The 7th International Electronic Conference on Medicinal Chemistry (ECMC 2021)
01–30 NOVEMBER 2021 | ONLINE

Phytochemical constituents from *Indigofera conferta* unravels the lacuna in the treatment of snake bites: A pivotal study on snake venom phospholipase A_2 and metalloprotease with the aid of molecular dynamics studies

Isah Maryam[1]*, Hamza Asmau Nasiru[1], Abdullahi Maryam[1], Ahmed Elrashedy[2], Mahmoud Soliman [2,3], Aliyu Muhammad Musa[1]

[1] Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria;
[2]Molecular Bio-computation and Drug Design Laboratory, School of Health Sciences, University of KwaZulu-Natal, Westville Campus, Durban 4001, South Africa;
[3]College of Pharmacy and Pharmaceutical Sciences, Florida Agricultural and Mechanical University, FAMU, Tallahassee, Florida 32307, USA.

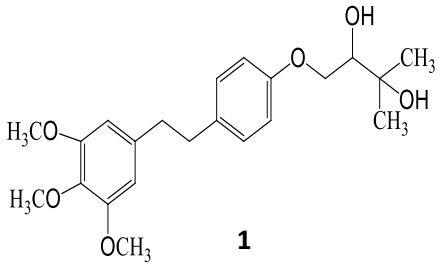
*Corresponding author: maryamisah01@gmail.com

Institution logos:

1 2 3

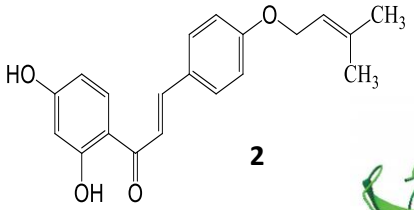
Phytochemical constituents from *Indigofera conferta* unravels the lacuna in the treatment of snake bites: A pivotal study on snake venom phospholipase A_2 and metalloprotease with the aid of molecular dynamics studies

- **Graphical Abstract**

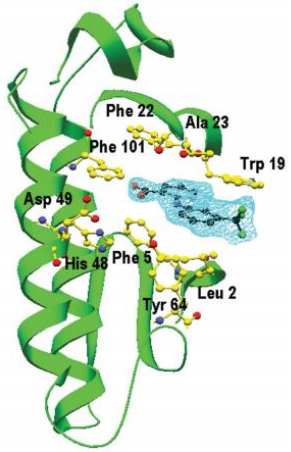


1

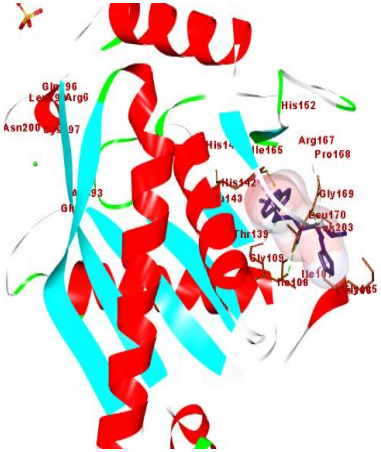
In silico anti snake venom activity



2



Crystal structure of PLA2 (1TD7)



Crystal structure of Metalloprotease (2AIG)

Molecular dynamics simulation

Abstract: Envenomation resulting from snakebite is an important public health problem particularly in rural areas of Africa, Asia, and Latin America. Phospholipase A_2 and metalloprotease are some of the principal toxic components of snake venom. Hence, the inhibition of these enzymes is of pharmacological and therapeutic interest as they are involved in several hemorrhage and inflammatory diseases. This study employed *in silico* methods to provide insights into the inhibitory mechanism of 2',4'- dihydroxy-4-prenyloxychalcone and 3,5-dimethoxy-4'-O-(2,3-dihydroxy-3-methylbutyl)-dihydrostilbene isolated from the aerial parts of *I. conferta* on PLA_2 and metalloprotease snake venom. The method includes; predicting their ADME properties, molecular docking, molecular dynamics simulation, and binding free energy calculations. The result of the MD simulation revealed the average RMSD values for the C-α backbone atoms of PLA_2 in complex with the prenylated chalcone and the prenylated stilbene to be 1.14 and 1.16 Å while that of metalloprotease in complex with prenylated chalcone and the prenylated stilbene were 1.37 and 1.18Å. Also, the electrostatic forces and van der Waals forces made a greater contribution to the total binding free energy in the PLA_2 complexes than in the metalloprotease complexes implying that the compounds exerted a greater inhibitory effect on the PLA_2 than on the Metalloprotease. The design of specific inhibitors of PLA_2 could help in the development of new pharmaceutical drugs, more specific antivenom, or even as alternative approaches for treating snakebites

Keywords: Indigofera conferta; Metalloprotease; Molecular dynamics simulation; Phospholipase A_2; Phytochemicals.

INTRODUCTION

- Envenomation resulting from snake bite is an important public health problem particularly in rural areas of tropical and sub-tropical countries situated in Africa, Asia and Latin America.
- Ethnobotanical information indicates that *Indigofera conferta* is used in northern Nigeria for the management of poisonous snakebites and the plant was previously reported to have antivenin activity
- In search for bioactive constituents responsible for the antivenin properties, we report the *In silico* evaluation of the antivenin potential of the phytochemical constituents isolated from the aerial parts of *Indigofera conferta*

AIM

- To evaluate the antivenin potential of 2' 4'-dihydroxy-4-prenyloxychalcone and 3,5-dimethoxy-4'-O-(2,3-dihydroxy-3-methylbutyl)-dihydrostilbene isolated from the aerial parts of *Indigofera conferta* using computational methods.

OBJECTIVES

- To predict the ADME properties of the characterized compounds
- To investigate via computational methods possible mechanism of inhibition of snake venom activity of the phytochemical constituents

RESULTS AND DISCUSSION

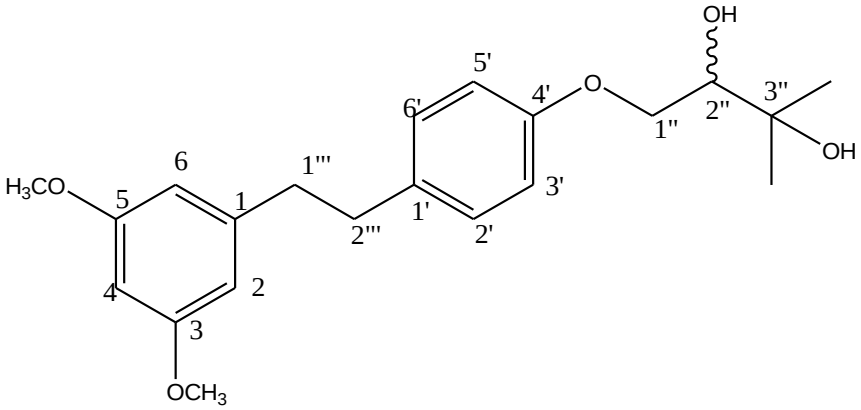


Fig. 1: 3,5-dimethoxy-4'-O-(2, 3-dihydroxy-3-methylbutyl)-dihydrostilbene (2D structure)

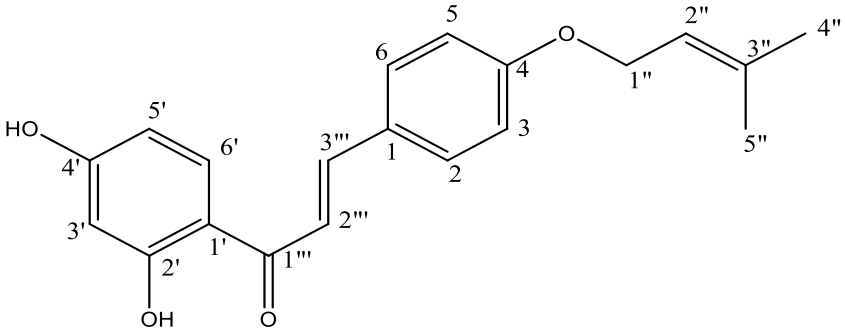
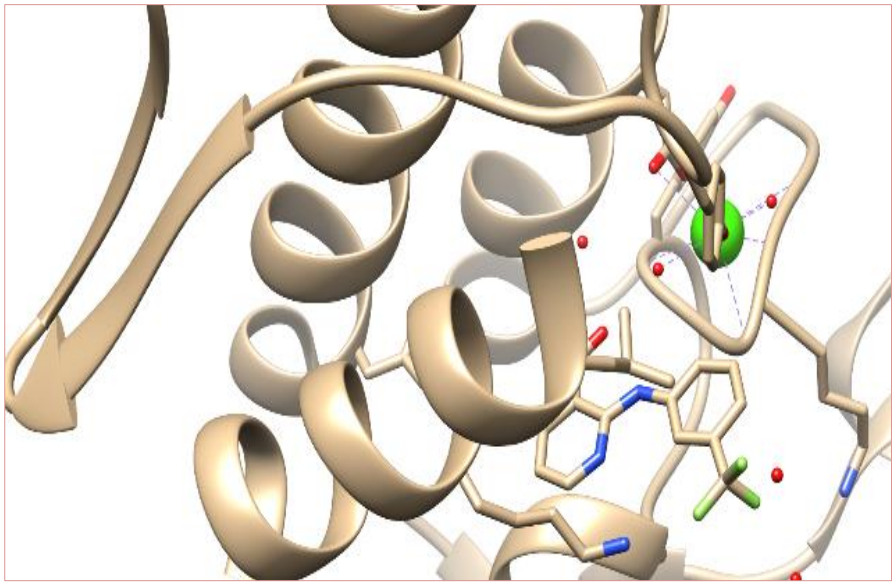


Fig. 2: 2' 4'- dihydroxy-4-prenyloxychalcone (2D structure)

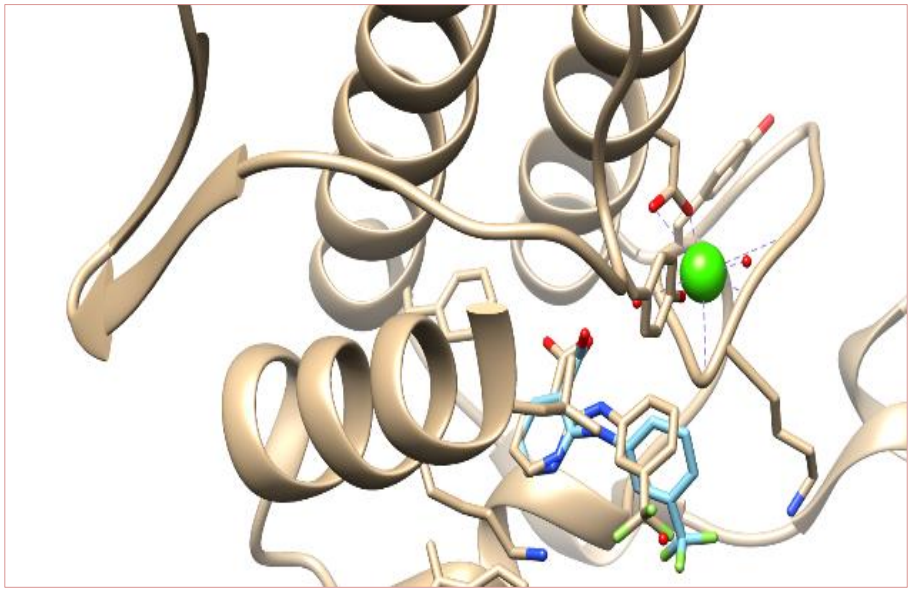
RESULTS AND DISCUSSION

Validation of Docking Procedure

Crystal structure of 1TD7 (PLA_2 and Ligand)



Crystal structure complex with Re-docked ligand (Validation)



RESULTS AND DISCUSSION

Table 1: Analysis of theoretical oral bioavailability of DDMDS and DPC based on Lipinski's rule of five

Compound ID	Lipinski's rule of five[b]					
	Mol.Wt[a]	HbA	HbD	nRB	MLogP	Inference
DDMDS	360.44	5	2	9	2.33	Pass
DPC	324.37	4	2	6	2.73	Pass

(a) Molecular weight in g/mol, (b) Lipinski *et al.,* 2001 (Mwt≤500, MLogP≤4.15, N or O≤10, NH or OH≤5 and number of rotatable bonds≤ 10), nRB: Number of rotatable bonds, LogP: Partition co-efficient, HbA: Hydrogen bond acceptor, HbD: Hydrogen bond donor, DDMDS: 3,5-dimethoxy-4'-O-(2, 3-dihydroxy-3-methylbutyl)-dihydrostilbene, DPC: 2' 4'- dihydroxy-4-prenyloxychalcone

Table 2: Showing the binding energy result of the co-crystallized ligands, DDMDS and DPC against PLA_2 and Metalloprotease

ENZYME	Affinity (kcal/mol)	Lig1	Lig2
	Lig		
PLA_2	-6.2	-7.3	-6.5
Metalloprotease	-7.0	-7.1	-6.3

Lig: Niflumic acid for PLA_2, Lig: N-(furan-2-ylcarbonyl)-L-leucyl-L-tryptophan for Metalloprotease, Lig1: 2' 4'- dihydroxy-4-prenyloxychalcone, Lig2: 3,5-dimethoxy-4'-O-(2, 3-dihydroxy-3-methylbutyl)-dihydrostilbene

RESULTS AND DISCUSSION

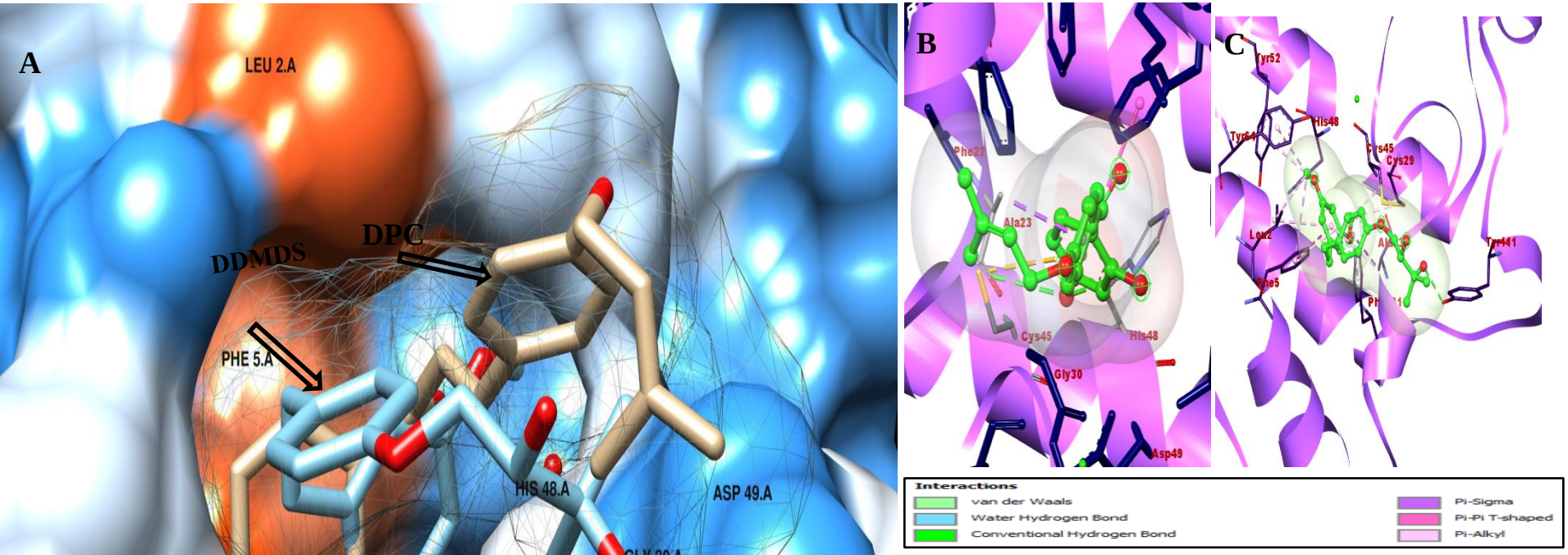


Figure 3: Molecular representation of PLA_2 with docked compounds. (A) 3D binding poses of DPC and DDMDS in the binding pocket of PLA_2, (B) 3D binding interaction of DPC in the binding cavity of PLA_2 and (C) 3D binding interaction of DDMDS in the binding cavity of PLA_2

RESULTS AND DISCUSSION

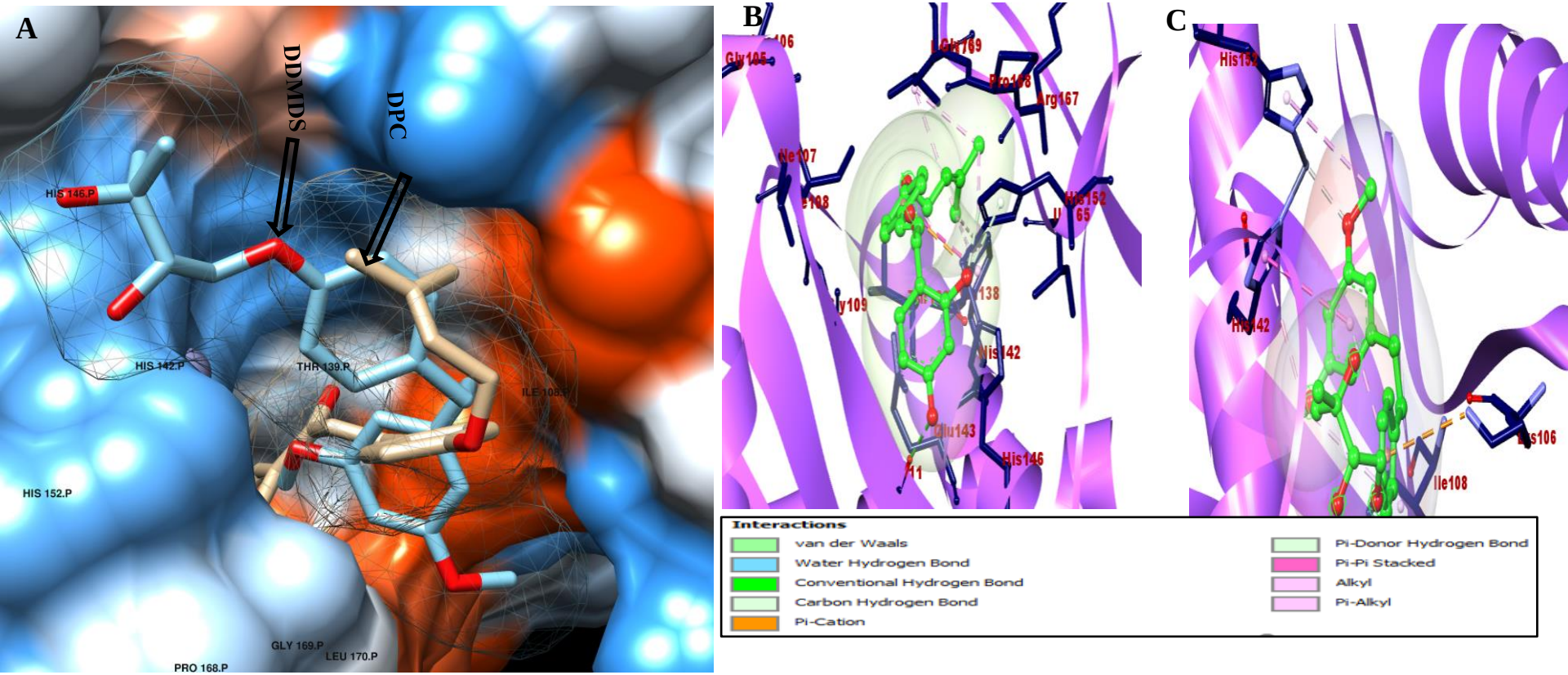


Figure 4: Molecular representation of Metalloprotease with docked compounds. (A) 3D binding poses of DPC and DDMDS in the binding pocket of Metalloprotease, (B) 3D binding interaction of DPC in the binding cavity of Metalloprotease and (C) 3D binding interaction of DDMDS in the binding cavity of Metalloprotease

RESULTS AND DISCUSSION

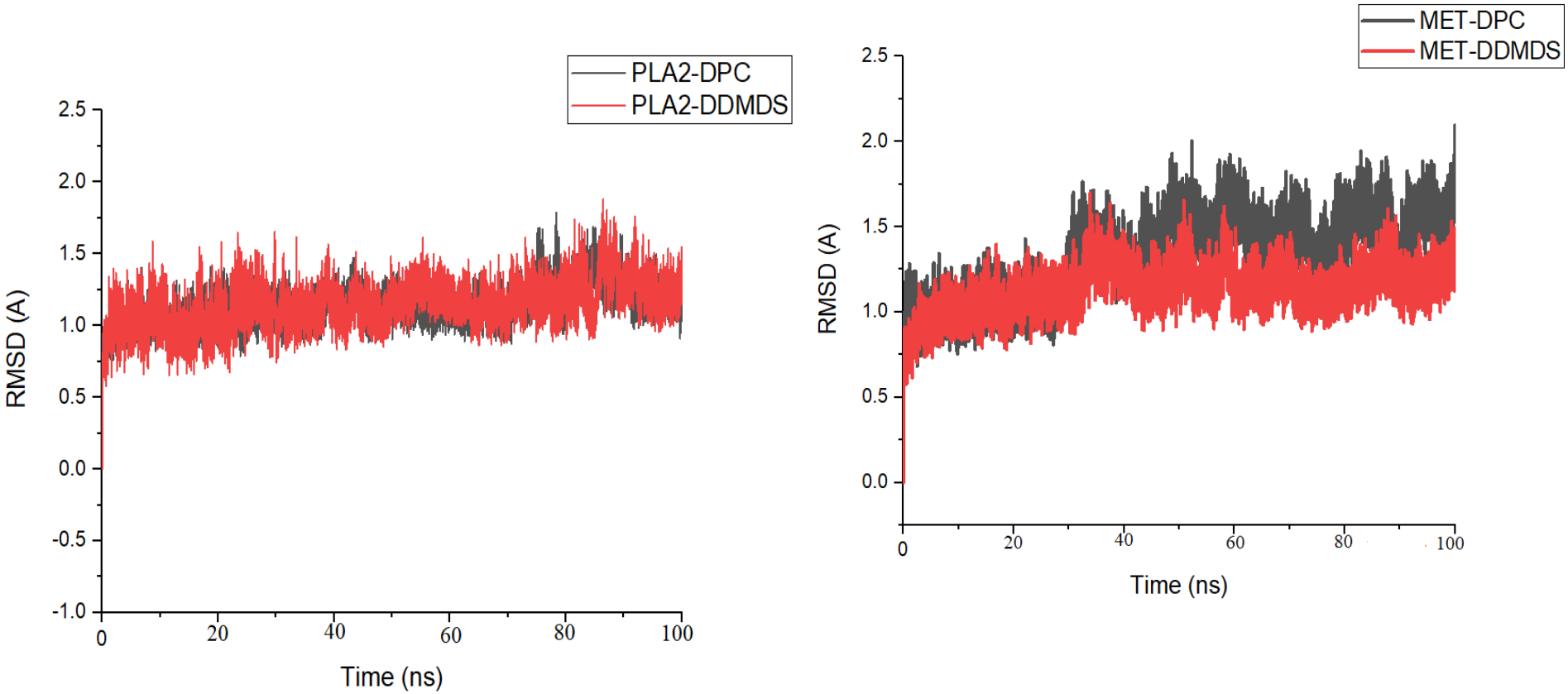


Figure 5: Comparative C-α RMSD plots of (A) PLA_2; (B) Metalloprotease bound to compound DPC and DDMDS during a 100ns simulation

RESULTS AND DISCUSSION

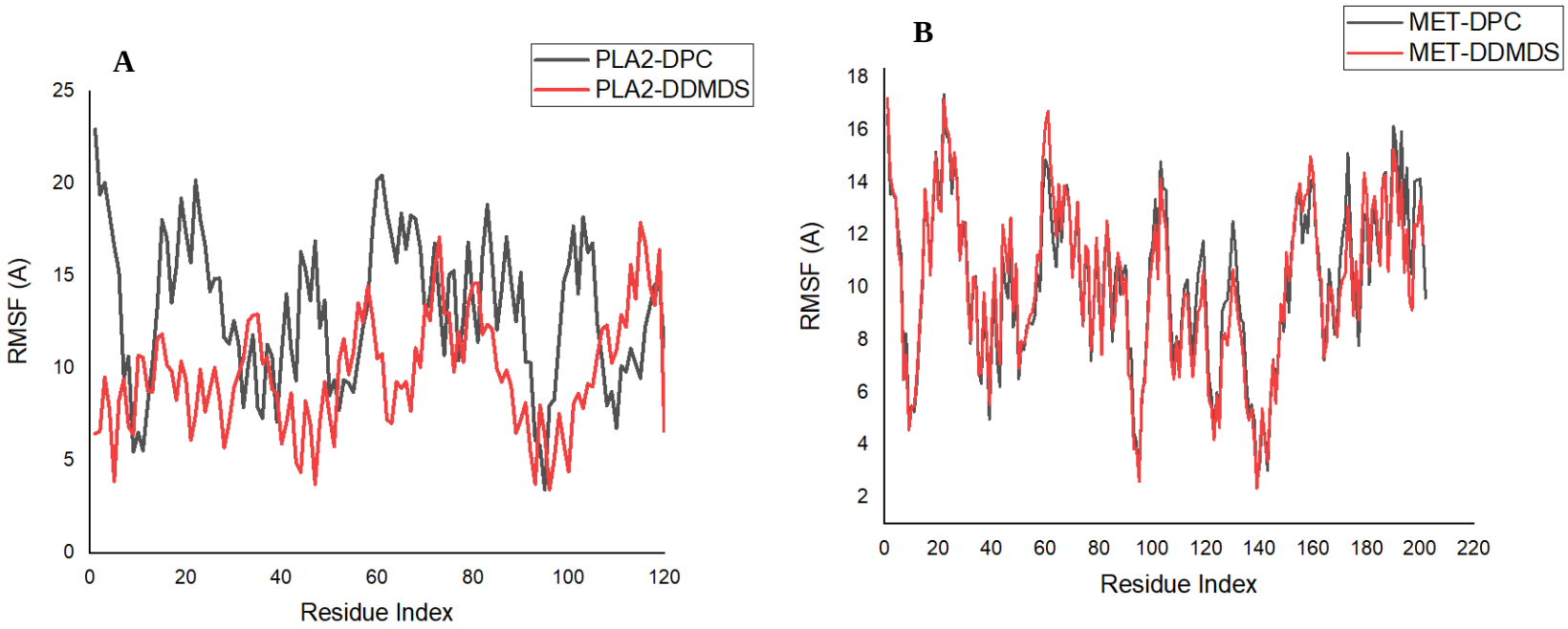
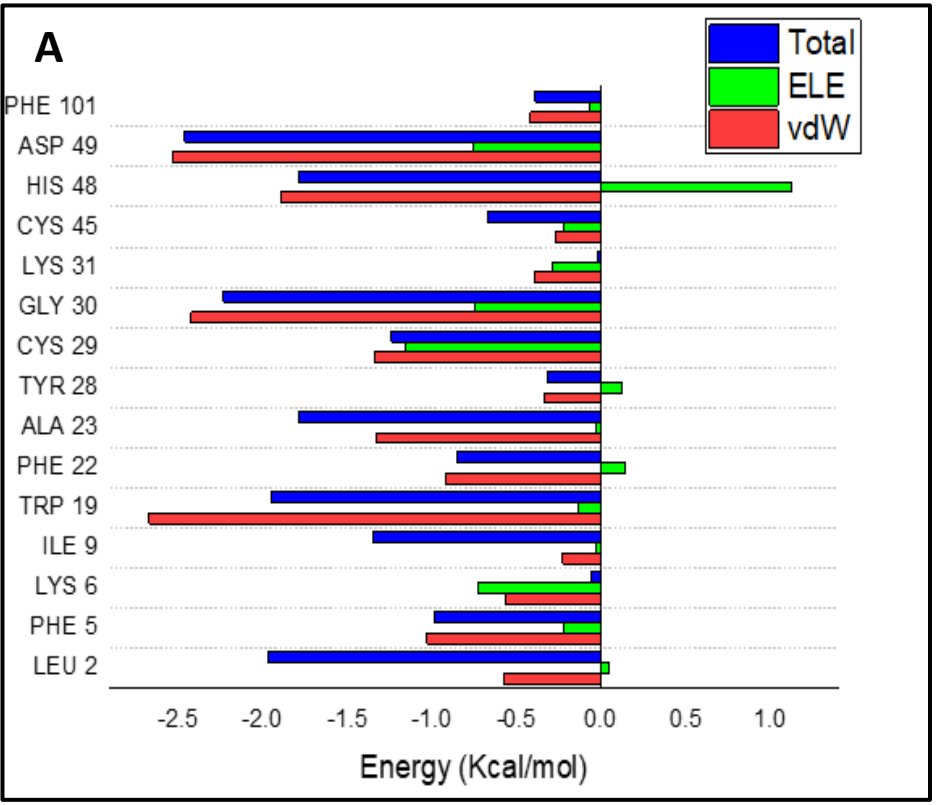


Figure 6: Root mean square of fluctuation plots of (A) PLA_2 (B) Metalloprotease bound to compound DPC and DDMDS

RESULTS AND DISCUSSION



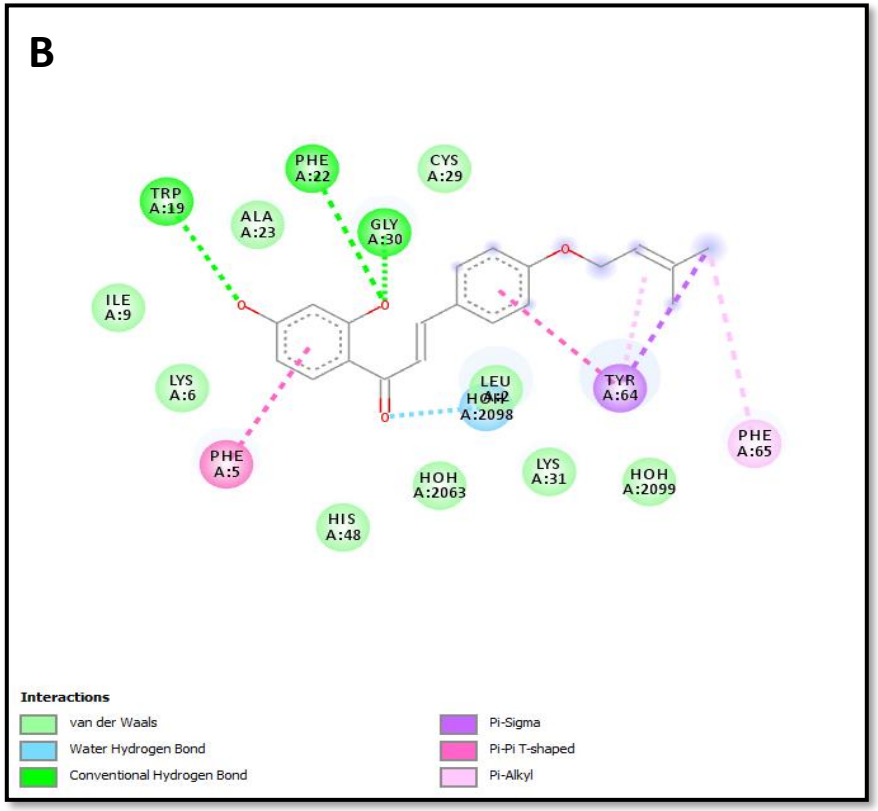


Figure 7: (A) Per residue energy decomposition plot of PLA_2 in complex with DPC and (B) 2D interactions between the active site residues of the enzyme and the compound

RESULTS AND DISCUSSION

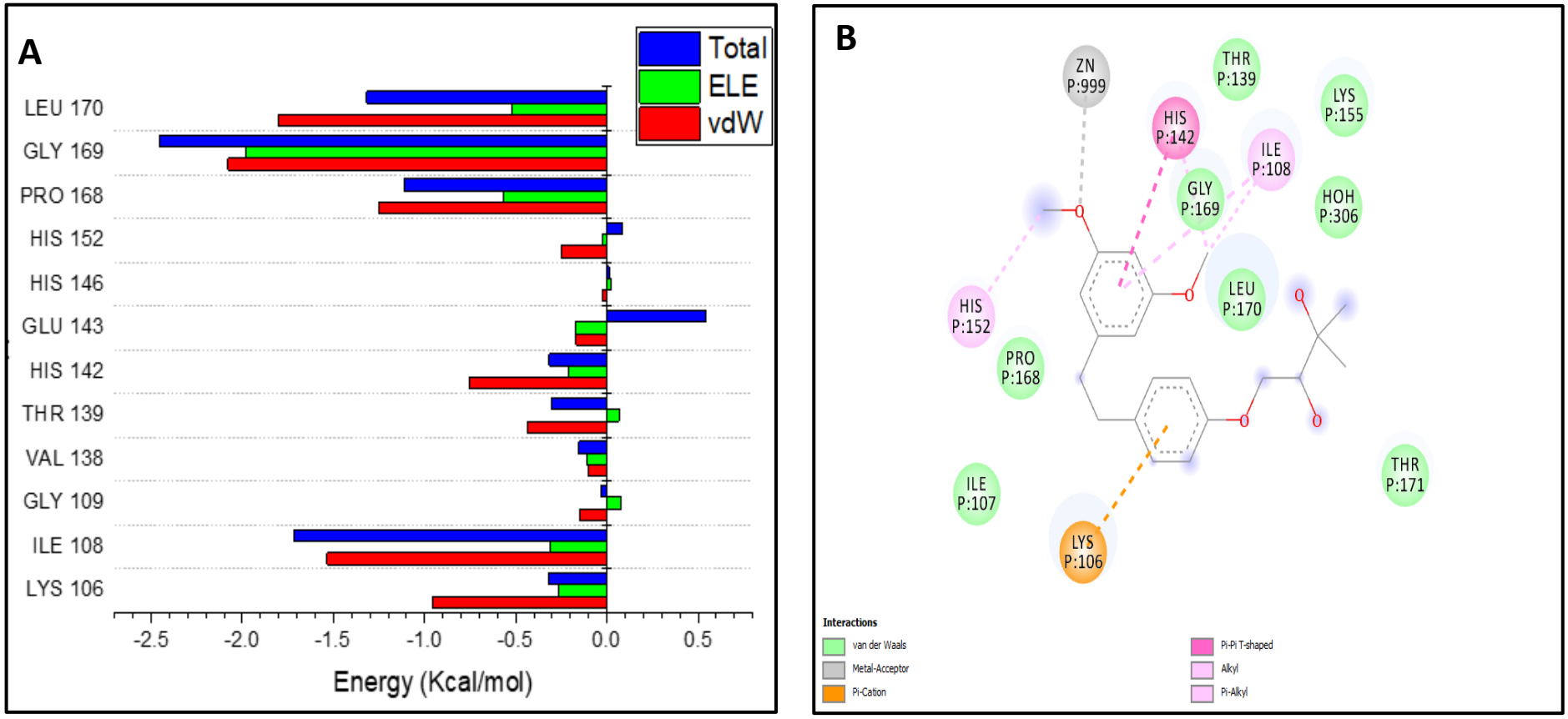


Figure 8: (A) Per residue energy decomposition plot of Metalloprotease in complex with DDMDS and (B) 2D interactions between the active site residues of the enzyme and the compounds

RESULTS AND DISCUSSION

Table 3 : MM/GBSA based binding free energy profile of inhibited PLA_2 and Metalloprotease enzymes of snake venom

Complexes	ΔE_{vdW}	ΔE_{ele}	ΔG_{gas}	ΔG_{solv}	ΔG_{bind}
PLA_2-DPC	-35.92	-26.82	-62.74	28.60	-34.13
MET-DPC	-12.99	3.30	-19.27	8.69	-10.59
PLA_2-DDMDS	-22.57	-10.48	-37.24	14.61	-19.96
MET-DDMDS	-26.75	-9.79	-22.78	17.27	-8.17

ΔEele = electrostatic energy; ΔEvdW = van der Waals energy; ΔGbind = calculated total binding free energy; ΔGsol = solvation free energy ΔG = gas phase free energy

CONCLUSIONS

- 3, 5-dimethoxy-4'-O-(2,3-dihydroxy-3-methylbutyl)-dihydrostilbene and 2' 4'-dihydroxy-4-prenyloxychalcone were isolated from the methanol aerial parts extract of *Indigofera conferta* for the first time.
- The compounds strongly inhibited PLA_2 and metalloprotease and could be the active principles in neutralizing the snake venom, thereby disclosing the molecular evidence of *Indigofera conferta*'s activity against snake venom

Acknowledgments

The authors are grateful to Mr Mzozoyana Vuyisa of the School of Chemistry and Physics, University of KwaZulu-Natal, Westville, Durban, South Africa for assisting with the NMR analysis