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IN SILICO STUDIES OF COMPOUNDS ISOLATED FROM METHANOL EXTRACT OF *ELEPHANTOPUS SCABER* BY GCMS AGAINST PROTEIN PPAR- Γ FOR ITS HYPOGLYCEMIC ACTIVITY

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ABSTRACT

PPAR-γ is the abbreviation of peroxisome proliferator-activated receptor gamma, is also called as glitazone receptor, therapeutically used to fight against hyperglycemia associated with the metabolic syndrome and type -2 diabetes since the currently used PPAR- γ –agonist from the thiazolidine dione type shown serious side effects and hence making the discovery of novel ligands which are highly relevant, effective in normalization of blood glucose levels .The present study evaluated the binding of certain GCMS derived compounds of the methanol extract of the *Elephantopus scaber* leaves for its hypoglycemic activity of PPAR - γ. Docking scores were compared with their binding energy as well as affinity using the Schrodinger, 2015.1.The docking or glide score of the above seven compounds are -5.0,-8.7, -4.55,-3.7,-6.1,-5.8,-4.3(kcal/Mol) and the glide score were -33.43,-16.91,-29.21,-17.54,-29.79,-28.87 and -23.07 (kcal/Mol). The results of the study showed that out of the 9 isolated compounds, 7 of them may act as good agonist for PPAR- γ and the compounds can be re-designed for better hypoglycemic activity for synthesis.

INTRODUCTION

PPARs in structurally was identical to the steroid or thyroid hormone receptor and are stimulated in response to small lipophilic molecules. The PPARs exist in three subtypes; α, β, δ, and γ, each of which mediates the physiological actions of a large variety of FAs and FA derived molecules. Activated PPARs are also capable of transcriptional repression through DNA-independent protein-protein interactions with other transcription factors such as NFκB signal activators and transducers of transcription STAT-1 and AP-1 signaling (Olivera, 2007). Peroxisome proliferator-activated receptor gamma (PPAR-γ or PPARG), also called as glitazone receptor or NR1C3 (nuclear receptor subfamily 1, group C, member 3). It is a type II nuclear receptor that in humans is encoded by the PPARG gene. The molecular agonists of the nuclear receptor PPARγ are therapeutically used to fight against hyperglycemia associated with the metabolic syndrome and type II diabetes. Since, the

currently used PPARγ agonists from the thiazolidinedione type shown serious side effects and hence, making the discovery of novel ligands which are highly relevant, effective in normalization of blood glucose levels (Wang, 2014). The naturally identified PPARγ ligands showed various binding modes to the receptor in comparing to the thiazolidinedione agonists. Moreover a number of *in vivo* studies recommend that some of the natural product activators of PPARγ improved the metabolic parameters in diabetic animal models, partly with reduced side effects in comparison to full thiazolidinedione agonists. Hence based on this idea the bioactive compounds can be a better target to modulate the PPARγ activation for its hypoglycemic activity (Wang, 2014). *Elephantopus scaber L*inn is a small herb from the family Asteraceae, order Asterales and the subclass Asteridae. The whole plant of *E. scaber L*inn is well known as a herb of Chinese folk medicine which is widely used in the treatment of nephritis, edema, dampness, pain in the chest, fever and cough of pneumonia, scabies and arthralgia due to wounding (Peer, 1980 and Tsai, 1999) It is also commonly used in China as a remedy for the treatment of gastropathy, hepatitis, nephritis, edema, chest pain, fever and cough of pneumonia, bronchitis, arthritis, and carbuncle. The root decoction of is widely used to treat diarrhoea, dysentery, stomach troubles and blood vomiting in tuberculosis in Nepal (Ahamed *et al.,* 2009 and Ho *et al*., 2009). The interest in herbal medicines originate mainly from the efficacy of ethnomedicinal plants in curing diabetes, scavenging free radicals and protecting liver from toxicity of biotic and abiotic origin. *E. scaber L*inn is one of such plants which have tremendous reputation in indigenous traditional system of medicine in India by virtue of which it has drawn the attention and concern of scientists for validation of its medicinal properties through phytochemical and pharmacological evaluation (Moumitadas, 2014). In the present study nine compounds were identified using GC-MS analysis from the methanol of *E. scaber*. The compounds were tested as agonist to the PPARγ receptor in targeting the hypoglycemic activity using *in silico* analysis. The docking analysis was used to predict the binding orientation of the ligands to the protein targets in order to predict the affinity and activity of the small molecules.

MATERIALS AND METHODS

Plant collection: The plant materials were collected from the local areas. It was authenticated by Dr. D.V. Swami, Assistant Professor from Dr. Y.S.R. Horticulture University, Venkataramannagudem-534101, W. G. District from Andhra Pradesh.

Extraction: The leaves of *Elephantopus scaber Linn* were dried under shade and then coarsely powdered. The powder was passed through sieve no.40 and stored in an air tight container for further use. The powder was then extracted with methanol using Soxhlet apparatus for 72 hrs. The extract was dried and stored in dessicator.

GCMS ANALYSIS: GC-MS analysis was performed using The JEOL GCMATE II GC-MS with Data system is a high resolution, double focusing instrument. Maximum resolution: 6000 Maximum calibrated mass: 1500 Daltons equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 μm ID × 0.25 μm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/minute, and an injection volume of 2 μl was employed (a split ratio of 10:1). The injector temperature was maintained at 250° C, the ion-source temperature was 200° C, the oven temperature was programmed from 110° C (isothermal for 2 minutes), with an increase of 10° C/minute to 200° C, then 5° C / minute to 280° C, ending with a 9 minutes isothermal at 280° C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 minutes, and the total GC/MS running time was 36 min. The relative

percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrums of the components were compared with the database of known spectrum components stored in the NIST library.

Molecular Docking (*In Silico* study) Protein preparation: The protein data bank web is a collection of 3D structure of protein with more information about the experimentally established X-ray and NMR biomacromolecules and their complexes with or without ligands (Berman, 2008; Friesner *et al.*, 2006). The crystal structure of the hypoglycemic target protein with a resolution of 2.3 A^o (PDB entry 2PRG) was downloaded from the www.pdb website and customized to be biologically active (Berman H.M, 2008). The protein may contain heavy atoms, water molecules, cofactors, metal ions and can also be multimeric. Hence, to achieve a biologically active protein, the raw 3D structure should be made to fit and available for docking study. This includes the removal of the water molecules from the cavity, stabilizing charges, generating the side chains and missing hydrogen atoms and so on according to the default limitation available on the module of "protein preparation wizard" (Schrodinger Maestro). Followed by this process the protein was processed to minimize the energy by using the kollman charges (OPLS 2005). Finally the receptor was made to biologically active and stable (Wang, 2010).

Receptor grid generation: The grid was generated using the module "receptor grid generation" of Schrodinger maestro 2015-1. This will be performed if the prepared protein consists of ligand molecules. The ligand was identified by minimizing the protein and selecting the ligand. By using the grid generation the ligand was excluded and click "run" for the job to complete (Halgren, 2004).

Ligand preparation: The ligands were drawn using the chemdraw tool and converted into the 3D format and minimized using the OPLS-2005 using the "LigPrep" module of Schrodinger 2015-1.

Ligand docking: The ligands were set to be flexible and the docking was manually set to the "extra (XP) precision mode". Usually this method (Grid based Ligand docking) is the best choice for docking of less numbers of ligands in "Ligand docking" module of Schrodinger Maestro, Glide module (Schrodinger, 2015-1). All the biologically active compounds were docked against the binding sites of antihypoglycemic target receptor and the interactions were calculated using the glide score, which was generated by the best fit of the ligand and the receptor. The ligands docked using GLIDE was graded according to their glide scoring function (most negative value). The function of scoring of the GLIDE docking program is shown in the Glide Score or the docking score. The Glide score of each ligand is screened against the receptor protein hypoglycemic target (Sherman, 2006; Friesner, 2004). The docking scores or the glide score (G-score) of the bioactive compounds were recorded and discussed.

Prediction of ADME Properties: The compounds which showed highly negative glide scored against hypoglycemic protein was selected for their ADME (Absorption, Distribution, Metabolism and Excretion) study using QikProp module. QikProp helps in determining the pharmacokinetics and pharmacodynamics of the ligand by accessing the drug like properties for over half a million compounds per hour. The significant ADME properties are: Molecular weight (MW), H-Bond donor, H-Bond acceptor and log P (O/W) were calculated using the QikProp module of Schrodinger, 2015.1) (QikProp, 2015-1).

RESULTS

GC-MS analysis: The GC-MS results showed presence of nine bioactive compounds in methanol leaf extract of *E. scaber*. The identification of the compounds was confirmed based on the peak area, retention time (RT) and molecular formula. The active principle with their RT, molecular formula, MW.

Fig 1. Chromatogram of GC-MS analysis with the methanolic extract of the plant *Elephantopus scaber*

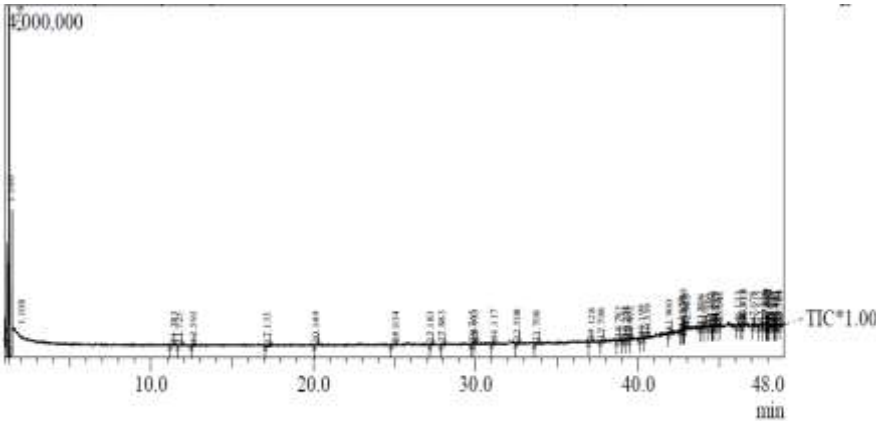


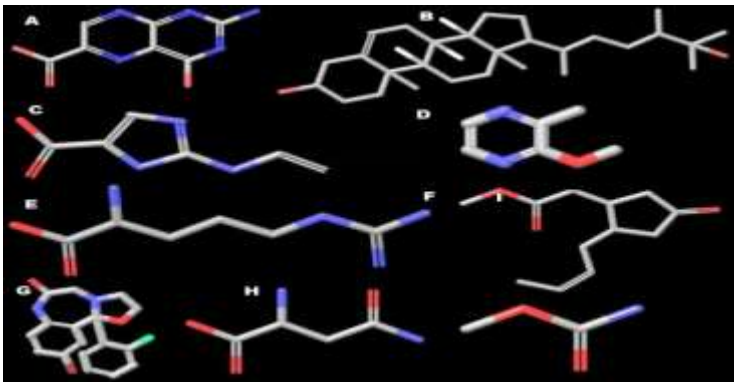
Table 1. Compounds identified in the methanolic extract of E. scaber by GC-MS analysis

Sl. No	R.T	Name of Compound	Molecular Formula	Molecular Weight	Peak Area %	Peak Height %
1	41.900	2-Amino-4-hydroxypteridine-6-Carboxylic acid	$C_7H_5N_5O_3$	207	1.11	0.12
2	42.850	25-Hydroxy-24-methylcholesterol	$C_{34}H_{64}O_2Si_2$	560	0.70	0.18
3	1.108	Imidazole-2-aminovinyl-5-carboxylic acid	$C_6H_7N_3O_2$	153	2.38	0.76
4	44.600	3-Methyl-2-Methoxy pyrazine	$C_6H_8N_2O$	124	0.60	0.14
5	39.275	Arginine	$C_6H_{14}N_4O_2$	174	0.65	0.13
6	43.806	Methyl Jasmonate	$C_{13}H_{20}O_3$	224	0.71	0.08
7	45.041	Haloxazolam	$C_{17}H_{14}BrFN_2O_2$	379	0.81	0.19
8	40.199	Asparagin	$C_4H_8N_2O_3$	132	0.56	0.11
9	40.559	Carbamic acid methyl ester	$C_2H_5NO_2$	75	0.99	0.13

***In silico* studies**

Fig 2 The 3D structures of various ligands: 2-Amino-4-hydroxypteridine-6- Carboxylic acid (**A**); 25-Hydroxy-24-methylcholesterol (**B**); Imidazole-2-aminovinyl-5-carboxylic acid (**C**); 3-Methyl-2-Methoxy pyrazine (**D**); Arginine (**E**); Methyl Jasmonate (**F**); Haloxazolam (**G**); Asparagin (**H**); and Carbamic acid methyl ester (**I**).

Table 2 Docking results of Anti-hypoglycemic Protein PPAR-γ with 9 bioactive compounds identified using GCMS analysis of Methanol extract of *E. scaber*



S.No	Compound	Glide score (kcal/mol)	Glide energy (kcal/mol)
1	2-Amino-4-hydroxypteridine-6-Carboxylic acid	**-5.0**	-33.43
2	25-Hydroxy-24-methylcholesterol	**-8.7**	-16.91
3	Imidazole-2-aminovinyl-5-carbo-xylic acid	**-4.5**	-29.21
4	3-Methyl-2-Methoxy pyrazine	**-3.7**	-17.54
5	Arginine	-1.2	-25.45
6	Methyl Jasmonate	**-6.1**	-29.79
7	Haloxazolam	**-5.8**	-28.87
8	Asparagin	**-4.3**	-23.07
9	Carbamic acid methyl ester	-3.3	-21.42

Table.3. ADME (Lipinski) properties of selected six bioactive compounds identified from the GCMS analysis of methanol extract (*E. scaber)*

S.No	Compound	Molecular Weight	H-Bond Donor	H-Bond Acceptor	Log P O/W	Rule of 5
1	25-Hydroxy-24-methyl-cholesterol	416.686	2	2.45	6.546	1
2	Methyl Jasmonate	210.272	0	4	1.767	0
3	Haloxazolam	377.212	1	4.25	3.168	0
4	2-Amino-4-hydroxy-Pteridine-6-Carboxylic acid	207.148	4	8	1.598	0
5	Imidazole-2-aminovinyl-5-carboxylic acid	153.14	2	4.5	0.213	0
6	Asparagin	132.119	4	4.5	4.086	0

The three dimensional (3D) and two dimensional (2D) ligand interaction diagram shows the interaction between the amino acids of PPAR-γ protein and the ligands. The lead ligand/ molecules selected were namely, 25-Hydroxy-24-methyl-cholesterol, Methyl Jasmonate, Haloxazolam, 2-Amino-4-hydroxypteridine-6- Carboxylic acid, Imidazole-2-aminovinyl-5-carboxylic acid and Asparagin. (Fig. 3A-F).

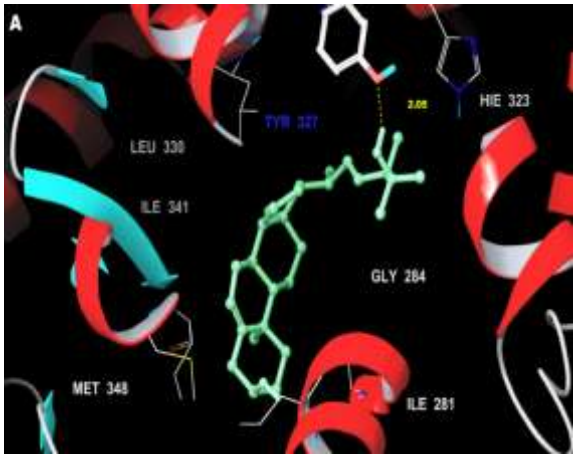


Figure. 3A. 25-Hydroxy-24-methyl-cholesterol

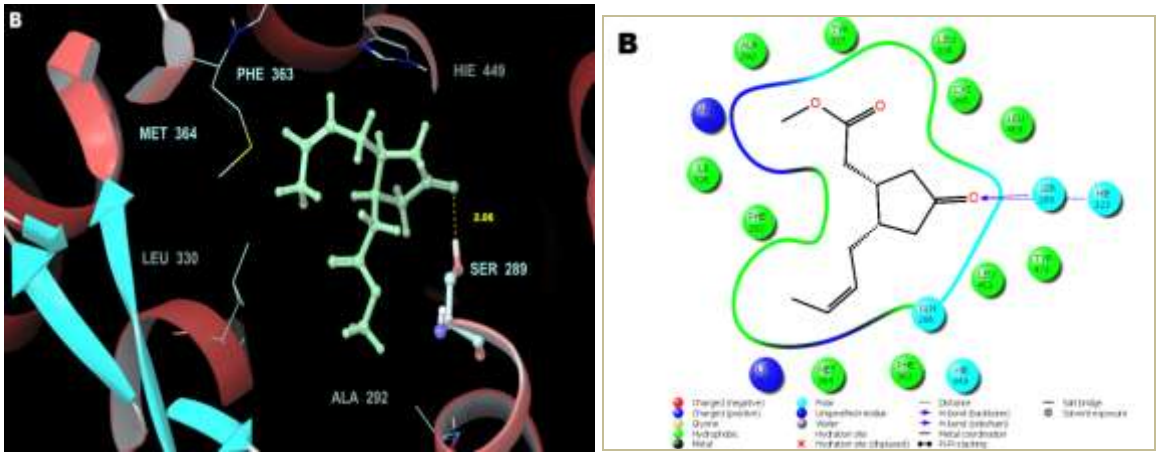


Figure. 3B. Methyl Jasmonate

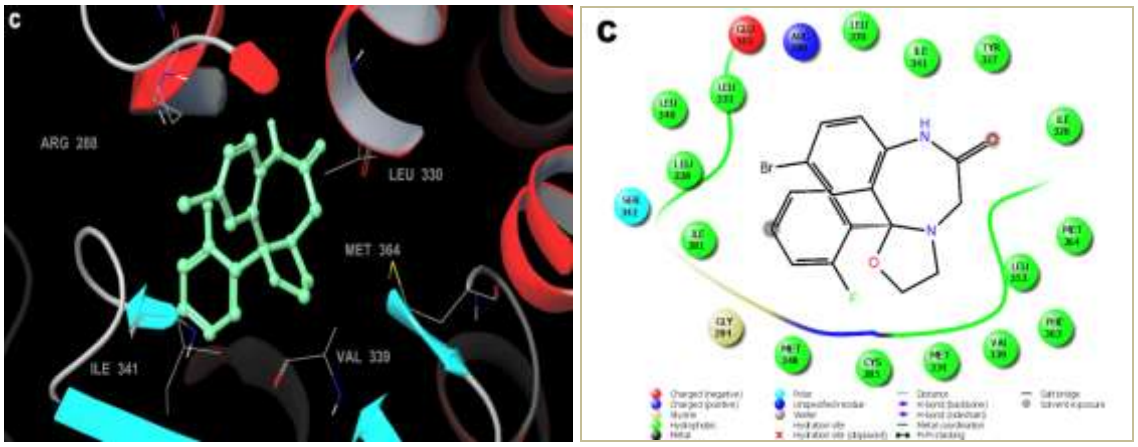


Figure. 3C. Haloxazolam

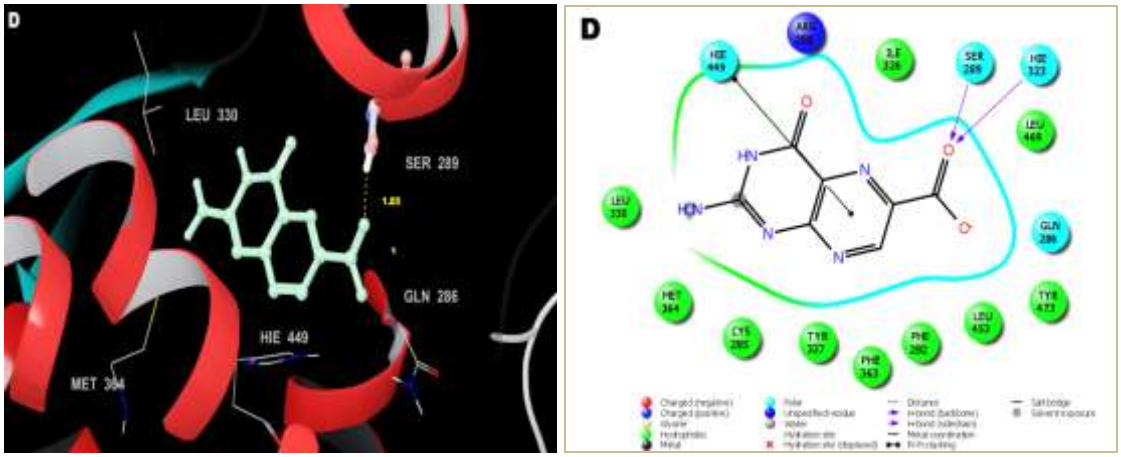


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Figure. 3D. 2-Amino-4-hydroxypteridine-6-Carboxylic acid

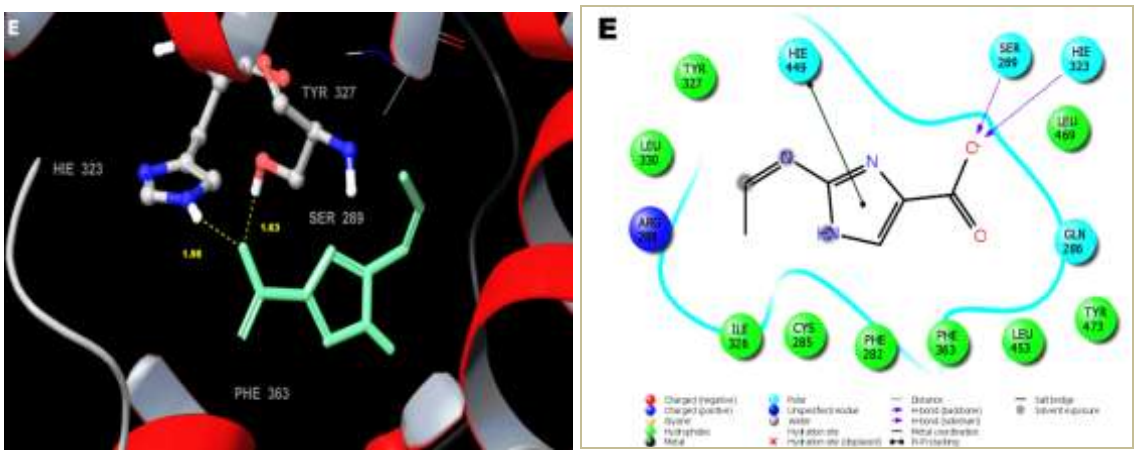

Figure. 3E. Imidazole-2-aminovinyl-5-carboxylic acid

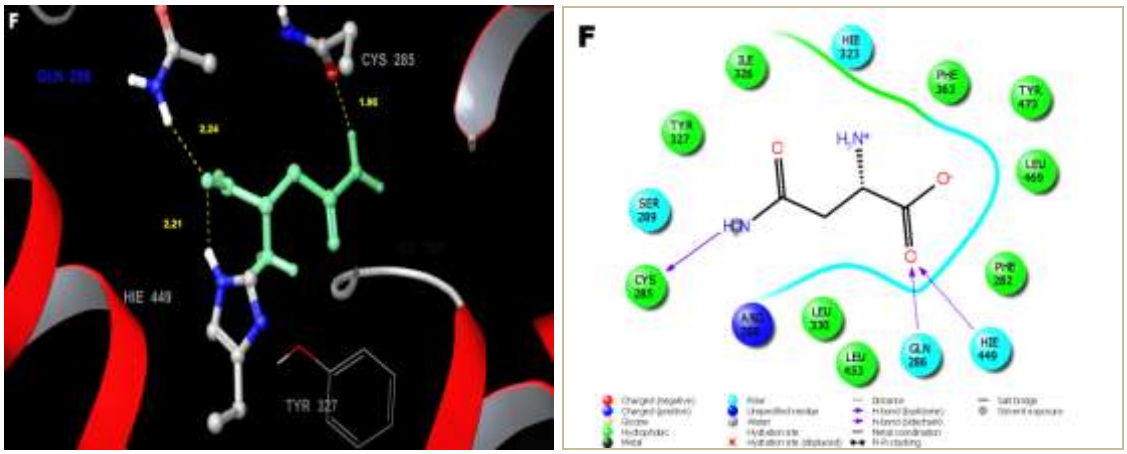

Figure. 3F. Asparagin

DISCUSSION:

The methanol, of *Elephantopus scaber* was processed for the GCMS analysis and identified 9 compounds from the methanol extract. The identified compounds from the methanol extract were named 2-Amino-4-hydroxypteridine-6- Carboxylic acid, 25-Hydroxy-24-methylcholesterol, Imidazole-2-aminovinyl-5-carboxylic acid, 3-Methyl-2-Methoxy pyrazine, Arginine, Methyl Jasmonate, Haloxazolam, Asparagin and arbamic acid methyl ester. . The total nine compounds were further used for the docking study analysis to predict the binding affinity towards the hypoglycemic PPAR-γ protein. The docking analysis was used to predict the binding orientation of the ligands to the protein targets in order to predict the affinity and activity of the small molecules. The nine molecules showed their binding interaction in the active site region (amino acids) of the PPAR-γ protein. The interaction was confirmed by using GLIDE module of Schrodinger Maestro 2015-1. The results of the docking study for the nine bioactive compounds obtained from the methanol extracts of *E. scaber* were complexed with the PPAR-γ protein are shown in the table. Among these bioactive compounds, 25-Hydroxy-24-methyl-cholesterol, Methyl Jasmonate, Haloxazolam, 2-Amino-4-hydroxy-Pteridine-6-Carboxylic acid, Imidazole-2-aminovinyl-5-carboxylic acid and Asparagin showed better Glide/docking score such as -

8.7, -6.1, -5.8, -5.0, -4.5, and -4.3 kcal/mol respectively. Also the glide energy was found to be -16.91, -29.79, -28.87, -33.43, -29.21 and -23.07 kcal/mol. respectively (Gellibert *et al.*, 2004; Fang *et al.*, 2013). The high value in negative characters of the glide score indicates that these complexes might have good affinity (Ethiraj, 2013). The 2D and 3D interaction diagram/images of 25-Hydroxy-24-methyl-cholesterol, Methyl Jasmonate, Haloxazolam, 2-Amino-4-hydroxy-Pteridine-6-Carboxylic acid, Imidazole-2-amino-vinyl-5-carboxylic acid and Asparagin (ligands) with the active site amino acids PPAR-γ protein are shown in Fig. 3 (A-F). The result indicates that the compounds namely, 25-Hydroxy-24-methyl-cholesterol, Methyl Jasmonate, Haloxazolam and 2-Amino-4-hydroxy-Pteridine-6-Carboxylic acid from the methanol extract of *Elephantopus scaber* was responsible for the hypoglycemic activity. All the high docking score compounds (6 compounds) were tested for the Pharmacokinetcic properties using QikProp module of Schrodinger 2015-1. The minimized molecules were predicted for the ADME properties (Lipinski rule factor) and the results were shown in Table 3. All the 6 compounds showed the values in the acceptable range except the compound 25-Hydroxy-24-methyl-cholesterol with one violation. Since one violation can be acceptable as per the LipinSki rule (Choy, 2011) hence, all the 6 molecules can be considered as the drug molecule for further design in future for targeting the PPAR-γ protein for the treatment of diabetes mellitus

CONCLUSION

In this study, the nine bioactive compounds were identified and isolated from the methanol extract of *Elephantopus scaber* using GCMS analysis. The identified compounds were drawn using the ChemDraw, energy minimized and converted into 3D structures using LigPrep module of Schrodinger maestro 2015-1. The compounds were tested using *in silico* analysis to identify their potential hypoglycemic activity. The molecular docking studies were performed with the help of Schrodinger Maestro software (Version 2015.1). The results of the study showed that out of these 6 bioactive compounds namely 25-Hydroxy-24-methyl-cholesterol, Methyl Jasmonate, Haloxazolam, 2-Amino-4-hydroxy-Pteridine-6-Carboxylic acid, Imidazole-2-aminovinyl-5-carboxylic acid, Asparagin showed better interaction in binding with the PPAR-γ protein. The bioactive compounds with high negative value in GLIDE score were chosen (decending order) as the best score. The docking/Glide scores were helpful in predicting that the bioactive compounds would act as good agonist for PPAR-γ protein. The research findings from the present study have shown 6 compounds for future research in the development of novel PPAR-γ agonist to treat diabetes mellitus.

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