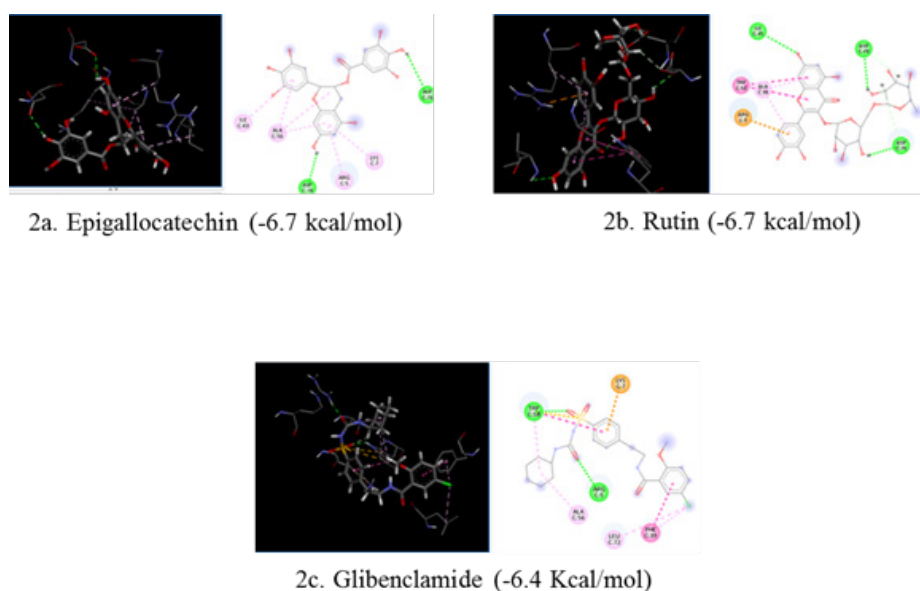
metabolism in the liver. Diabetes is related to the inactivation of GK, while hypoglycemia is linked to an increase in GK operation. The possibility of controlling GK activity with small chemical compounds acting as allosteric activators has piqued scientists' interest in studying the process of new allosteric GK activators are being developed<sup>[16]</sup>. The first phase in the complicated mechanism that regulates GK function is the interaction of GK with ligands. Molecular docking was used to investigate the interaction of the GK promoter (PDB ID: 1VA5) with natural compounds contained in the extract and the standard drug glibenclamide in this study (fig. 1).

**Selection of PDB ID-1Z2C:** When proteins are exposed to reducing sugars, they undergo nonenzymatic glycation, which results in Advanced Glycation End products (AGEs). Glycation causes enzymatic function loss, protein crosslinking and aggregation. The accumulation of AGEs plays an important role in many health disorders including DM, immunoinflammation, cardiovascular and neurodegenerative diseases. AGEs mediate their pathological effects by activating signaling cascades *via* the Receptor for Advanced Glycation End products (RAGE), a 45 kDa transmembrane receptor of the immunoglobulin superfamily prevalent at low concentrations in a variety of healthy human tissues, including the lungs, kidneys, liver, cardiovascular, nervous and immune systems<sup>[17]</sup>. As a receptor for AGE and other proinflammatory ligands, RAGE has been investigated as a potential biomarker of numerous pathological conditions. So in the present study RAGE inhibitor (PDB ID: 1Z2C) was selected and subjected to molecular docking (fig. 2).

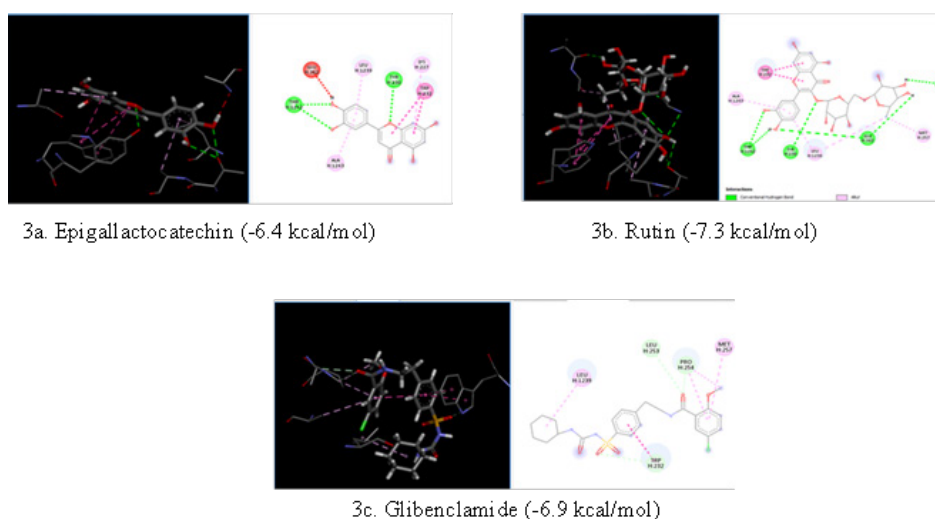
**Selection of PDB ID-5WUA:** The Adenosine Triphosphate (ATP)-sensitive K<sup>+</sup> channel (K<sub>ATP</sub> channel) in the pancreatic cell is critical for coupling membrane excitability with Glucose-Stimulated Insulin Secretion (GSIS)<sup>[18]</sup>. Increased glucose metabolism causes an increase in the intracellular [ATP]/[ Adenosine Diphosphate (ADP)] ratio, K<sub>ATP</sub> channel closure and membrane depolarization. Consequent activation of voltage-dependent Calcium (Ca<sup>2+</sup>) channels causes a rise in Ca<sup>2+</sup>, which stimulates insulin release. Conversely, a decrease in the metabolic signal is predicted to open K<sub>ATP</sub> channels and suppress the electrical trigger of insulin secretion. Sulfonylurea drugs promote and diazoxide suppresses, insulin secretion by binding to the regulatory Sulfonylurea Receptor-1 (SUR1) subunit and, inhibiting or activating K<sub>ATP</sub> channel current respectively<sup>[19]</sup>. So in the present study an ATP sensitive K<sup>+</sup> channel inhibitor



**Fig. 1:** Docking studies of isolated compounds against PDB: IVA5 with good scores, (a): Epigallocatechin (-10.1 kcal/mol); (b): Rutin (-8.0 kcal/mol) and (c): Glibenclamide (-8.6 kcal/mol)



**Fig. 2:** Docking studies of isolated compounds against PDB: 1Z2C with good scores, (a): Epigallocatechin (-6.7 kcal/mol); (b): Rutin (-6.7 kcal/mol) and (c): Glibenclamide (-6.4 kcal/mol)



**Fig. 3:** Docking studies of isolated compounds against PDB: 5WUA with good scores, (a): Epigallocatechin (-6.4 kcal/mol); (b): Rutin (-7.3 kcal/mol) and (c): Glibenclamide (-6.9 kcal/mol)

protein (PDB ID: 5WUA) is selected and subjected to molecular docking (fig. 3).

## RESULTS AND DISCUSSION:

MECI was screened for its anti-diabetic activity and antioxidant activity. All the results obtained in this study were included. The MECI was prepared by Soxhlation technique. The percent yield of the extract was calculated by using the following formula.

$$\text{Percent yield of extract} = \frac{\text{Amount of powder used (gm)}}{\text{Amount of extract obtained (gm)}} \times 100$$

$$= \frac{60}{500} \times 100$$

$$= 12 \% \text{ w/w}$$

MECI showed the presence of flavonoids, phenolic compounds, steroids, triterpenoids, glycosides, carbohydrates, proteins and volatile oils. The results are showed in Table 1.

Acute toxicity studies of MECI were performed as per OECD 425 guidelines. The oral administration of MECI did not exhibit any signs of toxicity and mortality even upto 2000 mg/kg. bd. wt. All animals were safe even after 14 d of observation.

The MECI was tested to determine its effects on blood glucose level in dexamethasone induced diabetic rat model.

Results obtained in dexamethasone induced diabetic rat model were tabulated in the Table 2. From the results, it is clear that the blood glucose levels of diabetic rats were elevated when compared to normal rats. Upon treatment with MECI at 200 mg/kg, bd. wt, 400 mg/kg; bd. wt. and glibenclamide 0.5 mg/kg, bd. wt. significant reduction in blood glucose levels was observed.

The MECI was subjected to *in vitro* antioxidant activity by reducing power assay and hydrogen peroxide scavenging assay.

The *in vitro* antioxidant activity was performed using reducing power assay. The half-maximal Inhibitory Concentration ( $IC_{50}$ ) value of the MECI was 36  $\mu\text{g/ml}$  and the standard drug ascorbic acid was 30  $\mu\text{g/ml}$  respectively. From the results it is clear that the MECI showed antioxidant activity. The results were expressed in Table 3.

The MECI has shown antioxidant activity in reduced power assay. The reducing ability of a compound generally depends on the presence of reductants which have been exhibiting antioxidative potential by breaking the free radical chain and donating a hydrogen atom.

The *in vitro* antioxidant activity was performed using hydrogen peroxide scavenging assay. The  $IC_{50}$  value of the MECI was 20  $\mu\text{g/ml}$  and ascorbic acid was 15  $\mu\text{g/ml}$  respectively. From the results it is clear that the MECI showed antioxidant activity. The results were expressed in Table 4. The MECI has shown antioxidant activity in hydrogen peroxide radical scavenging assay. The scavenging capacity of a compound may serve as a significant indicator of its potential antioxidant activity<sup>[14]</sup>.

Dexamethasone, a corticosteroid, is similar to a natural hormone produced by your adrenal glands. Glucocorticoids are known to oppose insulin action at the level of the liver by promoting gluconeogenesis and at the periphery, especially in muscle, by inhibiting glucose uptake and reducing glycogen synthesis and glucose storage. In addition the glucocorticoids are believed to attenuate insulin secretion, particularly the extent of the anticipated hyperinsulinaemic response to the insulin resistant state. Together these mechanisms are postulated to cause the development of glucose intolerance and diabetes observed in patients exposed to glucocorticoid therapy<sup>[14]</sup> (Table 5).

In the present study phytochemical screening has revealed the presence of flavonoids, phenolic compounds, steroids, triterpenoids, glycosides, carbohydrates, volatile oils as major chemical constituents. Flavonoids like epigallocatechin, quercetin, myricetin, luteolin, apigenin, kaempferol, rutin and vitex are majorly present in the extract as reported in Gas Chromatography–Mass Spectrometry (GC-MS) analysis<sup>[20]</sup>. Quercetin is 3,5,7,3',4'-pentahydroxyflavone or quercetin dihydrate ( $C_{15}H_{10}O_7$ ) is the most abundant flavonoid in human dietary nutrition. It is another epidrug present in citrus fruits and buckwheat. This drug acts as a DNA Methyltransferase 1 (DNMT1) inhibitor (*via* the repression of Tumor Necrosis Factor (TNF) induced Nuclear Factor-kappa B (NF- $\kappa$ B) transcription factor) and promotes Fas ligand-related apoptosis *via* histone H3 acetylation and potential Histone Deacetylase Inhibitors (HDACi). Quercetin was shown to be involved in the stimulation of glucose uptake through MAPK insulin-dependent mechanism. This is accomplished in the muscle *via* the translocation of Glucose Transporter-4 (GLUT4) transporters and in the liver *via* the downregulation of key gluconeogenesis enzymes. Treating Streptozotocin (STZ) induced diabetic rats with quercetin decreases the activity of GK, hyperglycemia stimulating GLUT4, hepatic gluconeogenesis and glycogenolysis while it increases glucose liver uptake<sup>[21]</sup>. Quercetin

supplementation for 2 w lowered the blood glucose level, upregulated the expression of genes involved in cell survival and proliferation in a liver, and enhanced the serum insulin in STZ induced diabetic mice<sup>[22]</sup>. Quercetin blocks the activities of a tyrosine kinase inhibitor, which has shown an effect against diabetes. The regulatory effect of quercetin to NF- $\kappa$ B also helps in improving glucose stimulated insulin secretion<sup>[23]</sup>. The co-treatment of quercetin and sitagliptin (a selective dipeptidyl peptidase-IV inhibitor) demonstrated an improvement in its oxidative and inflammatory status, metabolic profile, glycemic control,  $\beta$  cells function and islet structure in STZ induced DM in rats<sup>[24]</sup>. Quercetin intake is inversely associated with the prevalence of Type 2 DM (T2DM)

**TABLE 1: PRELIMINARY PHYTOCHEMICAL ANALYSIS**

Phytochemical constituents	Results
Flavonoids	++
Phenolic compounds	++
Steroids	++
Triterpenoids	+
Glycosides	+
Carbohydrates	+
Volatile oils	+
Saponins	-

Note: (+): Present and (-): Absent

**TABLE 2: ANTI-DIABETIC ACTIVITY OF MECI ON DEXAMETHASONE INDUCED DIABETIC RATS**

Treatment	Blood glucose level (gm/dl)		
	0 <sup>th</sup> d	5 <sup>th</sup> d	10 <sup>th</sup> d
Normal control	84.5 $\pm$ 1.124	92.33 $\pm$ 0.565	90.167 $\pm$ 0.723
Negative control (dexamethasone 10 mg/kg)	154.5 $\pm$ 0.964 <sup>a,**</sup>	162.5 $\pm$ 0.5652 <sup>a,**</sup>	234.5 $\pm$ 0.905 <sup>a,**</sup>
MECI (200 mg/kg)	152.62 $\pm$ 0.451 <sup>a,**</sup>	144.5 $\pm$ 0.807 <sup>A,a,**</sup>	125.83 $\pm$ 0.796 <sup>A,a,**</sup>
MECI (400 mg/kg)	151.67 $\pm$ 0.4513 <sup>a,B,**</sup>	137.17 $\pm$ 0.280 <sup>A,a,**</sup>	104.33 $\pm$ 0.652 <sup>A,a,**</sup>
Glibenclamide (0.5 mg/kg)	152.5 $\pm$ 0.656 <sup>A,**</sup>	107.67 $\pm$ 0.693 <sup>A,**</sup>	94.5 $\pm$ 0.697 <sup>A,**</sup>

Note: Values are expressed as mean $\pm$ standard error of mean, (n=6). Statistical analysis was performed by using analysis of variance followed by Dunnett's test. Results were compared with control group (\*\*p<0.01), diabetic control (<sup>A</sup>p<0.01, <sup>B</sup>p<0.05) and standard (<sup>a</sup>p<0.01)

**TABLE 3: ANTI-OXIDANT ACTIVITY OF MECI ON REDUCING POWER ASSAY METHOD**

S. No	Compound	Concentration ( $\mu$ g/ml)	% Inhibition	IC <sub>50</sub> value ( $\mu$ g/ml)
I	MECI	10	10.79 $\pm$ 1.352	36
		20	31.80 $\pm$ 0.637	
		30	45.39 $\pm$ 0.202	
		40	56.55 $\pm$ 0.129	
		50	68.97 $\pm$ 0.066	
		10	18.46 $\pm$ 1.576	
II	Ascorbic acid	20	35.59 $\pm$ 0.562	30
		30	54.37 $\pm$ 0.157	
		40	66.78 $\pm$ 0.330	
			72.71 $\pm$ 0.348	

Note: Values are expressed as mean $\pm$ standard error of mean

**TABLE 4: ANTI-OXIDANT ACTIVITY OF MECI ON HYDROGEN PEROXIDE RADICAL SCAVENGING ACTIVITY**

S. No	Compound	Concentration ( $\mu\text{g/ml}$ )	% Inhibition	IC <sub>50</sub> value ( $\mu\text{g/ml}$ )
I	MECI	10	40.23 $\pm$ 0.340	20
		20	48.23 $\pm$ 0.388	
		30	56.49 $\pm$ 0.173	
		40	63.77 $\pm$ 0.129	
		50	74.60 $\pm$ 0.07	
II	Ascorbic acid	10	46.14 $\pm$ 0.421	15
		20	53.61 $\pm$ 0.183	
		30	64.14 $\pm$ 0.461	
		40	73.31 $\pm$ 0.490	
		50	77.06 $\pm$ 0.117	

**TABLE 5: GLIDE SCORE FOR DIFFERENT NATURAL COMPONENTS AND GLIBENCLAMIDE**

Components	Glide score (Kcal/mol)		
	PDB ID: 1VA5	PDB ID: 1Z2C	PDB ID: 5WUA
Glibenclamide	-8.6	-6.4	-6.9
Epigallocatechin	-10.1	-6.7	-6.4
Kaempferol	-7	-6	-6.2
Rutin	-8	-6.7	-7.3
Apigenin	-7.4	-6.3	-6.3
Quercetin	-7.6	-6	-6.2
Myricetin	-7.5	-6	-6.4
Luteolin	-7.7	-6.1	-6.3

in the Chinese population which suggests its preventive activity against T2DM.

Rutin is also known as glycosylated quercetin, sophorin and quercetin-3-O-rutinoside. The anti-diabetic effects of rutin includes the reduction of carbohydrates absorption from the small intestine, the improvement of glucose uptake by tissues, the suppression of tissue gluconeogenesis, the activation of insulin secretion from  $\beta$ -cells and the protection of the islets of Langerhans from degenerative changes. Rutin also lowers the formation of reactive oxygen species, advanced glycation end-product precursors, sorbitol and pro-inflammatory cytokines<sup>[25]</sup>. The oral or Intraperitoneal administration of rutin (50 mg/kg, bd.wt or 100 mg/kg, bd. wt) into a STZ model of type 1 diabetic rats significantly decreased glycated hemoglobin/A Hemoglobin A1c (HbA1c) and Fasting Blood Glucose (FBG)<sup>[26]</sup>.

Kaempferol (3,4',5,7-tetrahydroxyflavone) is a natural flavonol, found in a variety of plants and plant-derived foods, has several antidiabetic effects, like improving Adenosine Monophosphate (AMP) activated cellular protein expression and activation, reducing cellular apoptosis by suppressing caspase 3 activities, and increasing the production and secretion of insulin from  $\beta$ -cells. The extracts of kaempferol from *Bauhinia forficata* leaves reduce hyperglycemia and enhance

glucose uptake, mimicking the action of insulin<sup>[27]</sup>. *In vitro* studies confirmed that treating with 10  $\mu\text{M}$  of kaempferol enhances cellular viability and represses apoptosis. Apigenin is a trihydroxyflavone that is flavone substituted by hydroxy groups at positions 4', 5 and 7. The administration of apigenin (4 mg/kg, bd. wt) in STZ induced diabetic rats, showed a significant anti-hyperglycemic effect<sup>[28]</sup>. Apigenin treatment could prevent induced apoptosis through the inhibition of NF- $\kappa\text{B}$  activation. In alloxan induced insulin dependent diabetic mice, the oral administration of apigenin for 10 d reduced hepatic antioxidants, like catalase, glutathione and superoxide dismutase. The treatment with apigenin helps in reducing hyperglycemia, serum cholesterol and Glucose-6-Phosphatase (G6Pase) activities in the liver<sup>[29]</sup>. Luteolin 3',4',5,7-tetrahydroxyflavone, is a common flavonoid and was reported to initiate insulin action and to enhance the expression of Peroxisome Proliferator-Activated Receptor gamma (PPAR- $\gamma$ ) target genes in primary mouse adipose cells. In damaged pancreatic cells,  $\beta$  cells, this flavonoid improves insulin secretion in uric acid by decreasing Micro-Autologous Fat Transplantation (MAFT), a transactivator of insulin gene through NF- $\kappa\text{B}$  signaling pathway<sup>[30]</sup>.

Myricetin is a hexahydroxyflavone that is flavone substituted by hydroxy groups at positions 3, 3', 4', 5, 5' and 7. It has been isolated from the leaves of *Myrica*

*rubra* and other plants. It has a role as a cyclooxygenase 1 inhibitor, an antineoplastic agent, an antioxidant, a plant metabolite, a food component and a hypoglycemic agent. Myricetin has been observed to increase the activity of glycogen synthase 1 in the hepatocytes of rats with diabetes<sup>[31]</sup>. Recent evidence suggests that myricetin administration might be beneficial for increasing insulin sensitivity and inhibiting islet  $\beta$ -cell apoptosis<sup>[32]</sup>. Epigallocatechin Gallate (EGCG), also known as epigallocatechin-3-gallate, is the ester of epigallocatechin and gallic acid, and is a type of catechin. Hepatic glucose production was regulated by epigallocatechin through Phosphoinositide 3-Kinase (PI3K) dependent manner<sup>[33]</sup>. L-EGCG could markedly inhibit the activity of  $\alpha$ -glucosidase *in vitro* as well as *in vivo* and therefore exerted its significant effect on postprandial hyperglycemia improvement. Glibenclamide acts to increase secretion of insulin from the pancreas, probably by interacting with SURs on  $\beta$  cells or by interfering with ATP-sensitive potassium channels on pancreatic  $\beta$  cells, which increases secretion of insulin. These drugs also may increase sensitivity of existing insulin receptors.

The results have indicated that MECI at 200 mg/kg, bd. wt. and 400 mg/kg, bd. wt. significantly reduced the elevated FBG levels in diabetic animals. The possible mechanism by which MECI reduced the elevated blood glucose levels might be due to the presence of flavonoids and phenolics. These phytochemical constituents might be increasing either the pancreatic secretion of insulin from  $\beta$  cells of islets of Langerhans or increased peripheral utilization of glucose<sup>[34]</sup>. Like the standard drug glibenclamide, the flavonols like kaempferol, rutin and quercetin were able to reduce blood glucose levels by acting as insulin secretagogue. The presence of these flavonols in the extract might have worked synergistically in the reduction of blood glucose levels.

Free radical scavenging action is considered to be one among the various mechanisms for antioxidation. The MECI has shown antioxidant activity in reducing power assay and hydrogen peroxide radical scavenging assay.

Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium  $\text{Fe}^{3+}$  to form potassium  $\text{Fe}^{2+}$ , which then reacts with ferric chloride to form ferric-ferrous complex that has an absorption maximum at 700 nm.

The reducing capacity of a compound can be known by measuring  $\text{Fe}^{3+}$ - $\text{Fe}^{2+}$  complex due to electron donating activity of MECI, which is an important mechanism of antioxidant action and this might be due to the presence of flavonoids and phenols in the extract with adequate

number of hydroxyl groups<sup>[35]</sup>.

Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly and cross cell membranes rapidly; once inside the cell, it can probably react with  $\text{Fe}^{2+}$  and possibly  $\text{Cu}^{2+}$  ions to form hydroxyl radicals and this may be the origin of many of its toxic effects. The scavenging capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The MECI have shown reducing capacity in reducing power, assay hydrogen peroxide and good scavenging ability which was comparable to ascorbic acid. It might be due to the presence of flavonoids and phenols in the extract with adequate number of hydroxyl groups as reported<sup>[36]</sup>.

The application of antioxidants especially of natural origin is one of the strategies in treating DM<sup>[37]</sup>. Triterpenes play an important role as plant antioxidants. Ursolic acid exhibited hydroxyl radical scavenging activity, perhaps through its hydrogen donating ability. Some triterpene compounds possess the ability to suppress formation of AGEs and are promising agents in the prevention and treatment of DM.

MECI have shown reducing capacity in reducing power assay and also scavenged the toxic hydroxyl free radical produced from hydrogen peroxide. And this may be used as an indicator of the potential anti-oxidant activity.

Molecular docking continues to hold great promise in the field of computer based drug design which screens small molecules by orienting and scoring them in the binding site of a protein. The docking analysis of isolated compounds from MECI and standard drug like glibenclamide were carried out using Molecule database. The various constituents identified in the plant extract are epigallocatechin, quercetin, kaempferol, apigenin, myricetin, rutin, luteolin and standard drug glibenclamide were subjected to docking against PDB ID: 1VA5, PDB ID: 1Z2C and PDB ID: 5WUA. The proteins identified namely PDB ID: 1VA5, PDB ID: 1Z2C and PDB ID: 5WUA are modeled and the qualities of the 3D model were evaluated using the PROCHECK program and assessed using the Ramachandran plot (fig. 4). It is evident from the Ramachandran plot that predicted models have most favorable regions, additionally allowed regions, generally allowed regions and disallowed regions. Such a percentage distribution of the protein residues determined by Ramachandran plot shows that the predicted models are of good quality. According to Ramachandran plot, a good quality model would be expected to have over 90 % in most favored regions. So in the present study also, all the proteins models selected showed more than 90 %



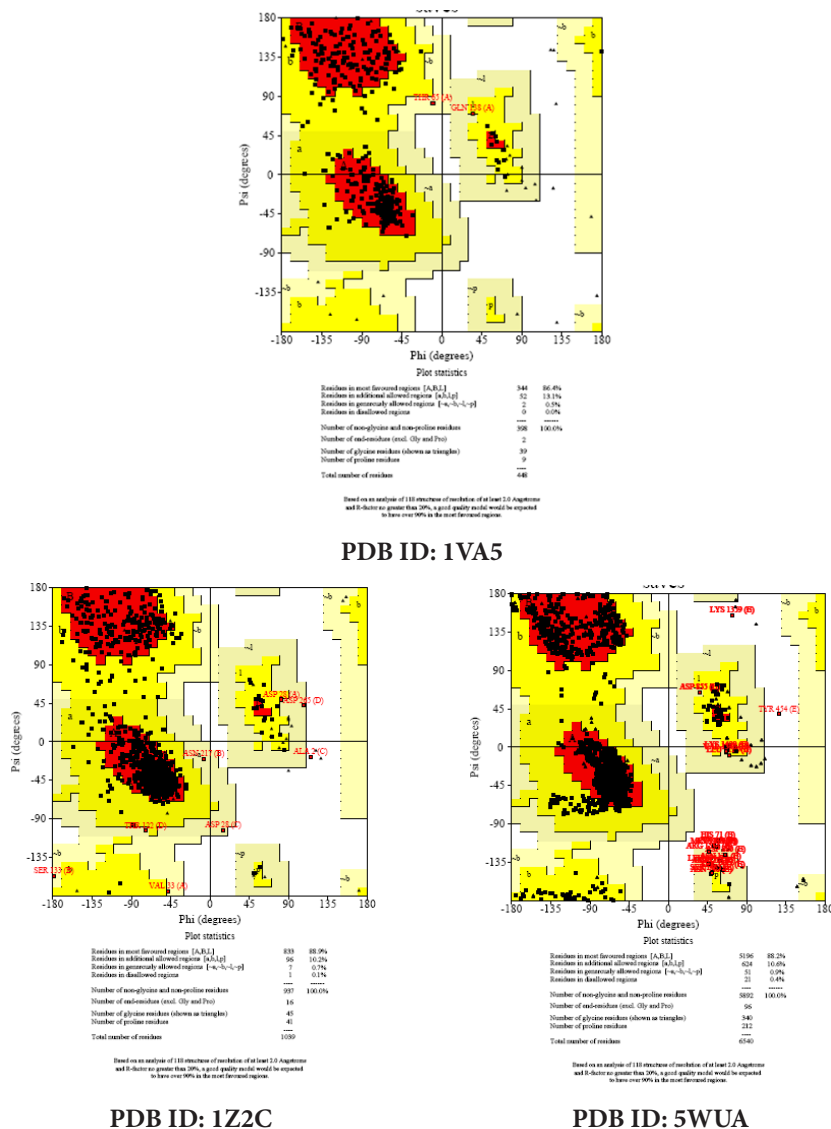


Fig. 4: The PDB ID selected for docking are validated using Ramchandran plot

most favored regions clearly indicating that these models are of good quality.

The results revealed that epigallocatechin and rutin had shown highest glide scores among the various phytochemical constituents present in the extract. The highest glide scores were observed with epigallocatechin, glibenclamide and rutin against PDB ID: 1VA5 and PDB ID: 1Z2C whereas the rutin, epigallocatechin and glibenclamide against PDB ID: 5WUA. The glide scores of the epigallocatechin and rutin were found to be similar with the glide score of standard drug glibenclamide stating that the compounds have same affinity to bind to the proteins. These results clearly indicate that the chemical constituents' epigallocatechin and rutin might have shown similar mechanism to that of the standard drug glibenclamide in reducing diabetes. The MECI possess anti-diabetic and anti-oxidant activity in rodent models.

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## Conflict of interests:

All authors have no conflicts of interest to declare.

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