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ABSTRACT

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Antihyperlipidemic Activity of *Rhynchosia Beddomei* whole Plant: An *in Vitro*, *in Vivo* and *in Silico* Approach

N V L Suvarchala Reddy[α], Ganga Raju M[σ], M, Mamatha[ρ], M. Lakshmi Madhuri [CO]
Shabnam Kumari Thakur[¥], Pavani K[§] & G. Kinnera Ratnasri[χ]

ABSTRACT

Obesity and hyperlipidemia are two widespread and difficult health issues that affect people all over the world. In these situations, elevated blood lipid levels are risk factors for atherosclerosis, coronary artery disease, and cerebral vascular disease. The goal of the current investigation was to evaluate the phytoconstituents' inhibitory effects on in vitro HMG-CoA reductase, in vivo HFD-induced hyperlipidemia, and dexamethasone-induced hyperlipidemia. High cholesterol diet was prepared by mixing cholesterol 2%, sodium cholate 1% and coconut oil 2%, with powdered chow diet which was prepared as pellets was placed in the cage and administered for 20 days. Dexamethasone, a glucocorticoid excess is known to evoke plasma lipid elevation in the dose of 10 mg/kg/day, s.c. was administered to Wistar rats for 8 days. The in vitro assessment of HMG -CoA reductase activity indicated an IC50 value of 150 and 75 µg/mL by the extract and a standard drug (Pravastatin), respectively. Additionally, an in-silico evaluation was made using appropriate docking software and results also indicated as significant interactions of the identified compounds with the target enzyme. Treatment of rats with the ethanolic extract of Rhynchosia beddomei whole plant resulted in significant ($p<0.05$) reductions in total cholesterol, LDL cholesterol, VLDL cholesterol, and triglyceride. It can be illustrating that the methanolic extract of Rhynchosia beddomei whole plant contains potent bioactive phytocompounds might be inhibit HMG – CoA reductase and have reduction potential of atherogenic index.

Author: Department of Pharmacology, Gokaraju Rangaraju College of Pharmacy, Nizampet Village, Bachupally, Hyderabad, Telangana State.

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I. INTRODUCTION

Hyperlipidaemia, commonly known as hypercholesterolemia or hyperlipoproteinemias, is a condition when there are abnormally high levels of fats in the blood. The structure and functionality of blood-circulating cholesterol is complex. According to (Ankur *et al.*, 2012), TGs are quickly recycled, deposited in adipocytes, absorbed from the intestine, or processed in hepatic tissue. When endothelial cells begin to suffer damage from atherosclerotic abrasion, cholesterol crystals are discovered. Increased blood lipids (LDL) have been proven to cause atherosclerosis, while epidemiological data has shown that high HDL levels have a protective effect. The greatest risk factor for artery hardening and narrowing is hyperlipidemia. Additional issues include thrombosis, elevated blood pressure, weight gain, and T2DM (Desu and Saileela, 2013). High fat diet and Dexamethasone are used in induction of hyperlipidaemia and Pravastation, Atorvastatin serves as standard in our present study. The healthcare system is structured in such a way that natural remedies are now widely perceived as inferior or something that people use when they cannot afford modern medicine.

Rhynchosia beddomei called as adavi kandi and vendaku, found in dry deciduous forests. It is rampant in South India; originate in AP and Karnataka. The leaves of *Rhynchosia beddomei* have abortifacient, antibacterial, antifungal, antidiabetic hepatoprotective properties and are also used for healing

wounds, cuts, boils and rheumatic pains by adivasi tribes. Root decoction is applied on chronic sores to keep off infection due to airborne diseases. A liniment prepared from the root is applied to reduce the pain near swollen wounds. The crushed stem parts are boiled in the sesame oil and used externally to cure sprains. The leaf paste is used as an antidote to treat insect bites. The aim of our study is to evaluate anti-hyperlipidemic activity of the whole plant extract of *Rhynchosia beddomei* in Wistar Albino rats and to execute *in-silico* analysis.

II. 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 steps.

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.

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.,

2.4 Antihyperlipidemic Activity

2.4.1 In vitro HMG-CoA reductase activity

The concentration of the purified human enzyme stock solution was 0.52–0.85 mg protein/mL. Pravastatin was castoff as reference. To characterize HMG-CoA reductase inhibition under defined assay conditions, reactions containing 4 µL of NADPH (to obtain a final concentration of 400 µM) and 12 µL of HMG-CoA substrate (to obtain a final concentration of 400 µM) in a final volume of 0.2 mL of 100 mM potassium phosphate buffer, pH 7.4 (containing 120 mM KCl, 1 mM EDTA, and 5 mM DTT), were initiated (time 0) by the addition of 2 µL of the catalytic domain of human recombinant HMG-CoA reductase and incubated at 37 °C in presence or absence (control) of 1 µL aliquots of drugs dissolved in DMSO. The rates of NADPH consumed were monitored every 20 sec up to 15 min spectrophotometrically. IC_{50} value was calculated and the % inhibitory enzymatic activity was calculated using the formula (Suvarchala *et al.,* 2015).

2.5 In vivo Anti-hyperlipidemic Activity

2.5.1 High fat diet induced hyperlipidaemia in rats

High cholesterol diet was prepared by mixing cholesterol 2%, sodium cholate 1% and coconut oil 2%, with powdered standard animal food. The diet which was prepared as pellets was placed in the cage and administered for 20 days. Adult Wistar albino rats were administered with corresponding treatments for one month. They were divided into 5 groups with 6 animals per group. Study design of High fat diet induced hyperlipidaemia method is Group –I serves as Control (Normal saline) were as Group –II received HFD, Group –III received HFD and methanolic extract of *Rhynchosia beddomei* (300mg/kg, *p.o*) and Group – IV received HFD and atorvastatin (10 mg/kg/day *p.o*) (Suvarchala *et al.*, 2015).

2.5.2 Biochemical investigations for body fat

The serum triglycerides and HDL (High Density Lipoproteins) and total cholesterol were measured using span diagnostic kit. TC, TG and High-Density Lipoproteins profiles were estimated using standard monograph.

2.5.3 Measurement of cardiac disorder threat issue

Atherogenic index (AI), which is a measure of the atherogenic potential of an agent, was calculated using the formula and the results were tabulated.

Atherogenic Index (AI) = LDL - cholesterol/HDL – cholesterol

$$\% \text{ Protection} = \frac{\text{AI of control-AI of treated group}}{\text{Atherogenic Index of control}} \times 100$$

2.5.4 Dexamethasone-induced hyperlipidaemia in rats

To induce hyperlipidaemia, dexamethasone, (a glucocorticoid excess is known to evoke plasma lipid elevation) in the dose of 10 mg/kg/day, *s.c.* was administered to Wistar rats for 8 days and animals were divided into five groups each group containing six rats. After the induction of hyperlipidaemia, MERB were given to the rats for 8 days at a dose of 300 mg/kg and on 9^{th} day, after overnight fasting blood was collected from retro-orbital plexus for the study of biochemical parameters. Study design of High fat diet induced hyperlipidaemia method is Group–I serves as Control (Normal saline) were as Group –II received dexamethasone 10 mg/kg, b.w., s.c., Group –III received dexamethasone 10 mg/kg, b.w., *s.c.* and methanolic extract of *Rhynchosia beddomei* (300 mg/kg, *p.o*) and Group – IV received HFD and atorvastatin (10 mg/kg/day *p. o*) (Suvarchala *et al.*, 2015).

2.6 In silico analysis

2.6.1 Molecular Docking Studies

Molecular docking is an attractive scaffold to understand drug bimolecular interactions for rational drug design and discovery. Molecular docking generates different possible adduct structures that are ranked and grouped together using a 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 (Suvarchala *et al.,* 2021). Molecular docking performed with mCule online tool.

2.6.1.1 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 3ASX, 6Q2T and 7S5G. Docking indicates that some of our compounds, docked with protein of Squalene synthase Inhibitor (PDB ID: 3ASX), Lanosterol 14 alpha demethylase Inhibitor (PDB ID: 6Q2T) and PCSK9 Inhibitor (PDB ID: 7S5G)

2.6.1.2 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 (Ganga raju *et al.,* 2021).

2.6.2 Ramachandran plot

The docked proteins of Squalene synthase Inhibitor (PDB ID: 3ASX), Lanosterol 14 alpha demethylase Inhibitor (PDB ID: 6Q2T) and PCSK9 Inhibitor (PDB ID: 7S5G) protein 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 (Ganga raju *et al.,* 2022). 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 by Morris. the darkest areas (here shown in *red*) correspond to the "core" regions representing the most favourable combinations of phi-psi values.

2.7 Statistical analyses

The Results were expressed as the AM ± S.E.M. The significance of the results was calculated using ANOVA and Dunnett's t-test and results were deliberated statistically noteworthy when significant $p<0.01$, $p<0.05$ ns- non significant.

III. RESULTS AND DISCUSSION

3.1 Preparation of Rhychosia beddomei methanolic 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.

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

$$= 35.43\%. \quad \text{w/w.}$$

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 Invitro antihyperlipidemic Activity

3.3.1 In vitro HMG CoA Reductase Inhibitor Activity

The data of percent inhibition and IC_{50} at two dose levels of methanolic extracts of two plants are presented in Table 1 indicated that the % inhibition is increased with increase in dose of plant extract, though proportionate increase was not obtained. Additionally, inhibition data were processed for relative inhibition compared to standard and reported. The processing is done on the lines of expressions of relative bioavailability (%). Higher the relative inhibition, the greater is the inhibition of HMG CoA reduction. In this study, standard is pravastatin. As expected, pravastatin produced inhibition at lower dose (100 µg/mL). Plant extracts also showed appreciable inhibition at greater doses (500 µg/mL). The methanolic extracts of both plants showed same extent of % inhibition of the HMG CoA reduction.

The IC_{50} values of standard and test extracts are compared and the ratio was found to be less by 55% respectively, for MERB.

Table 1: In vitro HMG-COA reductase inhibitory activity of MERB

Test extract/standard	Dose (µg/mL)	Percentage inhibition AM±SEM (n=3)	% Relative inhibition	IC_{50} (µg/mL)
Methanolic extract of *Rhynchosia beddomei*	100	43.33 ±0.76	33.34	150
	500	85 ±0.012	73.9	
Pravastatin (Standard)	100	66.86 ± 0.0788	100	75
	500	100±0.012	100	

3.4 In vivo Antihyperlipidemic Activity

Hyperlipidaemia was induced by giving high cholesterol diet and dexamethasone (10 mg/kg, b.w., *s. c*) and parameters measured were TC, triglyceride, low density lipoproteins, VLDL, high density lipoprotein levels. Atherogenic index and % protection were also measured.

3.4.1 High fat diet (HFD) induced hyperlipidaemia

Rats fed on HFD is a means for the induction of metabolic syndrome features including distinctive visceral adiposity, dyslipidaemia which are typically associated with human obesity. The rat model of diet-induced obesity is often used to investigate the effects of metabolic syndrome ameliorating agents. The data of cholesterol, triglycerides, LDL, VLDL and HDL levels upon the treatment of *Rhynchosia beddomei* methanolic extract at one dose level (300 mg/kg, b.w.) are presented in Table2. When HFD is provided to rats, cholesterol, triglycerides, LDL, VLDL levels increased and HDL level decreased from 80 to 100% within 20 days. MERB were administered and levels decreased within 30 days compared to Disease control group. The standard in the present study is atorvastatin (10 mg/kg, b.w.). As expected, standard produced lower levels of cholesterol, triglycerides, LDL, VLDL and increased HDL levels at 10 mg/kg. The MERB 300 also showed appreciable decrease of cholesterol levels and nearer to the standard value. In the present work, *Rhynchosia beddomei* was reported to contain β-sitosterol and stigmasterol. These might be responsible to lower cholesterol in animals fed with HFD.

Table 2: Effect of MERB on cholesterol, triglycerides, LDL, VLDL and HDL in high fat diet induced model

Groups	Treatment/Standard Dose mg/kg, b.w.	Lipid Profile (mg/dL) AM±SEM				
		Cholesterol	Triglycerides	LDL	VLDL	HDL
I	Control	104.2±0.47	79.83±1.68	42.93±0.30	15.8±0.33	46.83±0.15
II	Cholesterol control	219.66±0.1^{a,A}	185.5±1.94 ^{b,A}	162.96 ±0.05 ^{b,A}	34.3±0.16^{b,A}	31.33±0.6 ^{a,A}
III	MERB 300	112.6±1.7 ^{b,B}	109±1.54 ^{a,B}	48.7±0.6 ^{a,B}	18.1±0.2 ^{b,A}	45.8±0.9 ^{b,A}
IV	Atorvastatin 10	110.3±0.4	104.1±1.3	46.5±0.02	17.83±0.07	46.5±0.17

In view of reducing cholesterol by plant extracts and other related parameters, TG, LDL, VLDL was further evaluated. When HFD is provided to rats, triglyceride levels were increased by 80 to 100% within 20 days and LDL levels were amplified by 300% within 20 days, VLDL levels were doubled by within 20 days, HDL levels were decreased by 20% within 20 days in disease group. The plant extract was administered, the triglyceride levels, LDL, VLDL levels decreased compared to control, the test samples of significant in $p<0.05$, compared to standard, the samples have significantly different ($p<0.01$) and HDL increased within 30 days compared to control ($p<0.05$), when compared to standard the samples are significantly different ($p<0.05$).

Measurement of Cardiac Disorder Threat Issue

3.4.1.1 Atherogenic Index

The atherogenic index is obtained based on the knowledge of cholesterol, LDL and HDL values. Thus the index was calculated. The data of atherogenic index upon the treatment of plant extracts at one dose level (300 mg/kg, b.w.) shown in figure, indicated that the atherogenic index remained same for the control up to 30 days. When high fat diet is provided to rats, the atherogenic index was increased by 3 times (300%) within 20 days. The differences between control and cholesterol control are significant ($p<0.05$). The plant extracts were administered, the atherogenic index reached the value of control within 30 days, the test samples are significant ($p<0.05$), when compared to standard, the samples are significantly same ($p<0.01$). The lower the atherogenic index, the higher is the hypolipidemic activity (of the test extracts). The standard in the present study is atorvastatin (10 mg/kg, b.w.). As expected, atorvastatin produced lower levels of atherogenic index (1) at a dose level of 10 mg/kg. The plant extract also showed appreciable decrease of atherogenic index and nearer to the standard value ($p<0.05$).

3.4.1.2 Effect on % protection

The percent protection of heart is considered based on the levels are different parameters evaluated so far. In the calculation of atherogenic index, control and cholesterol control values are not included. But in the calculation of percent protection, control values are also included. % Protection was calculated for the groups treated with MERB 300 mg/kg, b.w. explained in table 3. As observed in the values of atherogenic index, the percent protection is 30 times lower than the standard, when dose-normalized.

Table 3: Effect of MERB on % protection in high fat diet induced model

Groups	Treatment/Standard Dose mg/kg, b.w.	% Protection, AM±SEM, (n=6)
I	Control	-
II	Cholesterol control	-
III	MERB 300	72.04±0.43 ^{b,A}
IV	Atorvastatin 10	72.6±0.56 ^{b}

Test groups were compared with control group and standard group. By using Dunnett's t-test significant values are expressed as control group (a=p<0.01, b=p<0.05) and standard (A = p < 0.01, B = p < 0.05), ns- non significant.

3.4.2 Dexamethasone induced hyperlipidaemia

Dexamethasone, triggering a disproportion in lipid metabolism leading to hyperlipidaemia (Wiesenberg *et al.,* 1998). Hyperlipidaemia was produced by administering dexamethasone (10 mg/kg, b.w., *s. c*). The parameters measured were total cholesterol; triglyceride, LDL, VLDL, HDL levels, atherogenic index and percent protection were also measured. The antihyperlipidemic activity was evaluated for MERB.

The cholesterol, triglycerides, LDL, VLDL and HDL levels upon the treatment MERB at one dose level (300 mg/kg, b.w.) are presented in Table 4 indicated that all levels remained same for the control upto 11 days. When dexamethasone is injected to rats, the cholesterol, TGL, VLDL levels were almost doubled, the LDL levels increased nearly 3½ times and HDL levels were decreased by 5 to 6% within 11 days. The Methanolic extract of *Rhynchosia beddomei* extract were administered, the cholesterol, triglycerides, LDL, VLDL levels decreased and HDL levels increased when compared to control, the results of test samples are significant (p<0.05) that indicates the higher is the hypolipidemic activity of the test extract may be due to the presence of phytosterol (Bopanna *et al* 1997). Any lipid reduction (1% cholesterol) produces a 2-3% reduction in CHD risk (Xu *et al* 2005). The standard, atorvastatin (10 mg/kg, b.w.) produced lower levels of cholesterol. In the present work, *Rhynchosia beddomei* was reported to contain β-sitosterol and stigmasterol. These might be responsible for lowering the Lipid levels, though the animals were injected with dexamethasone.

Table 4: Effect of MERB on cholesterol, triglycerides, LDL, VLDL and HDL in dexamethasone induced model

Group	Treatment/Standard Dose mg/kg, b.w.	Lipid Profile (mg/dL) AM±SEM, (n=6)				
		Cholesterol	Triglycerides	LDL	VLDL	HDL
I	Control	104±0.09	79±0.28	44±0.09	16±0.22	47±0.15
II	Cholesterol control	233±0.93 ^{a,B}	157±0.23 ^{b,A}	176±0.7 ^{b,B}	51±0.7 ^{b,A}	26±0.86 ^{b,B}
III	MERB 300	108±0.92 ^{a,B}	99±0.02 ^{a,A}	57±0.64 ^{a,A}	20±0.21 ^{b,B}	40±0.44 ^{a,A}
IV	Atorvastatin 10	105±0.69 ^{b}	92±0.46 ^{b,B}	53±0.46 ^{b,A}	18±0.5 ^{a,A}	43±0.08 ^{b,A}

Test groups were compared with control group, cholesterol control group and standard group. By using Dunnett's t-test significant values are expressed as control group (a=p<0.01, b=p<0.05), cholesterol control (**= p<0.01, *= p<0.05) and standard (A = p < 0.01, B = p < 0.05), ns- non significant.

Measurement of cardiac disorder threat issue

3.4.2.1 Effect on atherogenic index (AI)

When dexamethasone is provided to rats, the atherogenic index was increased by 3 times within 10 days. The data of atherogenic index upon the treatment of MERB at one dose level (300 mg/kg, b.w.) are presented in Table 5. Atherogenic index remained the same for the control upto 11 days. When the MERB was administered, the atherogenic index decreased within 11 days, compared to control and values are nearer to the standard value standard,

Table 5: Effect of MERB on atherogenic index in dexamethasone induced model

Group	Treatment/ Standard Dose mg/kg, b.w.	Atherogenic Index AM±SEM, (n=6)
I	Control	0.93±0.28
II	Cholesterol control	5±0.27 [b,A]
III	MERB 300	1.23±0.11 [a,A]
IV	Atorvastatin 10	1.09±0.04 [a]

Test groups were compared with control group, cholesterol control group and standard group. By using Dunnett's t-test significant values are expressed as control group (a=p<0.01, b=p<0.05), cholesterol control (**= p<0.01, *= p<0.05) and standard (A = p < 0.01, B = p < 0.05), ns- non significant.

3.4.2.2 Effect on % protection

Percent protection was calculated for the groups treated with MERB 300 mg/kg, b.w. As observed in the values of atherogenic index, the percent protection is 30 times lower than the standard, when dose-normalized.

Table 6: Effect of MERB on % protection in dexamethasone induced model

Groups	Treatment/Standard dose, mg/kg, b.w.	% Protection AM±SEM (n=6)
I	Control	-
II	Cholesterol control	-
III	MERB 300	81.2±0.41 [a,A]
IV	Atorvastatin 10	81±0.74 [a]

*Test groups were compared with control group, cholesterol control group and standard group. By using Dunnett's t-test significant values are expressed as control group (a=p<0.01, b=p<0.05), cholesterol control (**= p<0.01, *= p<0.05) and standard (A = p < 0.01, B = p < 0.05), ns- non significant.*

Dexamethasone induced hyperlipidaemia, the animal model effectively used for evaluating the antihyperlipidemic activity of natural products (Kumar *et al.*, 2007). In the present study, treatment with MERB (300 mg/kg) has reduced the serum cholesterol, triglycerides levels and atherogenic index, in dexamethasone treated animals. The extracts inverted the hyperlipidaemia produced by administration of dexamethasone. The low lipoprotein lipase activity in the liver may be responsible for low degradation of lipoprotein, triglycerides and cholesterol. Hence the hyperlipidemic effect of dexamethasone was found to be reversed by MERB, further this was also found to be comparable with that of atorvastatin. In this work, friedelin is proposed to decrease the TC, TG, and LDL-C and increase the HDL-C levels significantly. The methanolic extract of *Rhynchosia beddomei* contains

phytosterol and flavonoids. The high amount of flavonoids complemented the phytosterols which might be accountable for the hypolipidemic effect. It also improves HDL- cholesterol levels and lower atherogenic index. More specifically the presence of friedelin has the added benefit of stimulating the synergistic effect for eliciting the antihyperlipidemic activities.

3.5 In silico analysis

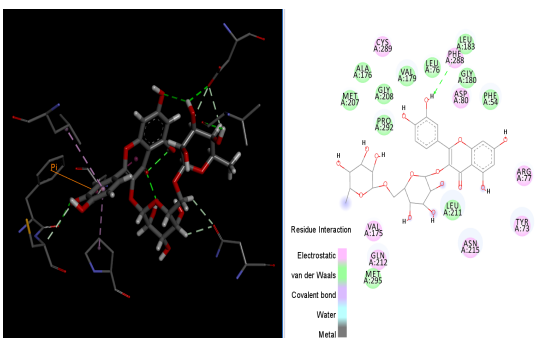
3.5.1 Molecular docking studies

Table 7: Docking score of chemical constituents and atorvastatin with protein 3ASX, 6Q2T and 7S5G

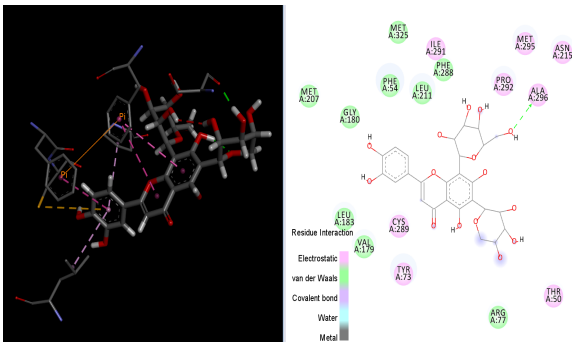
Compounds	3ASX	6Q2T	7S5G
Quercitin-7-O-methyl ether	-8.4	-6.2	-5.8
Isovitexin	-8.5	-6.3	-6.2
5,7,3',4'-tetrahydroxy 6-c- β -D-glucopyrynosyl flavone	-7.5	-5.6	-5.9
Apigenin	-8.2	-7.0	-6.0
Vitexin	-8.3	-6.2	-5.9
Vicenin	-8.3	-5.9	-6.3
Orientin	-8.1	-5.8	-5.7
Isoorientin	-7.9	-6.3	-6.5
Lucenin	-8.5	-5.4	-6.1
Rutin	-8.8	-5.6	-5.9
Rhynchosin	-7.9	-6.9	-6.0
Biochanin	-7.7	-7.3	-6.2
D-Pinitol	-4.8	-3.5	-3.9
D-Inositol	-4.6	-3.5	-4.0
Atorvastatin	-7.9	-8.7	-5.4

G score = glide score, Higher the negativity, the more favourable the binding.

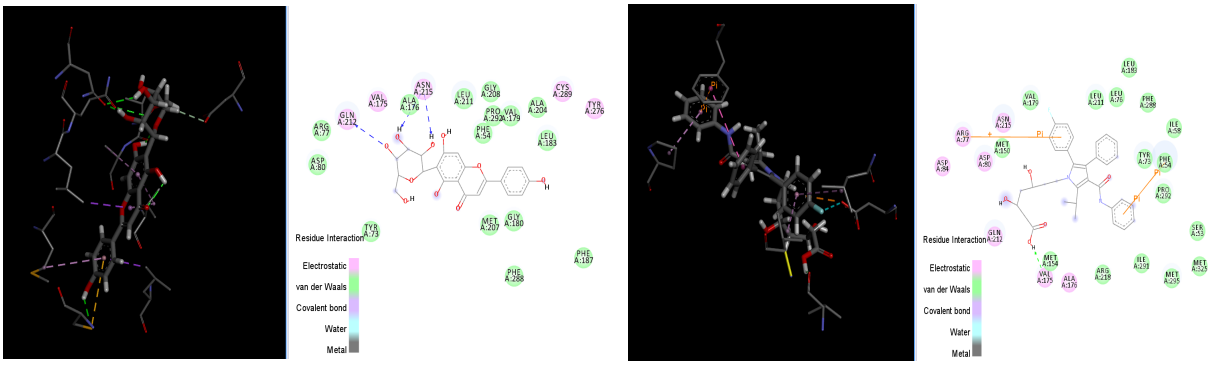
Hydrophobic bond interactions of ligands with 3ASX, 6Q2T and 7S5G PDB ID: 3ASX



a) Rutin -8.8

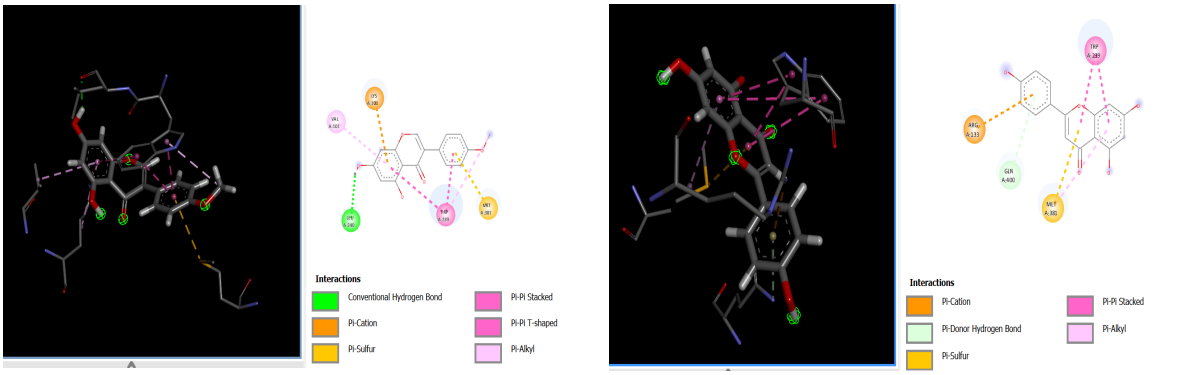


b) Lucenin -8.5

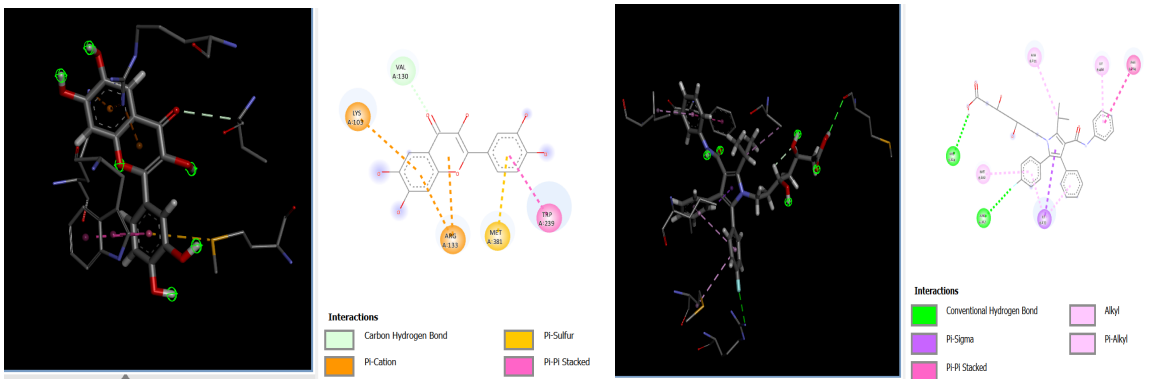


c) Isovitexin -8.5 d) Atorvastatin -7.9

Figure 3: 3D & 2D structures of 3ASX protein with Glide scores with interactions PDB ID: 6Q2T

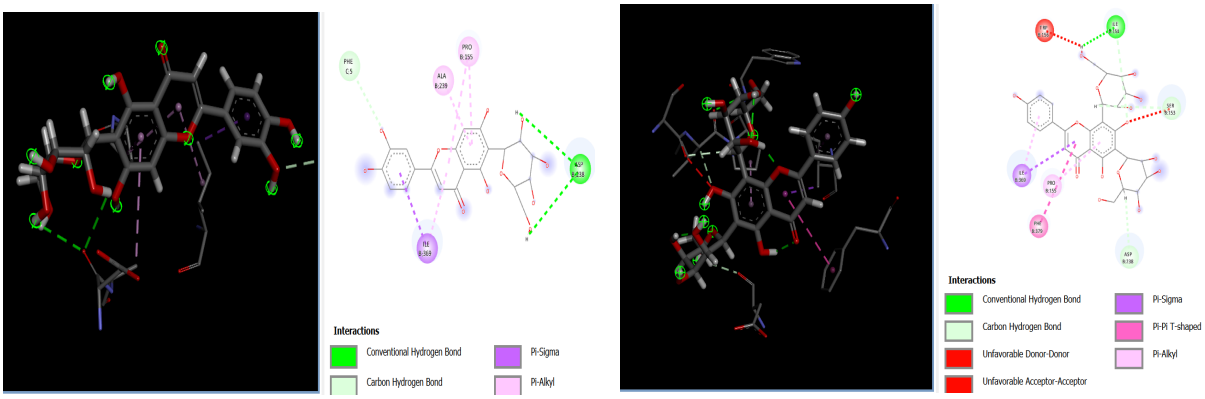


a) Biochanin -7.3 b) Apigenin -7.0

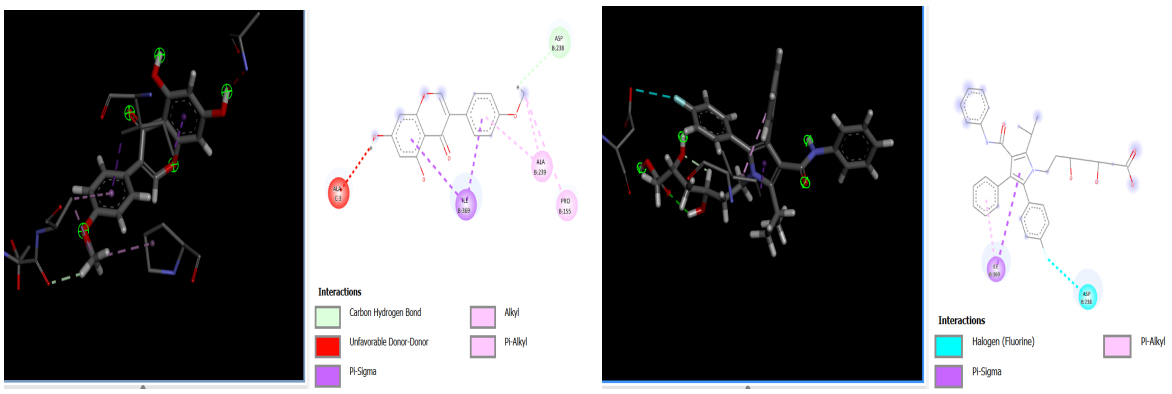


c) Rhynchosin -6.9 d)Atorvastatin -8.7

Figure 4: 3D & 2D structures of 6Q2T protein with Glide scores with interactions PDB ID: 7S5G



a) Iso orientin -6.5 b) Vicenin -6.3



c) Biochanin -6.2 d)Atorvastatin -5.4

Figure 5: 3D & 2D structures of 7S5G protein with Glide scores with interactions

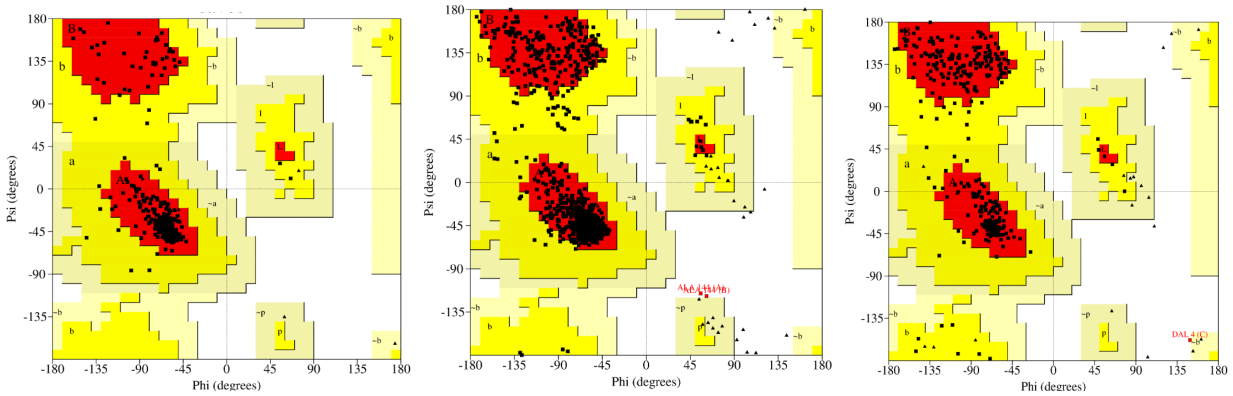
The compounds present in MERB, are docked with protein Squalene synthase Inhibitor (PDB ID: 3ASX), Lanosterol 14 alpha demethylase Inhibitor (PDB ID: 6Q2T) and PCSK9 Inhibitor (PDB ID: 7S5G) and Ramachandran plot is analysed. Rutin, Lucenin, Iso vitexin showed good score for protein 3ASX; Biochanin, Apigenin, Rhynchosin for protein 6Q2T, Isoorientin, Vicenin and Biochanin for protein 7S5G shown good docking score when compared to other compounds. Squalene synthase (SQS), a key downstream enzyme involved in the cholesterol biosynthetic pathway, plays an important role in treating hyperlipidemia. Compared to statins, SQS inhibitors have shown a very significant lipid-lowering effect and do not cause myotoxicity (Kourounakis *et al.*, 2020) Lanosterol 14α-demethylase (CYP51A1) is the animal version of a cytochrome P450 enzyme that is involved in the conversion of lanosterol to 4,4-dimethyl cholesta-8 (9),14,24-trien-3β-ol, demethylation of lanosterol has been implicated as a rate-limiting step in the post-squalene portion of cholesterol synthesis, suggesting the reaction as a potential focal point in sterol regulation (DeBose-Boyd, 2008). Proprotein convertase subtilisin kexin type 9 (PCSK9) inhibitors are novel agents indicated for the treatment of hyperlipidemia. Inhibition of PCSK9 produces an increase in surface low-density lipoprotein (LDL) receptors and increases removal of LDL from the circulation (Sible, 2016).

Ramachandran plot Analysis

Protein 3ASX, 6Q2T and 7S5G were analysed for Ramachandran plot to know amino acid presence in different regions of respective protein tabulated in table 8 and pictorial representation by figure 6.

Table 8: Ramachandran plot status with protein 3ASX, 6Q2T and 7S5G

Residues	3ASX	6Q2T	7S5G
Most favourable region (%)	94.8	90.9	88.4
Additional allowed regions (%)	5.2	8.9	11.2
Generously allowed regions (%)	0.0	0.00	0.0
Disallowed regions (%)	0.0	0.3	0.3



a) 3ASX b) 6Q2T c) 7S5G

IV. CONCLUSION

Preliminary phytochemical tests of MERB in the present work showed the presence of constituents such as flavonoids, glycosides, sterols, alkaloids and tannins. The whole plant extract of *Rhynchosia beddomei* inhibited HMG-CoA reductase by 43.33% at 100 µg/mL and 85% inhibition at 500 µg/mL. The standard pravastatin was found to possess 65.0% inhibition at 100 µg/mL. Graded response is an encouraging observation. The extract lowered the TC, TG, LDL and VLDL, results were consistently observed in two models, also showed appreciable decrease of atherogenic index and nearer to the standard value. Therefore, more research into the use of a methanolic extract of *Rhynchosia beddomei* whole plant as an alternative therapeutic agent to statins and other medications, but one that has no unwanted side effects, is necessary for the treatment of cardiovascular disorders.

REFERENCES

1. Ankur R, Nidhi D, Seema R, Amarjeet D and Ashok K. Hyperlipidemia- a deadly pathological condition. *Int J Curr Pharm Res*. 2012; 4(2): pp 15-18.
2. Bopanna KN, Kannan J, Gadgil S, Balaraman R and Rathod SP. Antidiabetic and antihyperlipidemic effects of neem seed kernal powder on alloxan diabetic rabbits. *Ind J Pharmacol*. 1997; 29: pp 162-167.
3. DeBose-Boyd, R. Feedback regulation of cholesterol synthesis: sterol-accelerated ubiquitination and degradation of HMG CoA reductase. *Cell Res* 18, 609–621 (2008). https://doi.org/10.1038/cr.2008.61
4. Desu BSR and Saileela CH. Anti-hyperlipidemic activity of methanolic extract of *Rhinacanthus nasutus*. *Int J Res Pharm Chem*. 2013; 3 (3): pp 708-711.
5. Ganga Raju M, Sony Priyanka G and Suvarchala Reddy NVL. Novel *In Silico* and *In Vivo* Insights of Flavonoids as Anti-Diabetic and Anti-Oxidant in Rodent Models. Indian J Pharm Sci 2022;84 (4):1041-1050
6. Ganga Raju M, Veena Yadav, N V L Suvarchala Reddy V. Natural Compounds as D2 receptor agonist, M4 receptor antagonist and ACHE modulator: Mechanistic and *in silico* modeling studies. Journal of research in medical and dental science. 2021;9(6): 26-35.
7. Kourounakis AP, Bavavea E. New applications of squalene synthase inhibitors: Membrane cholesterol as a therapeutic target. Arch Pharm (Weinheim). 2020 Sep;353(9):e2000085. doi: 10.1002/ardp.202000085. Epub 2020 Jun 18. PMID: 32557793.
8. Kumar BRP, Praveen TK, Nanjan MJ, Karvekar MD and Suresh B. Serum glucose and triglyceride lowering activity of some novel glitazones against dexamethasone-induced hyperlipidaemia and insulin resistance. *Ind J Pharmacol*. 2007; 39: pp 299-302.

9. Sible AM, Nawarskas JJ, Anderson JR. PCSK9 Inhibitors: An Innovative Approach to Treating Hyperlipidemia. Cardiol Rev. 2016 May-Jun;24(3):141-52. doi: 10.1097/CRD.0000000000000102. PMID: 26886466.
10. Suvarchala Reddy NVL, M. Ganga Raju, M. Mamatha, N Nandini. GC-MS analysis, In silico docking studies and diuretic activity of methanolic extract of Citrus medica l. Leaf in Wistar albino rat model. Bull. Env.Pharmacol. Life Sci., Vol 2021;10(3): 42- 50.
11. Suvarchala Reddy NVL, Pooja Raj GB, Ganga Raju M, Sneha J Anarthe. Antihyperlipidemic activity of *Cassia fistula* bark using high fat diet induced hyperlipidemia. International Journal of Pharmacy and Pharmaceutical Sciences. 2015; 7(10): 61-64.
12. Suvarchala Reddy NVL, Swetha Aveti, Anjum M, Ganga Raju M. Antihyperlipidemic activity of methanolic extract of Syzygium alternifolium bark against high-fat diet and dexamethasone induced hyperlipidemia in rats. Asian Journal of Pharmaceutical and Clinical Research. 2015; 8(6): 165-168.
13. Venugopal A and Ramakrishna S. Indirect assessment of hydroxymethylglutaryl-CoA reductase (NADPH) activity in liver. *Clin Chem*. 2005; **21**: pp 1523-1525.
14. Wiesenberg I, Chiesi M, Missbach M, Spanka C and Pignat W. Specific activation of the nuclear receptor PPARγ and RORA by the antidiabetic thiazolidinedione BRL 49653 and the antiarthiritic thiazolidinedione derivative GGP 52608. *Mol Pharmacol*. 1998; **53**: pp 1131-1136.
15. Xu Y, He Z and King GL. Introduction of hyperglycaemia and dyslipidaemia in the pathogenesis of diabetic vascular complications. *Curr Diabetic Rep*. 2005; **5(2)**: pp 91-97.