ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 13, Issue 12, 2020

Online - 2455-3891
Print - 0974-2441

Research Article

IN VITRO AND *IN SILICO* ALPHA-AMYLASE INHIBITION POTENTIAL (ANTI-DIABETIC ACTIVITY) OF *PSEUDERANTHEMUM BICOLOR* (SIMS) RADIK

RAMESH BS, LOKESH RAVI

Department of Botany, St. Joseph's College (Autonomous), Bengaluru, Karnataka, India. Email: Rameshbs87@gmail.com

Received: 20 September 2020, Revised and Accepted: 20 October 2020

ABSTRACT

Objective: Aim of this study is to evaluate theanti-diabetic activity of *Pseuderanthemum bicolor* commonly called limang-sugat by inhibiting alpha-amylase protein.

Methods: Leaves of *P. bicolor* were extracted with methanol, chloroform, and ethyl acetate. The extracts were subjected for alpha-amylase inhibition assay and gas chromatography–mass spectrometry (GC–MS) analysis. Phytochemical compounds identified by GC-MS were subjected for protein-ligand docking study against alpha-amylase protein. Acarbose was used as a positive standard drug.

Results: The major bioactive compounds obtained from methanol, chloroform, and ethyl acetate extracts were 1,6;2,3-Dianhydro-4-Deoxy-Beta-D-Ribo-Hexopyranose, Pseduosarsasapogenin-5,20-Dien, methyl ether/Hexatriacontane, Di-N-decylsulfone/Octadecanal, and squalene, respectively. A total of 19 secondary metabolites were subjected for protein–ligand docking study against the alpha-amylase protein. The reference drug acarbose demonstrated binding energy of −7.8 Kcal/mol and formed 20 hydrogen bonds with the enzyme. Acarbose signified high polar interaction with the amylase enzyme. Among the 19 test ligands, "2,2-Dibromocholestanone" from ethyl acetate extract exemplified the highest binding energy of −9.3 Kcal/mol. The next highest remarkable inhibition was showed by "Pseduosarsasapogenin-5,20-Dien Methyl Ether" present in the methanol extract, with a binding energy of -9.3 Kcal/mol with the formation of 2 hydrogen bonds.

Conclusion: From the result, it could be concluded that the *P. bicolor* leaves contain various bioactive compounds which are considered as a good anti-diabetic drug.

Keywords: Gas chromatography–mass spectrometry, Protein–ligand docking, Acarbose, *Pseuderanthemum bicolor,* 2,2-Dibromocholestanone, Pseduosarsasapogenin-5,20-Dien Methyl Ether, Methanol, Chloroform, Ethyl acetate, Phytochemicals.

 DOI: http://dx.doi.org/10.22159/ajpcr.2020.v13i12.39824

INTRODUCTION

The importance of insulin in the human body is conversion of glucose into glycogen for storage in liver and muscle tissues, proper oxidation of carbohydrates, and responsible for the regulation of the normal blood sugar level. Insulin is produced by an organ pancreas [1]. If this hormone is not produced in sufficient quantity, the disease known as diabetes mellitus develops [2,3]. One of the main symptoms of this complaint is glycosuria (an excess of glucose in the urine) because the renal threshold is maximum [4]. Due to this impairment of carbohydrate oxidation leads to the formation of excessive ketone bodies. Many of these substances are acidic in nature and cause acidosis that results in a lack of insulin prone to diabetic coma, which is very fatal to a patient. It has been reported that 108 million in 1980–422 million in 2014 peoples suffered from this condition. In 2016, an estimated 1.6 million deaths were recorded [5]. Ayurveda is one of the most promised medicines to control this affliction, it prevents the sugar level in blood [6]. Plant species have been used as curative agents to treat various ailments, including diabetes mellitus because plants are a rich source of phytochemical constituents which are of biologically active. *Pseuderanthemum bicolor,* commonly known as "limang-sugat" belonging to the family Acanthaceae, is an important ornamental undershrub, usually about 1 m in length. The pharmacological studies of *P. bicolor* suggest antimicrobial, antipyretic, anti-inflammatory, hepatoprotective, central nervous system depressant, and anticonvulsant properties have been reported [7]. However, anti-diabetic activity study of *P. bicolor* has not been done. Hence, during the present investigation methanol, chloroform, and ethyl acetate extracts obtained from leaf of *P. bicolor* to screen the alpha-amylase activity in detail and protein–ligand docking has done using bioactive compounds obtained through gas chromatography–mass spectrometry (GC–MS) analysis.

MATERIALS AND METHODS

Collection of plant material

P. bicolor plants were collected in June 11, 2020 (monsoon season) at Gudemaranahalli, Magadi Taluk, Ramanagara district. The latitude, longitude, and elevation are 13.051951°N,77.263177°E, and 900 m (2,953ft), respectively. The plant was authenticated by Shivanandabhat. S, Assistant Professor, Department of Botany, Government First Grade College, Honnavar, Karwar district, Karnataka, India. Voucher specimen was deposited in the form of herbarium at the Department of Botany, BUB. The plant material was made into herbarium.

Preparation of leaf extracts by decoction method

The freshly collected leaves were washed using tap water to remove dirt and shade dried for 8–10 days. The dried leaves were ground into fine powder using mixer grinder. Fifteen grams of powdered plant material were soaked into 90 ml of organic solvents, namely, methanol, chloroform, and ethyl acetate for 4–5 h in water bath at 50°C. The extracts were filtered through the Whatman No.1 filter paper. The extracts were then allowed to evaporate. The condensed extracts were stored in Eppendorf vials at 4°C till further investigation [8].

Alpha-amylase inhibition activity

Test tubes were ranging from 20 to 100 µg/ml of different concentrations, to this 200 µl of 0.02 M sodium phosphate buffer, 20 µl of amylase enzyme, and the plant extracts were added. The reaction mixtures were incubated at room temperature for 10 min. Pipette out 200µl of starch solution into the test tubes, to that add 400µl of DNS reagent and incubate in boiling water bath for 5min. After boiling, the reactants were cooled and diluted with 15 ml of distilled water. Optical density was measured at 540 nm wavelength. Acarbose was an anti-

diabetic drug used as control taken in another test tube without test samples. The percentage of inhibition was calculated using the formula as follows [9].

$$\text{Inhibition } (\%) = \frac{\text{Abs 540 (control)} - \text{Abs 540 (extract)}}{\text{Abs 540 (control)}} \times 100$$

GC–MS analysis

Crude extracts were sent to a sophisticated instrumentation facility, VIT University, Vellore. The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m×0.25 mm ID×250 μm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. During the chromatographic run, the injector temperature was adjusted at 260°C. One microliter of plant sample was administered into the instrument through injector, the oven temperature was regulated at 60°C for 2 min, succeeded by 300°C at the rate of 10°C/min, and it was clenched at 300°C for 6 min. The mass detector temperature was set at 230°C for transfer line, while the ion source temperature was also set at 230°C. Mass spectra of ionization mode electron impact were taken at 70 eV, a scan time of 0.2 s and scan interval of 0.1 s, and fragments from 40 to 600Da had been recorded. The relative percentage of spectrum of the components was calculated by comparing its average peak area to the total areas. The database of spectrum of known components was stored in the GC–MS NIST (2008) library [10-12].

Protein–ligand docking

Protein–ligand docking was done to conclude the binding affinity between phytochemicals obtained by GC–MS analysis and amylase enzyme. Nineteen bioactive compounds were taken for protein–ligand docking study against alpha-amylase protein. Acarbose was a standard drug [13].

RESULTS AND DISCUSSION

Alpha-amylase inhibition assay

Three different crude extracts of *P. bicolor* leaves were subjected for α-amylase inhibition assay to test for its anti-diabetic potential. The observed results were compared with acarbose, which was a reference standard drug. The plant samples were tested at four different concentrations (1 mg, 0.75 mg, 0.5 mg, and 0.25 mg/ml). The results of α-amylase inhibition are represented graphically in Fig. 1. Among the three extracts of *P. bicolor* (methanol, chloroform, and ethyl acetate extracts), most pronounced enzyme inhibition was exhibited by ethyl acetate extract, with the highest inhibition observed at 1 mg/ml concentration with 43.1% inhibition. The reference drug acarbose indicated 96% inhibition at 0.5 mg/ml concentration. The result of the reference drug was compared with the crude extracts since the active ingredients in the leaf extracts were present at microgram concentrations. Among the three different crude extracts, ethyl acetate extract was identified to contain noteworthy alpha-amylase inhibition activity [14], as shown in Fig. 1.

GC–MS analysis

To identify the bioactive molecules present in the crude extracts, the extracts were subjected for GC–MS analysis. The chromatograms of each extract are shown in Fig. 2. The NIST library matches of each separated peaks are tabulated in Table 1. Methanol extract demonstrated three known secondary metabolites with significant molecular weight. Chloroform extract consisted of three known secondary metabolites. Ethyl acetate extract consisted 13 known molecules match in the library. These molecules were identified based on their mass spectrum match (both forward and reverse) with known molecules in the NIST library. The GC–MS peak interpretations of the crude extracts are tabulated in Table 1. The GC–MS spectrum of all three leaf extracts is shown in Fig. 2.

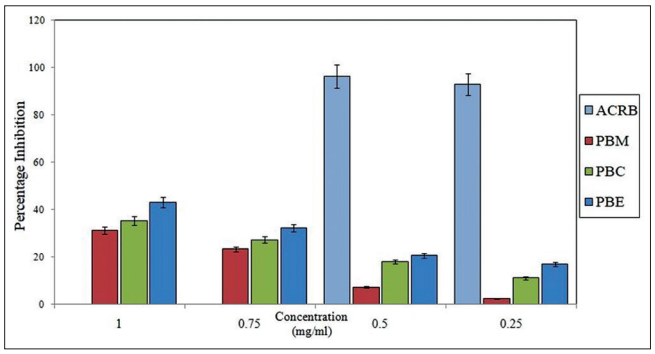

Fig. 1: Results of alpha-amylase inhibition assay for crude extracts of *Pseuderanthemum bicolor*. (ACRB: Acarbose, PBM: Methanol extract, PBC: Chloroform extract, PBE: Ethyl acetate extract)

Table 1: Phytochemical compounds identified in different solvent leaf extract of *Pseuderanthemum bicolor* by gas chromatography–mass spectrometry

No.	R.T	Name of the compound	PubChem ID	MW	Peak area %
Methanol extract					
1	24.6	1,6;2,3-Dianhydro-4-Deoxy-.Beta.-D-Ribo-Hexopyranose	545707	128	5.349
2	27.0	Pseduosarsasapogenin-5,20-Dien Methyl Ether	552194	428	5.618
3	27.2	2-[1,2-Dihydroxyethyl]-9-[.Beta.-D-Ribofuranosyl]Hypoxanthine	135599152	328	4.128
Chloroform extract					
1	26.0	2-T-Butylperoxy-2-Ethylbutan-1-Ol, Butyrate Ester	537771	260	3.288
2	26.4	Hexatriacontane	12412	506	48.874
3	27.6	Di-N-Decylsulfone	19089489	346	14.353
Ethyl acetate extract					
1	16.5	Decane, 1-Iodo-	16314	268	6.319
2	17.6	Hexadecane	11006	226	5-765
3	17.8	1-Decanol, 2-Ethyl-	545566	186	2.440
4	17.9	Octadecanal	12533	268	20.802
5	18.4	Phytol	5366244	296	6.420
6	19.0	Heptadecane, 2,6-Dimethyl-	545603	268	3.069
7	24.307	Squalene	545603	410	10.474
8	25.903	Heptacosane	11636	380	10.352
9	26.783	2-Propenoic acid, Oxybis(Methyl-2,1-Ethanediyl) Ester	3034434	240	4.692
10	27.028	Dotriacontane	11008	450	3.623
11	28.063	1-Hexyl-2-Nitrocyclohexane	544017	213	2.874
12	28.434	2,2-Dibromocholestanone	249903107	542	5.731
13	29.794	Hexadecane, 1,16-Dichloro-	544143	294	2.521

Table 2: Docking results of identified chemical compounds in the crude extracts with alpha-amylase protein

Ligand name	PubChem ID	Binding energy (Kcal/mol)
Methanol extract		
1,6;2,3-Dianhydro-4-Deoxy-.Beta.-D-Ribo-Hexopyranose	545707	−4.5
Pseduosarsasapogenin-5,20-Dien Methyl Ether	552194	−9.3
2-[1,2-Dihydroxyethyl]-9-[.Beta.-D-Ribofuranosyl]Hypoxanthine	135599152	−7.4
Chloroform extract		
2-T-Butylperoxy-2-Ethylbutan-1-Ol, Butyrate Ester	537771	−5.5
Hexatriacontane	12412	−4.7
Di-N-Decylsulfone	19089489	−5.1
Ethyl acetate extract		
Decane, 1-Iodo-	16314	−4.4
Hexadecane	11006	−4.5
1-Decanol, 2-Ethyl-	545566	−5.3
Octadecanal	12533	−4.6
Phytol	6420901	−4.8
Heptadecane, 2,6-Dimethyl-	545603	−5
Squalene	3034434	−5.3
Heptacosane	11636	−5.1
2-Propenoic acid, Oxybis (Methyl-2,1-Ethanediyl) Ester	5366244	−5.5
Dotriacontane	11008	−5.6
1-Hexyl-2-Nitrocyclohexane	544017	−5.7
2,2-Dibromocholestanone	249903107	−9.3
Hexadecane, 1,16-Dichloro-	544143	−5.2
Reference molecule		
Acarbose	41774	−7.8

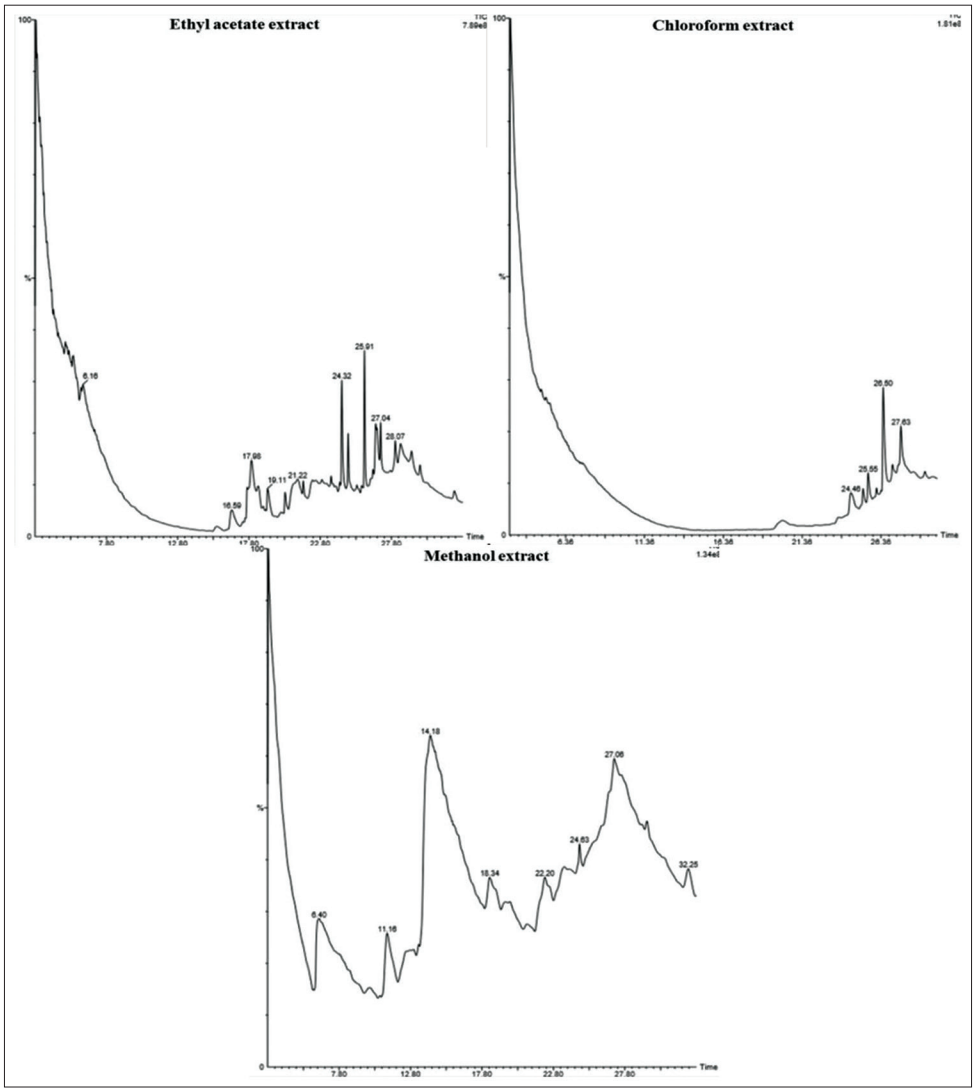

Fig. 2: Gas chromatography–mass spectrometry chromatogram of *Pseuderanthemum bicolor* leaf extracts

Protein–ligand docking

A total of 19 secondary metabolites were subjected for protein–ligand docking study against alpha-amylase protein. Positive standard drug used was acarbose. The results of the binding energy of test ligands and standard drug are tabulated in Table 2. A binding energy of acarbose was −7.8 Kcal/mol and formed 20 hydrogen bonds with the amylase enzyme. Acarbose displayed high polar interaction with the enzyme. Interactions between acarbose and amylase enzyme are shown in Fig. 3. Among the 19 test ligands, "2,2-Dibromocholestanone" from ethyl acetate extract showed the highest binding energy of −9.3 Kcal/mol and it was observed to be a potent inhibitor of alpha-amylase enzyme. Interaction between "2,2-Dibromocholestanone" and alpha-amylase enzyme is shown in Fig. 4. The second highest notable inhibitor was "Pseudosarsasapogenin-5,20-Dien Methyl Ether" present in the methanol extract having −9.3

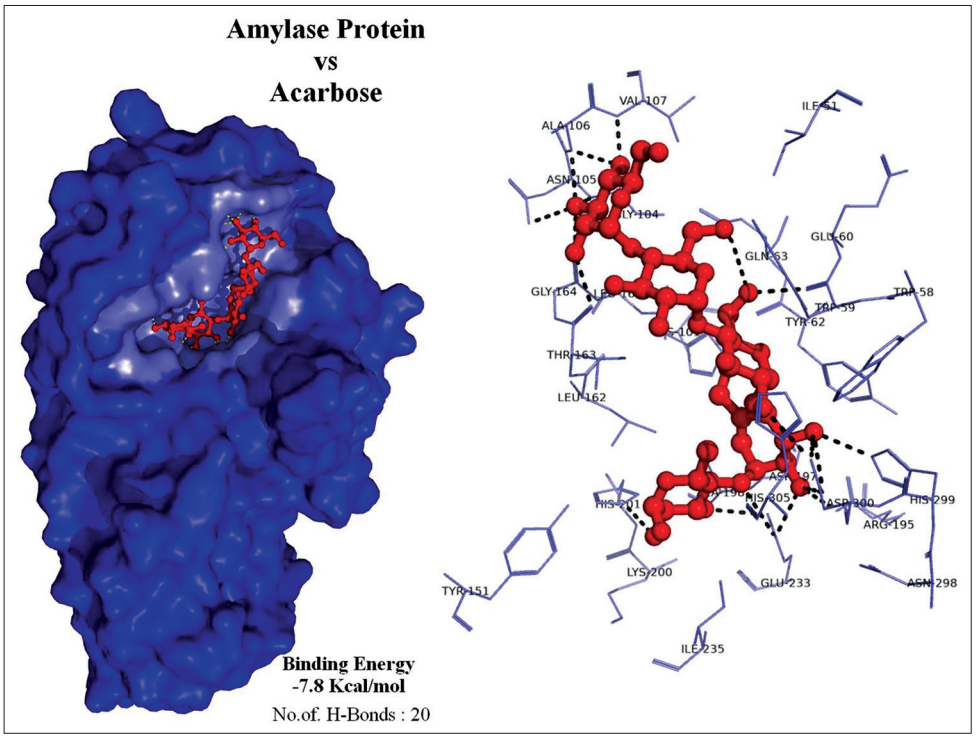

Fig. 3: Interaction between acarbose and alpha-amylase protein

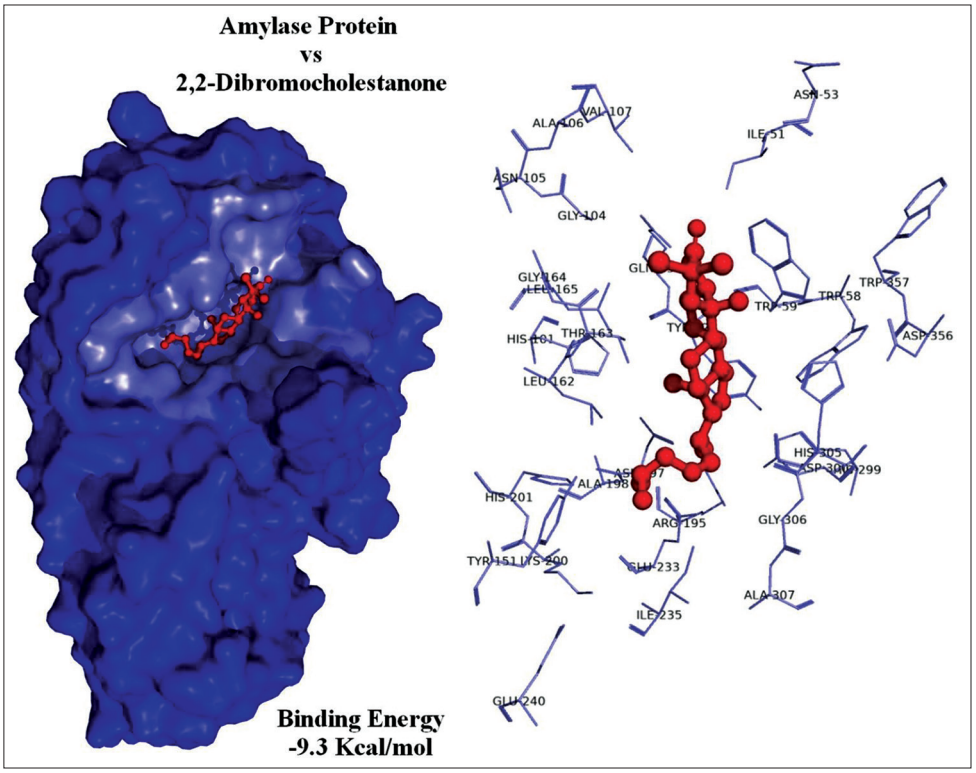

Fig. 4: Interaction between "2,2-Dibromocholestanone" and alpha-amylase protein

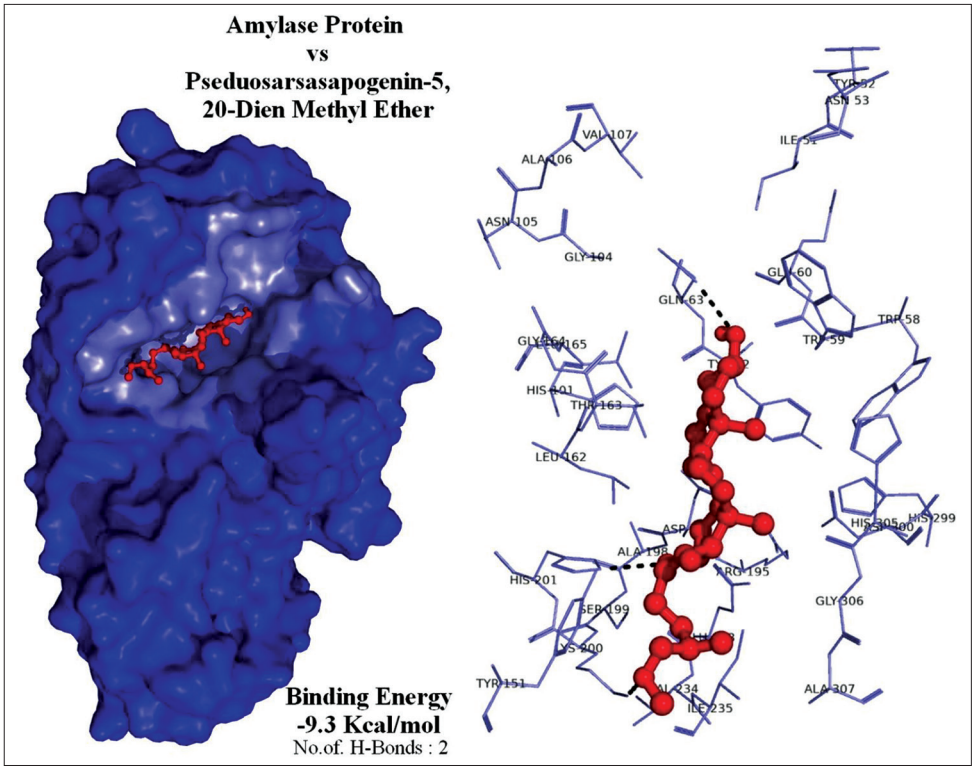

Fig. 5: Interaction between "Pseduosarsasapogenin-5,20-Dien Methyl Ether" and alpha-amylase protein

Kcal/mol of binding energy with the formation of two hydrogen bonds. Interaction between "Pseduosarsasapogenin-5,20-Dien Methyl Ether" and alpha-amylase enzyme [15,16] is shown in Fig. 5.

CONCLUSION

Results of this present study suggested that the ethyl acetate extract of *P. bicolor* was a consequential anti-diabetic source for alpha-amylase inhibition activity. Among the various secondary metabolites identified through GC–MS analysis in the ethyl acetate extract of *P. bicolor*, the compound "2,2-Dibromocholestanone" was identified to be the most potent inhibitor of alpha-amylase enzyme, using protein–ligand docking analysis. Hence, *P. bicolor* can be used in pharmaceutical industries for anti-diabetic applications.

ACKNOWLEDGMENT

The authors thank the management of St. Joseph's College (Autonomous), Bengaluru, for supporting this research work.

AUTHORS' CONTRIBUTIONS

All authors scientifically contributed in execution and preparation of this research manuscript.

CONFLICTS OF INTEREST

None declared.

AUTHORS' FUNDING

No funding was availed for this work.

REFERENCES

1. Routh JI. Fundamentals of Inorganic, Organic and Biological Chemistry. Philadelphia, PA: W.B. Saunders Company; 1955.
2. Poretsky L, editor. Principles of Diabetes Mellitus. Berlin, Germany: Springer Science and Business Media; 2010.
3. Lati MS, David MG, Kinuthia RN. The clinical significance of potential drug-drug interactions and their targets for minimization among hypertensive diabetic outpatients at a kenyan referral hospital. Int J Pharm Pharm Sci 2020;12:6-11.
4. Jacob R. Sickening sweet. Distillations 2015;1:12-5.
5. Narayan KV, Zhang P, Kanaya AM, Williams DE, Engelgau MM, Imperatore G, *et al*. Diabetes: The pandemic and potential solutions. In: Disease Control Priorities in Developing Countries. 2nd ed. United States: The International Bank for Reconstruction and Development, The World Bank; 2006. p. 591-601.
6. Sharma R, Amin H, Ruknuddin G, Prajapati PK. Efficacy of Ayurvedic remedies in Type 2 diabetes: A review through works done at Gujarat Ayurved university, Jamnagar. J Med Nutr Nutraceuticals 2015;4:63.
7. Sugat L. Philipine Medicinal Plants, *Pseuderanthemum bicolor* (Schrank) Radik. Ex Lindau. Available from: http://www.stuartxchange.org/Limang-sugat.html. [Last accessed on 2018 Nov 10].
8. Palakkal L, Hukuman NH, Mullappally J. Antioxidant activities and chemical composition of various crude extracts of *Lepidagathis keralensis*. J Appl Pharm Sci 2017;7:182-9.
9. Jung M, Park M, Chul HL, Kang Y, Seok-Kang E, Ki-Kim S. Antidiabetic agents from medicinal plants. Curr Med Chem 2006;13:1.
10. Mc Lafferty FW. Registry of Mass Spectral Data. 4th ed. New York: Wiley; 1986.
11. Sithara NV, Komathi S, Rajalakshmi G. Identification of bioactive compounds using different solvents through FTIR studies and GC-MS analysis. J Med Plants Stud 2017;5:192-4.
12. Otuokere IE, Amaku AJ, Igwe KK, Chinedum GC. Medicinal studies on the phytochemical constituents of *Justicia carnea* by GC-MS analysis. Am J Food Sci Health 2016;2:71-7.
13. Ravi L, Krishnan K. Benzoyloxy-ethyl-carbamic acid: A novel anticancerous secondary metabolite produced by *Streptomyces globosus* VITLGK011. Indian J Exp Biol 2017;55:411-20.
14. Tamil IG, Dineshkumar B, Nandhakumar M, Senthilkumar M, Mitra A. *In vitro* study on α-amylase inhibitory activity of an Indian medicinal plant, *Phyllanthus amarus*. Indian J Pharmacol 2010;42:280.
15. Ravi L. Procyanidin B2 of *Cassia fistula* a potent inhibitor of COVID19 protease: A molecular dynamic simulation analysis. Asian J Pharm 2020;14:274-81.
16. Arumugam A, Wong NK. Comparison study of antioxidant, antimicrobial and cytotoxic properties of secondary metabolite and protein extracts from *Clinacanthus nutans*. Int J Pharm Pharm Sci 2020;12:98-102.