Original Research Article

IN VITRO AND *IN SILICO* INHIBITORY ACTION OF METHANOL EXTRACT OF *RHYNCHOSIA BEDDOMEI* WHOLE PLANT ON *α*-GLUCOSIDASE AND *α*-AMYLASE

Abstract

The purpose of the current investigation was to explore the possible mechanisms through which *Rhynchosia beddomei* may be beneficial in managing diabetes and its associated complications by *in vitro* methods and to predict the potential bioactive constituent/s responsible for its anti-diabetic activity through *in silico* docking study. Soxhlet extraction resulted in extractive yield of 35.43% and *in vitro* anti-diabetic assays revealed that anti-hyperglycaemic activity of this plant can be attributed to its high efficiency to inhibit α-amylase (62.13%) and α-glucosidase (59.9%) enzymatic activity, which are well established targets for the management of diabetes. Further, through docking studies we predicted that flavonoids like rutin, lucenin, orientin, rhynchosin and isooreintin present in this plant might be responsible for the anti-diabetic properties. These results provide a scientific justification for the traditional anti-diabetic use of this plant. It may control diabetes through lowering dietary glucose uptake and may benefit in progression of diabetic complications. Predicted anti-diabetic molecules need to be screened further for the management of hyperglycemia.

Keywords: *Rhynchosia beddomei*, Anti-diabetic, Docking, α-glucosidase, α-amylase

1. INTRODUCTION

Diabetes Mellitus (DM) is a complex metabolic disorder which is characterized by the persistent hyperglycaemic state. Despite rapid advancement in the diabetic therapeutic, prevalence of DM is increasing at an alarming rate and is expected to double by the year 2040 [1]. DM is generally associated with several complications that arise secondary to diabetes affecting eyes, kidneys, hearts and nerves such as neuropathy, nephropathy, retinopathy, cardiovascular complications *etc* [2]. Available anti-diabetic drugs are focused on eliminating excessive glucose from the blood and have negligible effect on the mechanisms which leads to the development of various complications, besides several side effect and necessity to take lifetime medication is there. The majority of patients are overweight or obese at diagnosis and will be unable to achieve or sustain near normoglycaemia without oral antidiabetic agents; a sizeable proportion of patients will eventually require insulin therapy to maintain long-term glycaemic control, either as mono therapy or in conjunction with oral antidiabetic therapy [3].

Therefore an efficient therapeutic strategy for diabetes would be to supplement anti-diabetic drugs with certain additives which interfere with pathways leading to various complications. Human civilization has exploited natural resources for the treatment and prevention of several ailments since time immemorial. In recent time focus of the research has been inclined towards traditional medicinal plants and significant amount of evidence has been accumulated which scientifically justifies traditionally use of these plants. Considerably small amount of work have been focused on its effect on diabetic complications and none of the precious studies depicts possible mechanisms through which it may prove beneficial in countering diabetic complications [4]. Therefore, aim of the present study was to gain preliminary in site into the mechanism through which it can aid in controlling diabetes and its complications through *in vitro* and *in silico* tools. On this ground we evaluated the potential of methanolic extract of *R. beddomei* to neutralize oxidative stress which plays decisive role in the development and progression of diabetic complications. Further, potential antidiabetic mechanism of this plant was experimentally screened through *in vitro* assays and bioactive anti-diabetic constituents which may be responsible for anti-diabetic property were predicted from *in silico* docking studies.

2. MATERIALS AND METHODS

All the chemicals used in the present study were procured from Sigma-Aldrich, Loba Chemie, Merck, Sdfine-Chem, Himedia and Spectrochem.

2.1 Plant collection & drying:

Rhynchosia beddomei whole plant procured, prepared and referred for certification which were verified by Dr. K. Madhava Chetty, botanist, S.V University, Tirupati. Whole plant, cleaned under running water to remove debris and dried in shade. The dried plant material is then made into a coarse powder and was subjected to further step.

2.2 Preparation of plant extract

The powdered plant material was successively extracted in 500 ml of methanol using Soxhlet extraction and plant material was suspended in the round bottomed flask containing extraction solvent. This was then equipped by a condenser and flask was then heated; active constituents of extract get into the fluid. The finale of the extraction process source was filtered. The excess was vaporized and extracts were then kept in desiccators to remove remaining moisture, if extant, and finally stored in air tight ampoules at 4°C until used [5].

2.3 Preliminary phytochemical screening

Preliminary phytochemical screening of the methanolic whole plant extract of *R. beddomei* (MERB) was qualitatively tested for the presence of phytochemical constituents such as alkaloids, flavonoids, terpenoids, phenols, tannins etc., [6].

2.4 *In vitro* anti-diabetic activity

2.4.1 α-amylase inhibitory activity

In a test tube containing 0.2 mL of 0.5 M Tris-HCl buffer (pH 6.9) and 0.01 M calcium chloride (substrate), starch (2 mg) was added. Then the test tube was boiled for 5 min and pre-incubated at 37 ℃ for 5 min. One mL of 0.1% of gum acacia was used to dissolve 1 mg of dried MERB extract. 0.2 ml of MERB extract were added in the tube containing the substrate solution followed by 0.1 mL of porcine pancreatic amylase in Tris-HCl buffer (2 units/mL) was added and process was carried out at 37 ℃ for 10 min. The reaction was terminated by the addition of 0.5 mL of 50% acetic acid in each tube and the reaction mixture was centrifuged at 3000 rpm for 5 min at 4 °C. The absorbance was measured at 595 nm using UV spectrophotometer [7].

2.4.2 α-glucosidase inhibitory activity

α-glucosidase inhibitory activity for MESA was determined by incubating 0.1 mL of an enzyme solution and p-nitropheynyl glucopyranoside (pNPG) (substrate) with 0.2 M Tris buffer, pH 8.0 (1.0 mL) containing different concentrations of MERB extract at 37ºC for 60 min. The reaction mixture was boiled for two min in water bath to stop the reaction and amount of liberated glucose was measured by glucose oxidation method. (Assay condition 3 ºC±0.1 ºC, pH-8.0; optical density at 540 nm). Same protocol was repeated thrice [8].

2.5 *Insilico* analysis

2.5.1 Molecular Docking Studies

Molecular docking is an attractive scaffold to understand drug bimolecular interactions for the rational drug design and discovery. Molecular docking generates different possible adduct structures that are ranked and grouped together using scoring function in the software. The main objective of molecular docking is to attain ligand-receptor complex with optimized conformation and with the intention of possessing less binding free energy [9]. Molecular docking performed with mCule online tool.

2.5.2 Structure based drug design

Initially the protein downloaded in PDB format was prepared in discovery studio by generating attributes of sphere. Water molecules present in both the chains are removed. Later molecules are drawn using chemdraw/Chemsketch in mol format. Protein and ligand were docked against proteins like 1B2Y, 3TOP and 3RX2. Docking indicates that some of our compounds, docked with protein α amylase inhibitor (PDB ID: 1B2Y), α glucosidase inhibitor (PDB ID: 3TOP) and Aldose reductase (PDB ID: 3RX2).

2.5.3 Docking results visualization

The resulting docking poses were visualized through discovery studio. The best docked structures were chosen using glide score function. The more negative the glide score the more favourable the binding. Additionally, the docked ligand poses were visualized and the different ligand receptor interactions were studied [10].

2.5.4 Ramachandran plot

The docked proteins of α amylase inhibitor (PDB ID: 1B2Y), α glucosidase inhibitor (PDB ID: 3TOP) and Aldose reductase (PDB ID: 3RX2) were validated and evaluated by using

procheck by calculating the Ramachandran plot to access the quality of the model by looking into the allowed and disallowed regions of the plot [11]. The Ramachandran plot shows the phi-psi torsion angles for all residues in the ensemble (except those at the chain termini). The colouring/shading on the plot represents the different regions described in [12]. The darkest areas (here shown in *red*) correspond to the "core" regions representing the most favourable combinations of phi-psi values.

2.5.5 ADME analysis

Molinspiration (Calculation of Molecular Properties and Bioactivity Scores)

Lipinski's rule of five was used to evaluate the drug-likeness and calculate the molecular properties that are essential factors for a drug pharmacokinetics, including ADME (absorption, distribution, metabolism and excretion). Molinspiration website-based software (www.molinspiration.com) was employed to obtain certain molecular parameters. The values of miLogP, as (octanol/water partition coefficient) and TPSA of the investigated cytosolic PLA_2 were determined using the method developed by molinspiration. Drug-likeness scores were calculated to represent the amount of fragments based on contributions and correction factors [13]

The bioactivity score of selected agents were also evaluated using the tool Molinspiration Chem informatics server (http://www.molinspiration.com). In this computational chemistry technique large chemical databases are analysed in order to identify possible new drug candidates [15]. The prediction of bioactivity scores of these compounds of MERB were calculated by recording the activity scores of GPCR (G-protein coupled receptors ligand), KI (kinase inhibitor), PI (protease inhibitor), EI (enzyme inhibitor), ICM (ion channel modulator) and NRL (nuclear receptor ligand) [13].

3. RESULTS AND DISCUSSION

3.1 Preparation of *Rhychosia beddomei m*ethanolic extract

The methanolic extract of *Rhychosia beddomei* was prepared by soxhlation technique. The percentage yield of the extract was calculated by using the following formula.

$$\% \text{ yield of extract} = \frac{\text{Amount of extract obtained (grams)}}{\text{Amount of the powder used (grams)}} \times 100$$

$$= 35.43\%. \quad w/w.$$

3.2 Preliminary phytochemical screening

Crude extract was then subjected to preliminary phytochemical screening of *Rhynchosia beddomei* showed the presence of alkaloids, glycosides, steroids, flavonoids, carbohydrates, proteins and tannins.

3.3 *In vitro* anti-diabetic activity

3.3.1 α- Amylase inhibition assay

α-Amylase is an enzyme found in the salivary, intestinal mucosal and pancreatic secretions, functioning in the breakdown of the α-1-4-glycosidic bonds in starch. Thus, this enzyme increases the bioavailability of glucose in the blood. For a drug to be antidiabetic, it should be able to reduce the amount of glucose in the blood or inhibition of α-amylase. The data of percent inhibition and IC_{50} of methanolic extracts of *Rhynchosia beddomei* are represented in Table 1, indicated that the % inhibition is increased with increase in dose (for plant extracts), but the responses are not linear for the three dose levels. Further, the inhibition data are processed for relative inhibition compared to standard. The higher the relative inhibition, the greater is α- amylase inhibition assay.

Table 1: α-Amylase inhibitory activity of MERB

Test extract/ Standard	Dose (µg/mL)	Percent inhibition, AM±SEM (n=3)	% Relative inhibition	IC_{50} (µg/mL)
Methanolic Extract of *Rhynchosia beddomei*	50	28.90 ±0.0236	39.13	190
	100	45.04 ±0.0852	22.25	
	200	58.68 ±0.2967	18.86	
Acarbose	10	14.77 ± 0.0529	100	29.83
	25	50.59 ± 0.0688	100	
	50	77.78 ±0.1386	100	

The standard used is acarbose. As expected, acarbose produced inhibition at lower dose levels (10-50 µg/mL).

3.3.2 α- Glucosidase inhibition assay

The inhibition of α-glucosidase delay carbohydrate digestion caused a decrease in the rate of glucose absorption and consequently reducing the postprandial plasma glucose rise

[14]. α-Glucosidase inhibition is one of the beneficial methodologies for reducing postprandial hyperglycemia [8]. The IC_{50} values of standard and test extract are compared. In other words, the plant extract have nearly one-fourth activity of acarbose. At higher doses, the relative % inhibition is decreased; suggesting that complete dose is not utilized for eliciting the response. The % inhibition is augmented as the dose of standard is amplified.

Table 2: α-Glucosidase inhibitory activity of MERB

Test extract	Dose (µg/mL)	Percent inhibition, AM±SEM (n=3)	Relative inhibition	IC_{50} (µg/mL)
Methanolic extract of *Rhynchosia beddomei*	10	16.14± 0.0606	34.10	33
	25	44.71 ± 0.2810	25.65	
	50	71.84 ±0.4081	22.78	
Acarbose	2.5	11.83 ± 0.05292	100	9.22
	5	34.85 ± 0.0688	100	
	10	63.06 ±0.1386	100	

3.4 *Insilico* analysis

3.4.1 Molecular docking studies

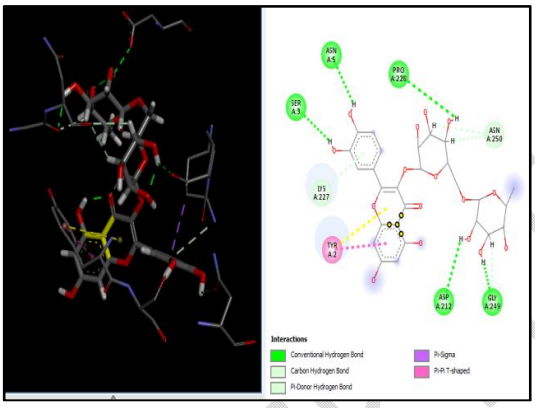
Table 3: Docking scores of compounds isolated from *Rhynchosia beddomei*

Compound	1B2Y	3TOP	3RX2
Rutin	-7.7	-8.1	-8.3
Rhynchosin	-6.2	-6.1	-10.0
Orientin	-7.2	-7.2	-8.1
Isoorientin	-6.8	-7.5	5.9
5,7 dihydroxy-4-methyoxy isoflavone	-6.1	-6.3	8.7
Quercetin	-6.2	-6.3	-9.4
Vitexin	-7.1	-7.3	-8.3

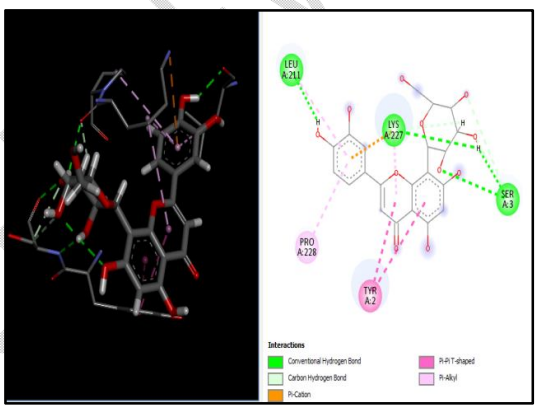
Isovitexin	-6.2	-7.0	-7.5
5,7,3,4 tetra hydroxy 6-C-β-D-glucopyronsyl flavone	-7.2	-7.8	-7.2
Apigenin	-6.4	-6.3	-8.7
Vicenin	-6.7	-6.1	-8.4
Lucenin	-6.1	-7.0	-6.3
Biochanin	-6.3	-6.1	-9.9
D-Pinitol	-4.4	-4.2	-6.0
D- inositol	-5.0	-4.3	-5.7
Acarbose	-5.6	-6.6	-3.9

G score = glide score, The more negative the Glide score, the more favourable the binding.

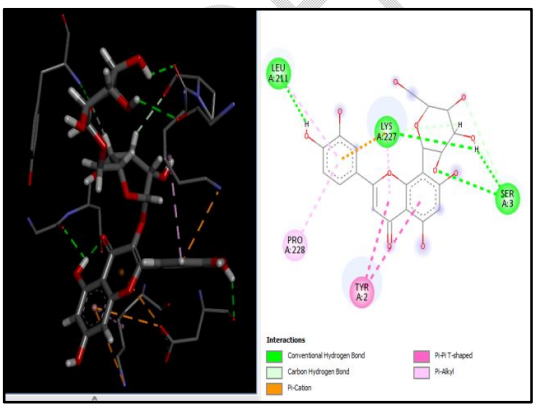
PDB ID: 1B2Y



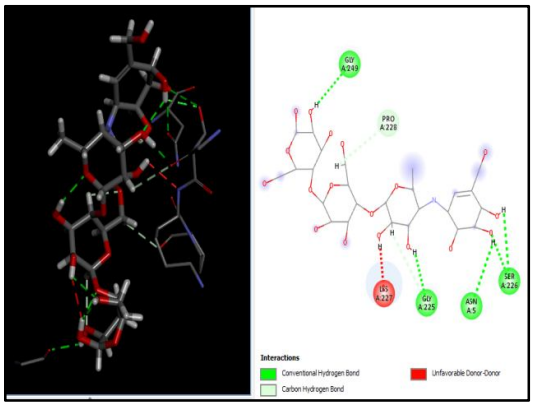
a) Rutin -7.7



b) Orientin -7.2

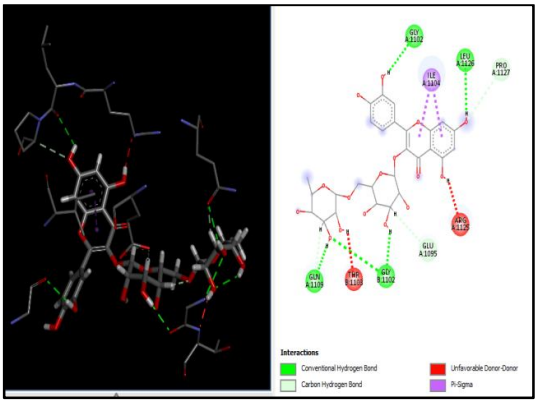


c) 5,7,3,4 tetrahydroxy 6-C-β-D glucopyransyl flavone -7.2

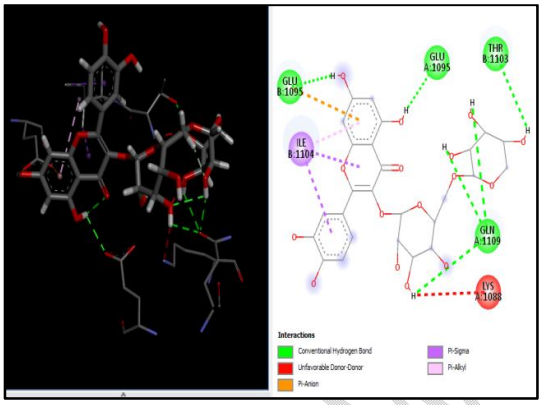


d) Acarbose -5.6

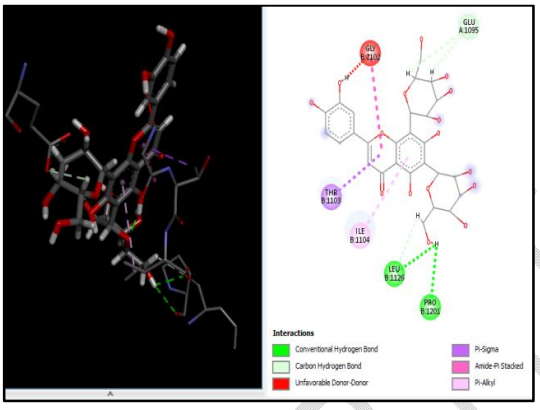
PDB ID: 3TOP



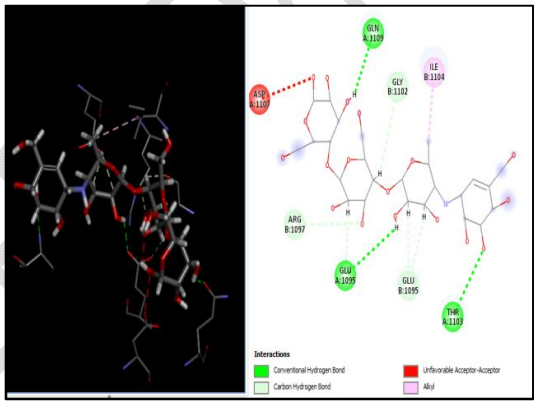
a) Rutin -8.1



b) 5,7,3,4 tetrahydroxy 6-C-β-D glucopyransyl flavone -7.8

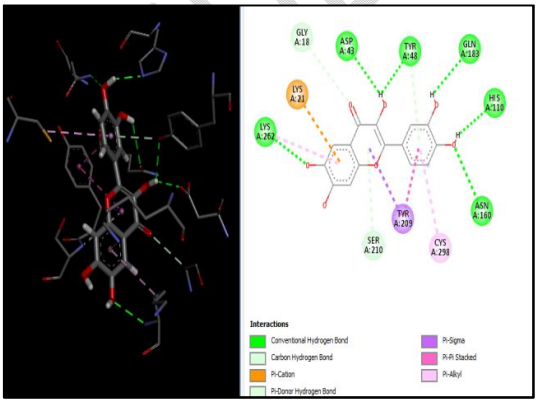


c) Iso orientin -7.5

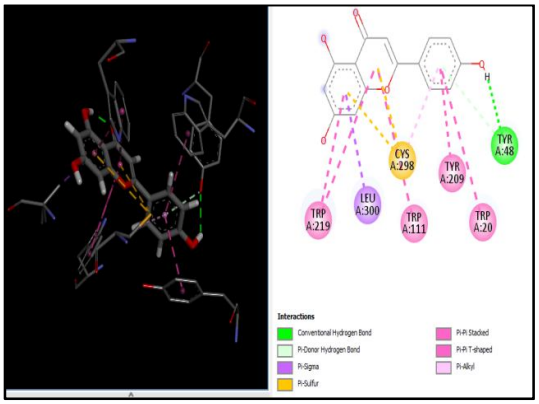


d) Acarbose -6.6

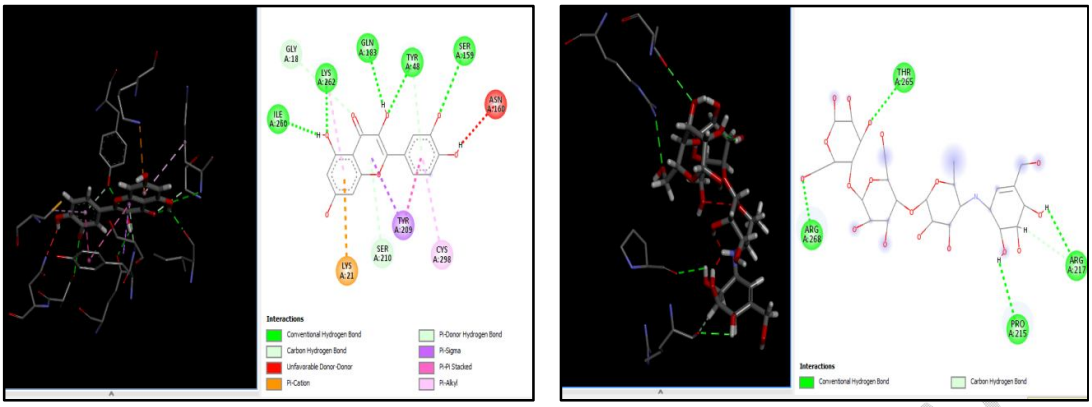
PDB ID: 3RX2



a) Rhynchosin -7.7



b)Apigenin -8.7



c) Quercetin -9.4 **d) Acarbose -3.9**

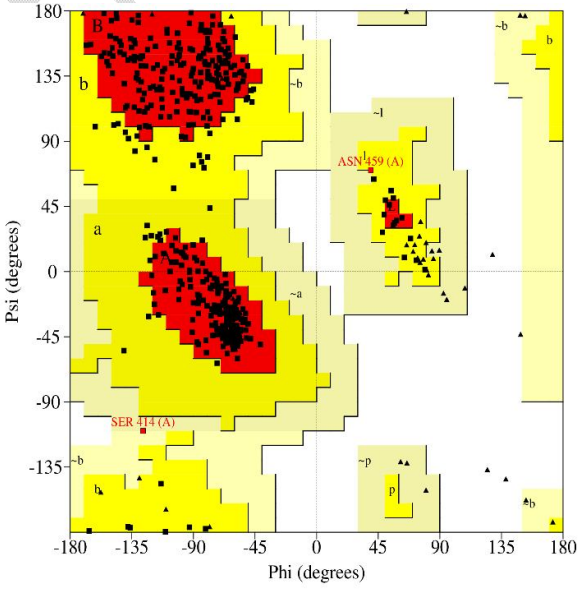
Figure 1: Compounds showing interactions with the binding site (PDB ID)

3.4.2 Ramachandran plot Analysis

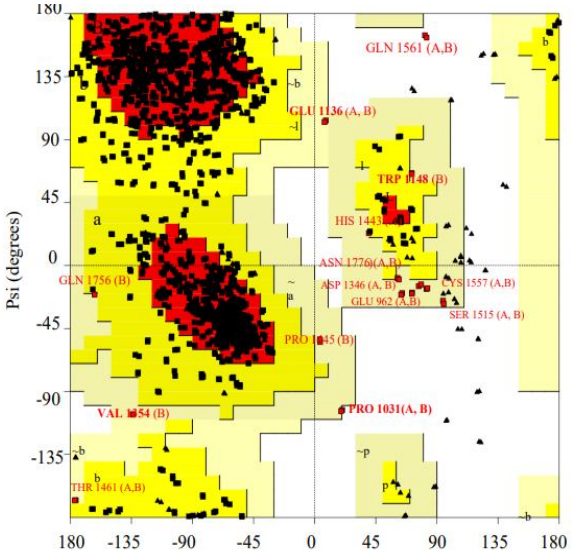
Protein were analysed for Ramachandran plot to know amino acid presence in different regions of respective protein tabulated in table 4 and pictorial representation by figure below.

Table 4: Ramachandran plot status with protein with 1B2Y, 3TOP and 3RX2

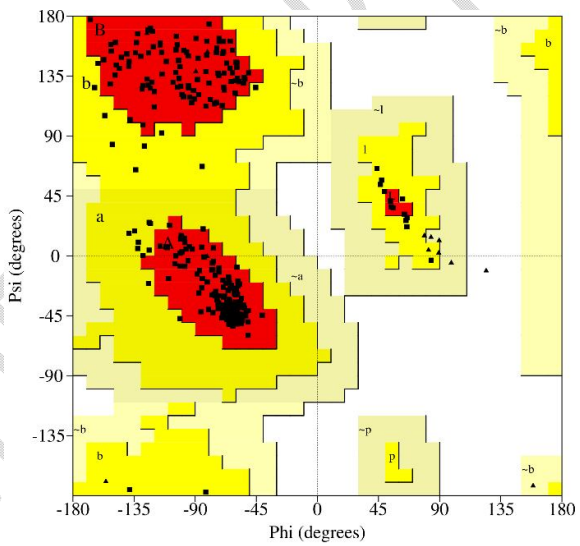
Residues	1B2Y	3TOP	3RX2
Most favourable region (%)	88.1	80.8	88.6
Additional allowed regions (%)	11.4	18.4	11.4
Generously allowed regions (%)	0.5	1.4	0.0
Disallowed regions (%)	0.0	0.1	0.0



a) 1B2Y



b) 3TOP



c) 3RX2

Figure 2: Ramachandran plot of protein 1B2Y, 3TOP and 3RX2.

3.4.3 ADME analysis

a. Calculation of molecular properties

Compounds obtained from MERB were subjected to docking with protein, were selected and ADME properties, drug likeness (Lipinski's rule of five) which are given in Table 5 (MWT ≤ 500).

Table 5: ADME properties of α amylase inhibitor, α glucosidase inhibitor and aldose reducatse Inhibitor by molinspiration

Compound	MW	nON	nOH	nV	nrotb	TPSA	miLogP
Quercetin	302.24	7	5	0	1	131.35	1.68
Isovitexin	432.38	10	7	1	3	181.64	0.52
Apigenin	270.24	5	3	0	1	90.89	2.46
Vitexin	432.38	10	7	1	3	181.04	0.52
Vicenin	594.52	15	11	3	5	271.19	-2.10
Orientin	448.38	11	8	2	3	201.27	0.03
Iso Orientin	448.38	11	8	2	3	201.27	0.03
Lucenin	580.50	15	11	3	4	271.19	-2.16
Rutin	610.52	16	10	3	6	269.43	-1.06
Rhynchosin	302.32	7	5	0	1	131.35	1.49
Biochanin	284.27	5	2	0	2	79.90	2.80
D-Pinitol	194.18	6	5	0	1	110.37	-1.99
D- Inositol	402.09	15	9	2	6	260.97	-4.71
5, 7, Dihydroxy-4-methoxy Isoflavone	624.55	16	9	3	8	258.43	-1.21
5, 7, 3, 4, tetrahydroxy-6-c-β-D Glucopyranosylflavone	490.63	9	3	0	7	124.67	3.81

MW = Molecular weight, nON = number of hydrogen bond acceptors, nOH = number of hydrogen bond donors, nV = number of violation of Lipinski's rule of five, nrotb = number of rotatable bonds, TPSA = Total Polar Surface Area and miLogP = Octanol-water partition coefficient logP.

3.4.4 Prediction of Bioactivity properties

Bioactivity of compounds was evaluated against six different protein structures. Biological activity is measured by bioactivity score that are categorized under three different ranges (Table 6)

1. If bioactivity score is more than 0.00, having considerable biological activity.
2. If bioactivity score is -0.5 to 0 having moderately activity.
3. If bioactivity score is less than -0.50, having inactivity [15].

Table 6: Bioactivity of α-amylase inhibitor, α-glucosidase inhibitor and aldose reductase Inhibitor by molinspiration

Compound	GPCR	Ion CM	KI	NRL	PI	EI
Quercetin	-0.06	-0.19	0.28	0.36	-0.25	0.28
Isovitexin	0.12	0.02	0.15	0.23	0.04	0.47
Apigenin	-0.07	-0.09	0.18	0.34	-0.24	0.26
Vitexin	0.13	-0.14	0.19	0.23	0.03	0.46
Vicenin	0.05	-0.41	-0.06	-0.03	0.03	0.20
Orientin	0.12	-0.14	0.20	0.20	0.01	0.45
Iso Orientin	0.11	0.01	0.16	0.20	0.01	0.46
Lucenin	0.07	-0.40	-0.03	-0.05	-0.02	0.28
Rutin	-0.05	-0.52	-0.14	-0.23	-0.07	0.12
Rhynchosin	-0.10	-0.28	0.20	0.19	-0.32	0.19
Biochanin	-0.23	-0.59	-0.07	0.23	-0.66	0.07

D-Pinitol	-0.54	-0.08	-0.69	-0.55	-0.51	0.05
D- Inositol	0.61	0.77	0.58	0.42	0.54	0.81
5, 7, Dihydroxy-4-methoxy Isoflavone	-0.14	-0.86	0.30	0.39	0.31	0.04
5, 7, 3, 4, tetrahydroxy-6-c-β-D Glucopyranosylflavone	0.15	-0.09	-0.37	-0.29	0.32	0.24

GPCR = G protein coupled receptor, Ion cm = Ion channel modulator, KI = Kinase inhibitor, sNRL = Nuclear receptor ligand, PI = Protease inhibitor, EI = Enzyme inhibitor.

4. DISCUSSION

The present work aimed with the objective of *in vitro, in silico* analysis with anti-diabetic activity of *Rhynchosia beddomei* methanolic extract. The percent yield of extract was found to be 35.43%. Preliminary phytochemical investigation of CMME showed the presence of alkaloids, glycosides, steroids, flavonoids, carbohydrates, saponins and terpenoids.

Skeletal muscle is the primary responsible site for postprandial glucose use, and it is the most abundant tissue in the whole body [16]. On a general basis, the uptake of glucose by skeletal muscles is due to the accumulation of functional glucose transporting molecules in the cell membrane. The glucose transporting molecules are regulated by leptocytes and/or myocytes in response to high secretion of insulin in blood, resulting in hypoglycemic effect [17]. It is well known that reduction of postprandial hyperglycemia can be achieved by inhibiting intestinal α-glucosidase and pancreatic α-amylase activity via delayed carbohydrate digestion [18]. Inhibitors of -glucosidase delay the breaking down of carbohydrate in the small intestine and diminish the postprandial blood glucose excursion in a person suffering from diabetes. One of the strategies and methods adopted to cure diabetes mellitus involves the inhibition of carbohydrate digesting enzymes such as α-amylase and α-glucosidase in the gastrointestinal glucose absorption thereby lowering postprandial glucose level [19].

In our study we screened MERB for its potential anti-diabetic activity through α-glucosidase and α-amylase inhibition assay. The MERB also showed noticeable inhibition at higher doses (50-200 µg/mL) have nearly one-sixth activity of acarbose. The drugs that inhibit carbohydrate hydrolysing enzymes have been proved to decrease post prandial hyperglycaemia and improve impaired glucose metabolism without promoting insulin secretion in non-insulin dependent diabetes mellitus (NIDDM) patients. Among natural

compounds, pentacyclic triterpenoids (PT) are a class of pharmacologically active and structurally rich metabolites with privileged motifs for further modifications, which are reported to act on glucose metabolism and flavanols might cause conformational changes in structure [20] in the study of α-amylase and of α-glucosidases inhibitory activities.

Acarbose and miglitol are competitive inhibitor of α-glucosidases and reduces absorption of starch and disaccharide. The α-glucosidase inhibition activities of the extracts are much higher than those obtained by α-amylase inhibition activities, in terms of dose levels. In fact, the IC_{50} values of α-glucosidase inhibition (9.22) is double to that of α-amylase inhibitory activity. This process is mimicked *in vitro* to screen plant extracts for their potential anti-diabetic activity and these are one of the best known and extensively used *in vitro* anti-diabetic assays. Extract treatment resulted in appreciable and concentration dependent inhibition of the activity of both enzymes. The methanolic extract of *Rhynchosia beddomei* was proved to possess prominent antidiabetic activity by α-amylase inhibition assay and α-glucosidase inhibition assay. Studies regarding interaction between Inhibitor and enzymes have significance in obtaining a better insight in the mechanism of enzyme inhibition. Thus, the molecular docking predictions were used to find the probable inhibition mechanism. *In silico* docking studies i.e., Molecular docking is a very efficient tool used for predicting receptor specific activity of molecules by evaluating their binding interaction with the target protein. Docking outcome provide us with the ligand-protein interaction energy (kcal/mol), number of interactions and amino acids involved in it, based on which biological activity of various molecules is predicted. mCule software is used for *in silico* docking studies and we used it to screen compounds present in *Rhynchosia beddomei* for their interaction to possess α-amylase inhibition (1B2Y), α-glucosidase inhibition (3TOP) and aldose reduction (3RX2). Compounds having minimum ligand-receptor binding energy and maximum interactions with the receptor (<6 Å bond lengths) were predicted to be most effective. Rutin, 5,7 dihydroxy-4-methoxy isoflavone, Quercetin, Apigenin, Orientin and Rhynchosin showed good docking score. Rutin shown the highest score -7.7 and -8.1 for α-amylase inhibitor protein and α- glucosidase inhibitor protein when compared to other compounds and Rhynchosin showed good score in aldose reductase protein. The docked compounds were found to occupy the same binding site and form hydrogen bonding interactions. These conditions show that can be used to predict noncompetitive *α*-amylase inhibitor, *α*-glucosidase inhibitor and aldose reductase mechanism. Aldose reductase is an enzyme involved in catalyzing the reduction of glucose into sorbitol. During hyperglycemia associated with diabetes, significant quantities of sorbitol are

produced due to the increased flux. Thus, targeting this enzyme is of great benefit in the amelioration of diabetes complications [21].

Ramachandran plot is analysed for protein α amylase inhibitor (PDB ID: 1B2Y), α glucosidase inhibitor (PDB ID: 3TOP) and Aldose reductase (PDB ID: 3RX2), the amino acids in the protein were 80% above in favourable region.

Molinspiration molecular properties were calculated on the bases of Lipinski's rule and its components. All the compounds that are docked has lower molecular weight so that they are easily absorbed, diffused and transported. Lipinski's rule of violation were 0 for Quercetin, Apigenin, Rhynchosin, Biochanin and D-pinitol, Lipinski's rule of violation 1 for Isovetexin, vitexin other compounds showed >1 violation. All the compounds showed good intestinal absorption. Values of other Molinspiration properties like nON, nOHNH, nrotb and octanol water partition coefficient logP under the acceptable limits. Molinspiration predicted bioactivity of protein structures. Isovitexin, Iso orientin, D-ionositol having good bioactivity score against all. Quercetin, Isovitexin, Apigenin, Vitexin, Vicenin, Orientin, Iso orientin, Lucenin, Rhynchosin, 5, 7, Dihydroxy-4-methoxy Isoflavone and 5, 7, 3, 4, tetrahydroxy-6-c-β-D Glucopyranosylflavone have having moderate bioactivity score against all receptors.

Overall results explain us that MERB might possess antidiabetic activity by alpha amylase and alpha glucosidase inhibitory actions and i*n silico* studies have proved better glide score of MERB compounds in comparison to standard acarbose. The application of molecular docking studies and molinspiration calculations, practical approach to calculate the molecular properties and predict the bioactivity scores for the compounds of MERB and docked with protein 1B2Y, 3TOP and 3RX2 are considered very useful.

5. CONCLUSION

These results provide a scientific justification for the traditional anti-diabetic use of this plant. Further, MERB inhibited α-glucosidase and α-amylase activity and thereby might control diabetes through lowering dietary glucose uptake. It may benefit in progression of diabetic complications through reducing oxidative stress. Anti-diabetic potential of MERB may be attributed to the presence of Rutin, 5,7 dihydroxy-4-methoxy isoflavone, Quercetin, Apigenin, Orientin and Rhynchosin as predicted through *in silico* docking study and needs to be screened further. From these results it can be concluded that plant extract possesses appreciable potential to inhibit the activity of these enzymes and thereby may be useful additive to diabetic therapeutic for managing dietary inflow of glucose.

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