

# ISOLATION AND PHARMACOLOGICAL SCREENING OF VARIOUS COMPONENTS OF ALOE BARBADENSIS AGAINST A PDE SUBCLASS

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**ABSTRACT** - Various components from *A. barbadensis* were isolated and evaluated for anti-inflammatory activity. Among the synthesized molecules, compound 12, 13 and 24 were found to possess optimum PDE4D inhibition. Further docking studies were also performed to determine the binding affinity; and the results reveal compound 12 has better binding energy value comparative to compound 13 and 14. These results suggest it can be used as a feed additive to achieve anti-inflammatory property.

**Key words:** *A. barbadensis*, isolation, PDE inhibition, molecular docking, anti-inflammatory action

## INTRODUCTION

Super family of PDE are large and complex. It represents 11 gene families (PDE1-11) each containing 1-4 genes generating multiple isoforms. <sup>[1]</sup> All members differ in regulation, tissue distribution, and specificity to inhibitor. PDE enzymes are found in various regions of cell organelles and the cytoskeleton of a cell. <sup>[2]</sup> These have been regulated via intracellular cyclic nucleotide concentrations, phosphorylation, regulatory protein interactions, compartmentalization, and binding of Ca<sup>2+</sup>/calmodulin, as well as by alterations in expression of genes. <sup>[3, 4]</sup> PDE3, 4, 7 and 8 hydrolyze specifically cAMP (cAMP-PDE). PDE5, 6 and 9 hydrolyze cGMP (cGMP-PDE) specifically, and isozymes PDE1 and 2 hydrolyze both nucleotides. <sup>[1]</sup> Structure of the PDE super family generally consists of the key features such as catalytic core, amino-acid terminal that imparts isoform specificity and regulatory region b/w the amino-acid terminal and catalytic core. <sup>[5]</sup> Literature survey have revealed various studies done on *Aloe barbadensis*. Qiu Z et al., in 2000; obtained polysaccharide by partially digesting with cellulose and purified by dialysis, precipitation and size exclusion chromatography and then evaluated for immunogenicity action. <sup>[6]</sup> Hu Y et al., in 2003 evaluated anti-oxidant activity of the extracts of *Aloe Barbadensis* and compared the results with BHT and  $\alpha$ -tocopherol via DPPH radical scavenging methodology. <sup>[7]</sup> In 2007, Chandan BK et al., evaluated for hepatoprotective function of *Aloe Barbadensis* against CCl<sub>4</sub> induced hepatotoxicity. <sup>[8]</sup> Egesie UG et al, in 2011, from their study suggests, extracts of *A. barbadensis* possess anti-inflammatory property via inhibiting PDE4D. <sup>[9]</sup> Hence, in the present study, we have isolated various components of *Aloe barbadensis* and evaluated the components for PDE4D inhibition and further docking studies were carried out to determine the binding pattern of the compounds with the active site of the protein.

## Plant collection

*Aloe barbadensis* is naturalized wild plants which has become native to across the world. The plant was taken from a botanical garden of Osmania University, Hyderabad and given for identification.

## Plant identification

The plant was authenticated by Prof. Sheik Mehmood Department of Botany, Osmania university.

## Drying

The leaf skin of *Aloe barbadensis* was dried under shade for few days. It was then size reduced and powdered. The powdered weight obtained was about 0.5kg.

## Extraction

The powdered material of *Aloe barbadensis* was extracted thrice in 95% Ethanol and was filtered under low pressure as the compounds are dissolved more efficiently under reduced pressure. This led to a dark residue of about 34.3g. The crude extract of *A. barbadensis* was then suspended in distilled water in a quantity of about 500ml and fractioned thrice in a separating funnel using petroleum ether, ethylacetate and n-butanol separately. This was then concentrated to obtain a dark viscous fluid which was then subjected for chromatography to isolate compounds.

## Isolation

### Column preparation:

Preheated column was taken and a cotton swab is kept at the bottom. The outlet knob is turned off. The silica gel-G was added from the top using a funnel up to 3/4<sup>th</sup> of the size of the column. After the silica gel was added, it was tapped to make the silica gel particles to set tight without any gap. Above it, another cotton swab was kept to prevent disturbance of the silica gel while sampling.

### Sample preparation:

The crude viscous fluid obtained was taken and to it, silica gel was added and with some quantity of ethyl acetate. It was then subjected for rotary evaporator to form silica gel-crude compound mixture.

### Sampling:

The silica gel-crude compound mixture was added from the top of the column such that it forms a thin layer on the silica gel bed. Above this, a cotton swap pe kept and the mobile phase which is the mixture of methanol:water (30:70 v/v).

### Collection of the fractions:

The mobile phase was allowed to run from the top through a reservoir and 10 fractions (E1 – E10) were collected each of different  $R_f$  values. Fractions E3, E5, E6, E7 and E9 were found to have various spots when observed on TLC under UV light. Fraction E3 was separately subjected for isolation to obtain compound 1 (321mg), 2 (362mg) and 3 (738mg) respectively using methanol:water 26:74 v/v. Similarly, other fractions were also subjected for isolation to obtain various compounds (compound 4- compound 16). All these isolated compounds were subjected for enzymatic and cytotoxic assay.

## Enzymatic assays

### Preparation of 0.2M zinc sulfate and 0.2N Barium hydroxide solution:

28.7 grams of hydrated zinc sulfate was dissolved in 100ml of water. It was mixed well and placed in sonicator to dissolve the residue. It was then filtered and diluted to 500ml with water.

32 grams of hydrated barium hydroxide was taken and dissolved in 250ml of water. It was sonicated and filtered followed by diluting it to obtain 500ml with water.

### Bioactivity test:

Enzymatic PDE4D was assayed via 3H-cAMP as substrate on PDE4D cell-based Report Assay Kit. <sup>3</sup>H-cAMP was primarily diluted with the buffer (50 mM Tris-HCl; pH 7.5), 10mM magnesium chloride and 0.5mM Dithiothreitol to 2×10<sup>4</sup>–3×10<sup>4</sup>cpm per assay. The reaction was performed on PDE4D enzyme kit, substrate as well as concentrations of inhibitor were mixed in DMSO at 37°C for 15min, and then terminated by addition of 0.2M of ZnSO<sub>4</sub>. The reaction product <sup>3</sup>H-AMP was precipitated out using 0.2N of Ba(OH)<sub>2</sub>, whereas unreacted <sup>3</sup>H-cAMP retained in supernatant. All isolates that were screen primarily at concentration of 10μM and IC50 values for active molecules were analyzed by nonlinear regression. Each of the test sample was analyzed in triplicate. Rolipram, a PDE4 inhibitor, was take as positive control.

## Molecular docking study

### Preparation of Protein

The protein 3D structure (PDB: 3LY2) was obtained from protein data bank (rscb.org), ligands present were removed followed by removal of water molecules and addition of gastegier charges. It was then saved as pdbqt format which was further used to perform docking study.

### Preparation of ligands

All the ligands (Comp 12, 13, 14 and rolipram) were sketched in chemBio3D Ultra (Version 14) on windows 10 operating system. The molecules after sketching, were energy-minimized using MM2 tool of chemBio3D software and saved in mol2 format.

### Molecular docking

To perform docking study, Autodock tool (4.2.6) was used. [10] The grid values for the study were taken by observing the co-ordinates of residue of the active site (GLN443) from protein viewer (spdv). These values were used to obtain grid box, and performed the study. Further visualization was done in Discovery studio visualizer.

### RESULTS AND DISCUSSION

The fractions obtained were subjected to rotary evaporation resulting in obtaining various compounds (Compound 1-16) in their purest forms. These compounds were identified from the literature as the components presents in *A. barbadensis* and were isolated based on their  $R_f$  values. The various compounds obtained include coumarin derivatives, cinnamoyl derivatives and some anthraquinone and anthrone glycosides.

#### In vitro studies (PDE4D inhibition)

All these compounds were individually screened for biological activity by performing in vitro inhibition against PDE4D. Positive standard, rolipram, showed  $IC_{50}$  of  $0.59\mu M$ , that correlates the literature data. Bioassay results show that compounds 13 and 14 to have impressive activity against PDE4D, with  $IC_{50}$  of  $9.25\mu M$  and  $4.42\mu M$ , respectively. The inhibitory curves for compounds 13, 14 as well as rolipram were represented in figure 1 and the  $IC_{50}$  values along with quantity of each compound obtained are shown in the table 1.

TABLE 1: COMPOUND WITH THEIR QUANTITY OBTAINED

S. No	Compound	Compound name	Weight (in mg)	$IC_{50}$ ( $\mu M$ )
1.	Compound 1	Isoaloesresin	321mg	$>100\mu M$
2.	Compound 2	Aloin B	362mg	$>100\mu M$
3.	Compound 3	Aloin A	732mg	$>100\mu M$
4.	Compound 4	Aloin dimer A	3mg	$>100\mu M$
5.	Compound 5	Aloin-dimer B	2mg	$>100\mu M$
6.	Compound 6	Aloin-dimer c	3mg	$>100\mu M$
7.	Compound 7	Aloin-dimer D	4mg	$>100\mu M$
8.	Compound 8	Aloeresin K	45mg	$>100\mu M$
9.	Compound 9	6'-O-acetyl-aloin	63mg	$>100\mu M$
10.	Compound 10	Aloeresin	23mg	$>100\mu M$
11.	Compound 11	6'-O-acetyl-aloin	82mg	$>100\mu M$
12.	Compound 12	Aloe-emodin	8mg	$55.68\mu M$
13.	Compound 13	Elgonica-dimer A	3mg	$9.25\mu M$
14.	Compound 14	Elgonica-dimer B	2mg	$4.42\mu M$
15.	Compound 15	Aloinoside B	36mg	$>100\mu M$
16.	Compound 16	Aloinoside A	45mg	$>100\mu M$
17.	Rolipram	--	--	$0.59\mu M$

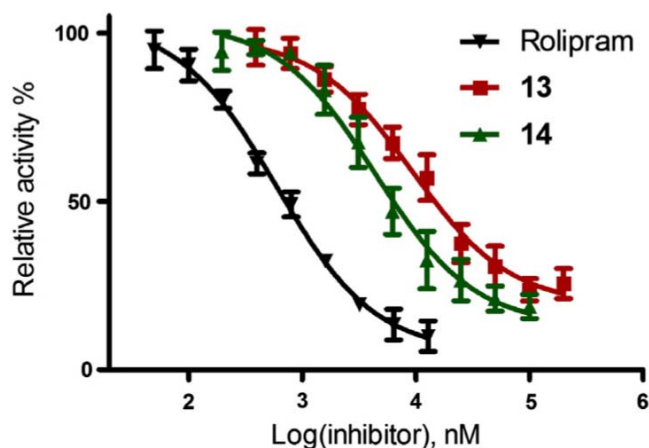


Figure 1: Inhibitory curves of active compounds

Structures of the active compounds against PDE4D (compound 12, 13 and 14) are shown in the figure 2. The Compounds 13 and 14 are observed to be a pair of diastereoisomers differing at C-10 in configuration.

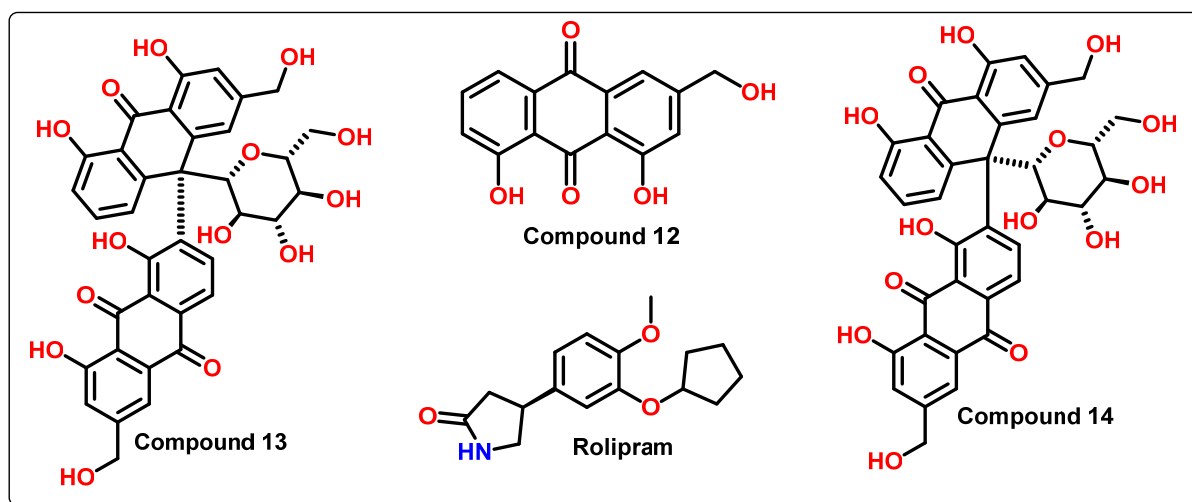


Figure 2: Structures of compound 12, 13 and 14

Interestingly, from the results obtained, it was found that anthraquinones with 10- carbonyl group like compounds 12, 13 and 14 showed the enzyme inhibitory activity especially compound 13 and 14, while compounds 2–7, 9, 11, 15 and 16 were observed to be inactive as they showed no response against PDE4D culture.

#### Docking results

The co-ordinates taken to perform docking study are: X = -6.581; Y = 45.194; Z = 46.462. From the docking results, it can be observed that, compound 13 and 14, due to their large size cannot get into the groove as shown in figure 4 and 5. However, compound 12 and rolipram, due to their small size, enters into the cavity as shown in figure 3 and 6 respectively. The greater activity of compound 13 and 14 could be as a result of a greater number of hydroxyl groups that helps in binding with the receptor. The prerequisite activity of compound 13 and 14 are due to Aloe-emodin moiety. All the docking results and their interacting amino acid are tabulated in table 2 while the docking images can be visualized in figure 3-7.

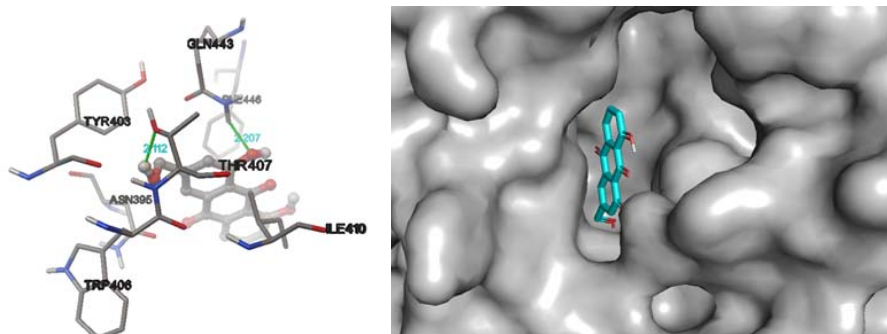


Figure 3: Docking image of C12

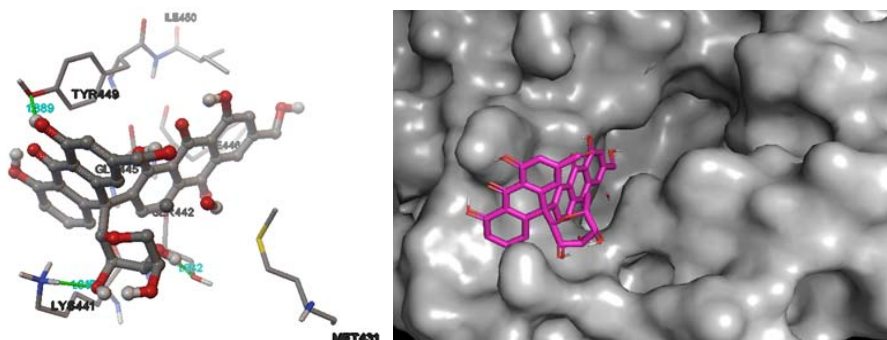


Figure 4: Docking image of C13

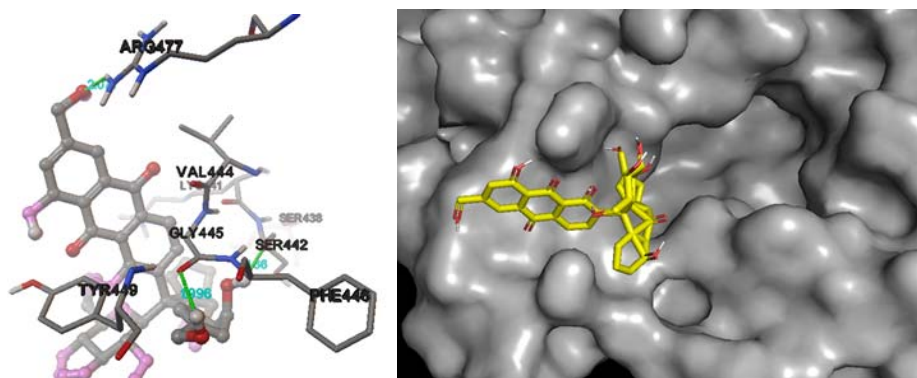


Figure 5: Docking images of C14

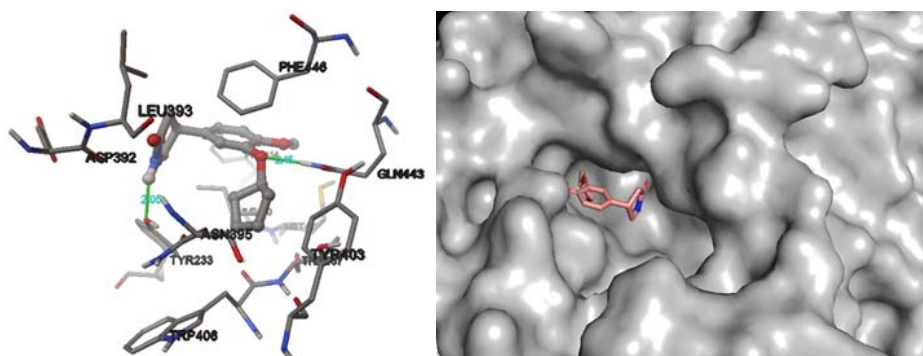


Figure 6: Docking images of rolipram

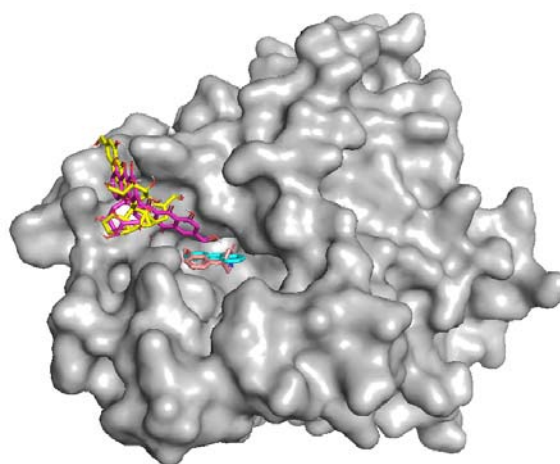


Figure 7: Binding of all molecules into the receptor (3LY2)

TABLE 2: DOCKING RESULTS OF ACTIVE COMPOUNDS

Compound	Binding energy	kI	RMSD (Å)	Interacting amino acids
Compound 12	-6.53kcal/mol	16.31μM	60.91	GLN443-2.207; THR407-2.112
Compound 13	-4.48kcal/mol	517.17μM	54.41	TYR449-1.889; LYS441-1.815; SER442-1.882
Compound 14	-5.2kcal/mol	155.29μM	57.08	ARG477-2.071; GLY445-1.996; SER442-1.66
Rolipram	-8.33kcal/mol	785.42nM	40.42	GLN443-2.45; TYR233-2.05

## CONCLUSION

In conclusion, various compounds were isolated from extract leaf skin of *A. barbadensis*. All the compounds were compared with the literature to identify the compound and subjected for bioactivity screening by enzymatic assay. In bioassay of PDE4D, active compounds 13 (IC<sub>50</sub> 9.25µM) and 14 (IC<sub>50</sub> 4.42µM) possess good PDE4D inhibition. However, from the docking study, it was observed that compound 13 and 14, due to bulkiness in their molecular structure, was unable to enter into the active site groove, compound 12 on the other hand, due to smaller size, was able to get within the groove, similar is in case of rolipram. These achievements provide evidences for the use of *A. barbadensis* leaf skin as functional feed additives for anti-inflammatory purpose. However, further investigation is necessary in order to explore their mechanism of inhibition.

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## CONFLICT OF INTREST

Nil

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