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IN SILICO STUDIES OF COMPOUNDS ISOLATED FROM METHANOL EXTRACT OF *ELEPHANTOPUS SCABER* BY GCMS AGAINST ANTI-ARTHRITIC PROTEIN PAD4

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ABSTRACT

Protein Arginine Deiminase4 is an enzyme responsible for the citrullination of antigens in rheumantoid arthritis. PADS are calcium dependent enzymes because PADS rely on increased concentration of calcium to citrullinate peptidyl arginine. PADS are not normally active until stimulated with calcium. C1 amidase, F4 amidase are the most potent PAD inhibitor. The present study, evaluated the binding of certain GCMS derived compounds of the methanol extract of "*Elephantopus scaber"* against the Antiarthritic protein PAD-4. 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 six compounds are -7.9, -5.2, -4.87, -3.77, -3.63, -3.33 kcaSl/mol respectively and the glide energy were -33.83, 24.80, -30.06,-34.67, -30.38 and -16.29 kcal/mol. The other properties such as Lipinski rule, absorption, distribution, metabolism, excretion (ADME), toxicity predictions, logP values and % of human oral absorption were analyzed using high-throughput screening. The results of our study showed that out of 9 compounds, six compounds of *E. scaber* may act as good inhibitors for arthritics and the compounds can be re-designed for better antiarthritic activity for synthesis.

INTRODUCTION:

Elephantopus scaber Linn. Commonly known as Elephant's foot, (Asteraceae) is a scabrescent aromatic herb distributed in the moist deciduous forests of central Western Ghats. According to the traditional claims, the roots were used as an antipyretic, cardiotonic, dysuria, diarrhea, dysentery, stomach pain, and diuretic. The leaf extract had already been evaluated for diuretic, anti-inflammatory, hepatoprotective effect, and also treatment for eczema and ulcers. *E. scaber* is used in Chinese medicine for the treatment of some types of cancer. Bioactivity screening of the extracts and their compounds had shown that *E. scaber* possess wound healing, anti-venom,

anti-microbial, antiinflammatory, anti-diabetic, cytotoxic and anti-tumour activities (Ho *et al.,* 2009). The "protein arginine deiminase 4" (PAD4) is an enzyme that can hydrolyze the peptidyl arginine residues and converted to citrulline and ammonia. This protein has been implicated in several disease states namely rheumatoid arthritis and therefore considred as a unique target for the development of a novel therapeutic anti-anthritic drug (Corey *et al.,* 2008). The pathway of Human peptidylarginine deiminase 4 (PAD4) includes are i) regulates histone Arg methylation by converting methyl-Arg to citrulline and releasing methylamine ii) PAD4 targets multiple sites in histones H3 and H4, including those sites methylated by coactivators CARM1 (H3 Arg17) and PRMT1 (H4 Arg3) (Wang *et al.,* 2004). *In silico* technique is an inexpensive technique that shortens the length of time spending in testing the efficacy drugs. Hence, the present study focused on the identification of bioactive compounds present in methanol extract of *E. scaber* through gas chromatography-mass spectrometry (GC-MS) analysis and to screen the potential bioactive compounds as an antiarthritic agent by molecular docking analysis studies against protein PAD4.

MATERIALS AND METHODS

Plant collection

The plant materials were collected from the local areas. It was authenticated by Dr. D.V. Swami, Assitant Professor from Dr. Y.S.R. Horticulture University, Venkataramanna gudem - 534101, West Godhavari. District from Andra 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 Antiarthritic target protein/receptor with a resolution of 2.3 Å (PDB entry **1WDA**) and 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 antiarthritic 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 Antiarthritic target and hypoglycemic **(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 Antiarthritic and hypoglycemic proteins were 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).

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

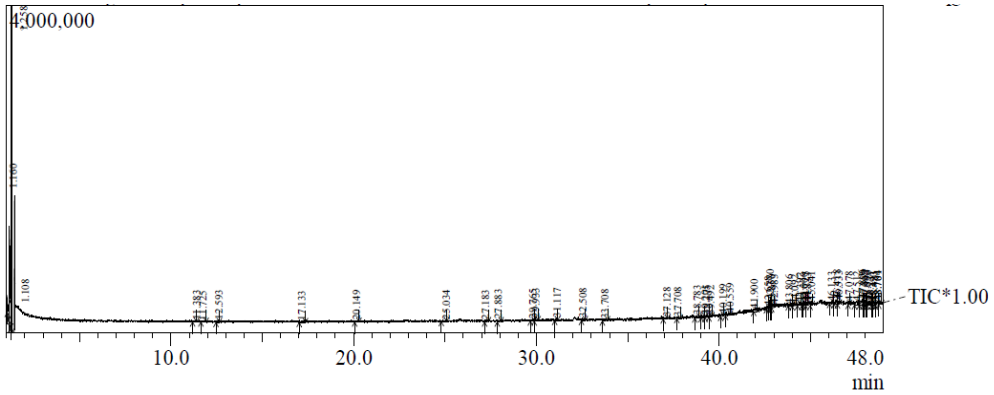
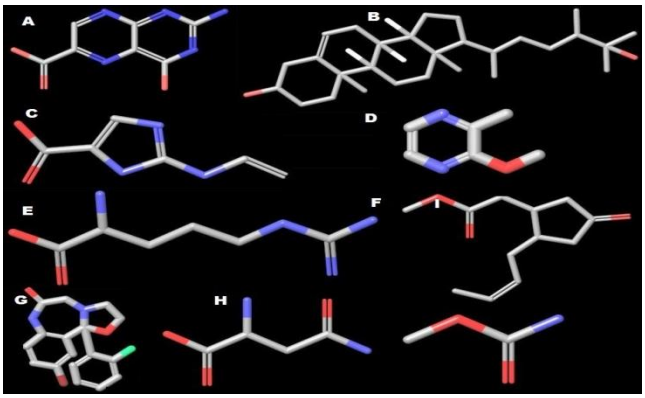


Table 1: The compounds with their molecular formula and its percentage calculated from the chromatogram of GCMS (methanolic extract of *Elephantopus scaber*) using NIST libraries

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

The 2D structures of ligands were converted into 3D structure using the LigPrep module of Schrodinger maestro is given in Fig 2.

Fig 2 The 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-Arthritic protein PAD4 with 9 bioactive compounds identified using GCMS analysis of Methanol extract of *Elephantopus scaber*

S.No	Compound	Glide score (kcal/mol)	Glide energy (kcal/mol)
1	2-Amino-4-hydroxypteridine-6-Carboxylic acid	**-4.87**	**-30.06**
2	25-Hydroxy-24-methylcholesterol	**-3.77**	**-34.67**
3	Imidazole-2-aminovinyl-5-carbo-xylic acid	**-3.33**	**-16.29**
4	3-Methyl-2-Methoxy pyrazine	-2.22	-13.32
5	Arginine	**-7.9**	**-33.83**
6	Methyl Jasmonate	-2.97	-23.64
7	Haloxazolam	**-3.63**	**-30.38**
8	Asparagin	**-5.2**	**-24.80**
9	Carbamic acid methyl ester	-1.91	-15.65

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

S.No	Compound	Molecular Weight	H-Bond Donor	H-Bond Acceptor	Log P O/W	Rule of 5
1	Arginine	174.202	7	5	3.594	1
2	Asparagin	132.119	4	4.5	4.086	0
3	2-Amino-4-hydroxy-pteridine-6- Carboxylic acid	207.148	4	8	1.598	0
4	25-Hydroxy-24-methyl-cholesterol	416.686	2	2.45	6.546	1
5	Haloxazolam	377.212	1	4.25	3.168	0
6	Imidazole-2-aminovinyl-5-carboxylic acid	153.14	2	4.5	0.213	0

The 3D and 2D ligand interaction diagram shows the interaction between the amino acids of protein PAD4 and the ligands namely, Arginine, Asparagin, 2-Amino-4-hydroxy- pteridine-6- Carboxylic acid, 25-Hydroxy-24-methyl-cholesterol, Haloxazolam and Imidazole-2-aminovinyl-5-carboxylic acid.

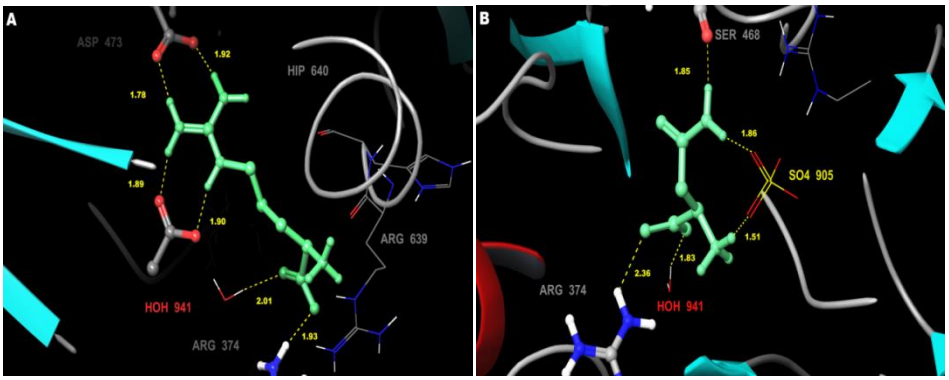


Fig. 2. A. Arginine interaction with the amino acids of PAD4 protein.
Fig 2. B. Asaparagin interaction with the amino acids of PAD4 protein.

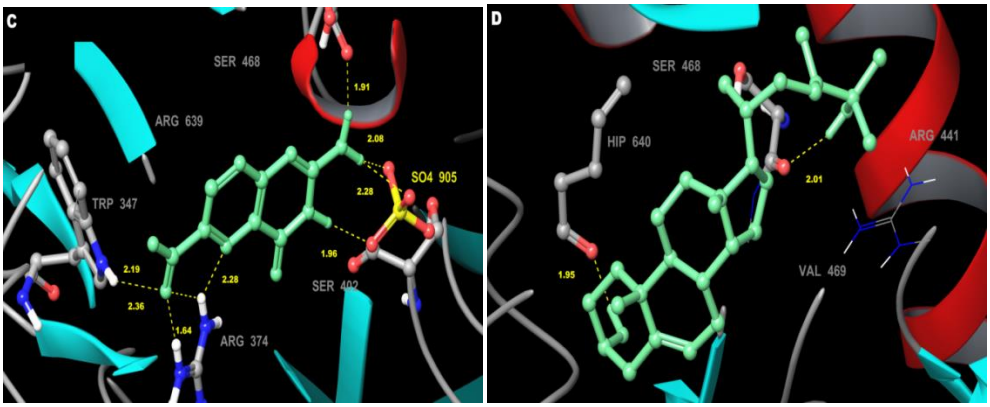


Fig. 2. C. 2-Amino-4-hydroxypteridine-6- Carboxylic acid interaction with the amino acids of PAD4 protein

Figure.2.D. 25-Hydroxy-24-methyl- cholesterol interaction with the amino acids of PAD4 protein.

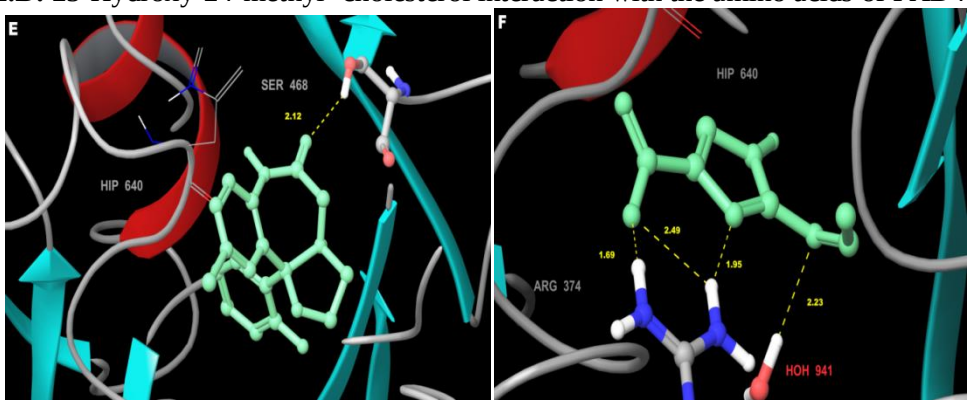


Fig. 2.E. Haloxazolam interaction with the amino acids of PAD4 protein

Fig. 2.F. Imidazole-2-aminovinyl-5-carboxylic acid interaction with the amino acids of PAD4 protein.

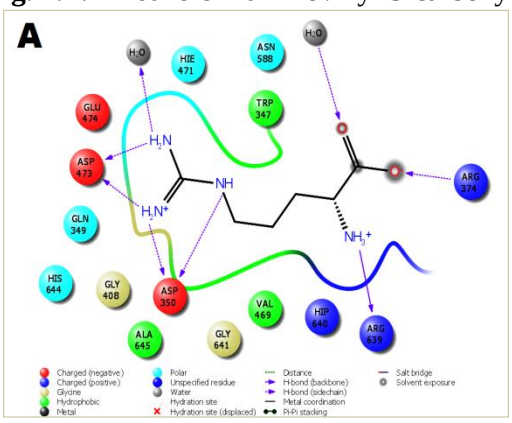
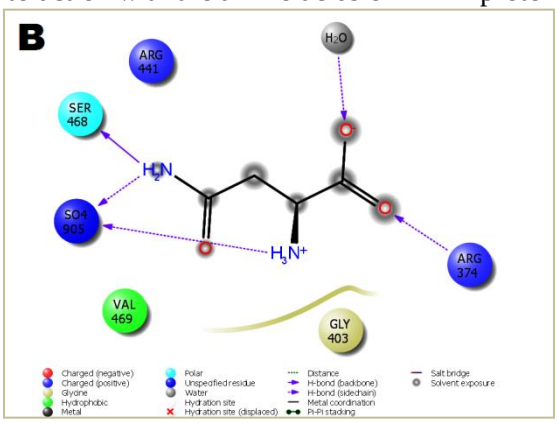


Fig 3.A.



2D docking poses of Arginine in the active site of PAD4 protein.

Fig. 3.B. 2D docking poses of Asparagin in the active site of PAD4 protein.

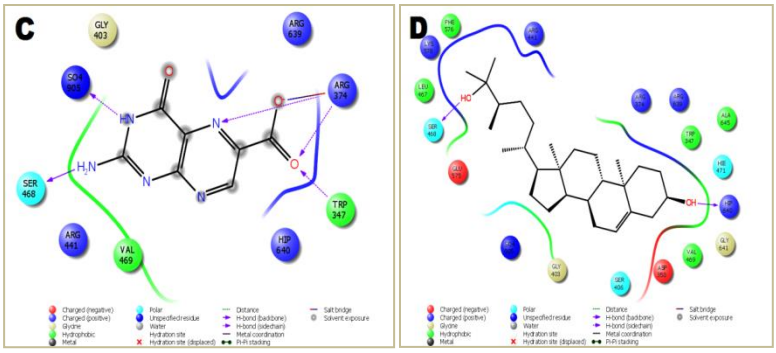


Fig.3.C. 2D docking poses of 2-Amino-4-hydroxypteridine-6-Carboxylic acid in the active site of PAD4 protein.

Fig.3.D. 2D docking poses of 25-Hydroxy-24-methyl- cholesterol in the active site of PAD4 protein.

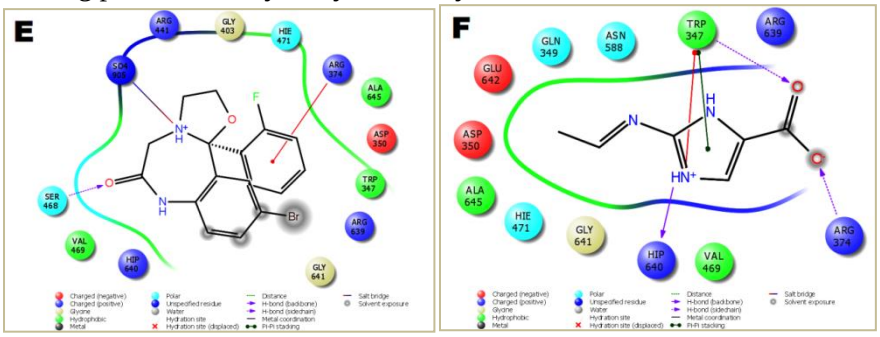


Fig.3.E. 2D docking poses of Haloxazolam in the active site of PAD4 protein.

Fig.3.F. 2D docking poses of Imidazole-2-aminovinyl-5-carboxylic acid in the active site of PAD4 protein.

RESULTS AND DISCUSSION:

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, and peak area in percentage are presented in Fig. 1 and Table 1. The compounds present in the methanol extract of *Elephantopus scaber* were docked with the active site amino acid of the Protein PAD4. Their affinity and interaction towards the Protein PAD4 were confirmed using Glide score (Maestro 9.4). The docking analysis was used to predict the binding affinity of the ligands to the protein targets in order to predict the interactions of the small molecules. The docking results of nine bioactive compounds from the methanolic extract of *Elephantopus scaber* were complexes with the protein PAD4 are shown in Table.8.1. Out of the nine compounds, six compounds namely, Arginine, Asparagin, 2-Amino-4-hydroxypteridine-6-Carboxylic acid, 25-Hydroxy-24-methyl-cholesterol, Haloxazolam and Imidazole-2-aminovinyl-5-carboxylic acid were showed better Glide score. The docking or Glide score of the above six compounds are -7.9, -5.2, -4.87, -3.77, -3.63, -3.33 kcal/mol respectively and the glide energy were -33.83, 24.80, -30.06,-34.67, -30.38 and -16.29 kcal/mol respectively. The ligand complexes with the Protein PAD4 are shown in Figure.8.3A-3I. The order of the highest negative glide score and the glide energy indicated that these complexes may have good affinity to PAD4 (Friesner, 2004). The 2D ligand interactions with active amino acid site of PAD4 are shown in Figure.8.4A-

4I. The selected six compounds namely, Arginine, Asparagin, 2-Amino-4-hydroxypteridine-6- Carboxylic acid, 25-Hydroxy-24-methyl-cholesterol, Haloxazolam and Imidazole-2-aminovinyl-5-carboxylic acid were evaluated for their ADME (Lipinski Rule factor) properties using QikProp module and the limitation of its properties (Walker, 2013) are: (a) not more than 5 hydrogen bond donors, (b) not more than 10 hydrogen bond acceptors, (c) the molecular weight less than 500 Daltons; (d) an octanol- water partition coefficient log P not greater than 5 (e). The QikProp module results showed that the ADME (Lipinski rule factor) properties of the selected five bioactive compounds were under acceptable range (Table.8.2).

CONCLUSION

Nine bioactive compounds were identified from the methanol extract of *Elephantopus scaber* using GCMS analysis. The molecular docking studies with six compounds namely, Arginine, Asparagin, 2-Amino-4-hydroxypteridine-6-Carboxylic acid, 25-Hydroxy-24-methyl-cholesterol, Haloxazolam and Imidazole-2-aminovinyl-5-carboxylic acid showed good interaction with the active site of the PAD4 protein. The results of the study further confirmed a successful docking, intermolecular hydrogen bonding and hydrophobic interactions with the ligand "PAD4" receptor. Interestingly these six compounds possess good inhibitor activity against PAD4 protein. In addition to these the ADME (Lipinski) properties of the six compounds were within the permitted limit. Hence these ligands could be used to develop a novel PAD4 inhibitor for the treatment of Arthritis.

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