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HOMOLOGY MODELLING OF α - AMYLASE; ITS MOLECULAR DOCKING BY FLAVONOIDS AS POTENTIAL LIGANDS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author SCB designed and conducted the study, wrote the protocol and wrote the first draft of the manuscript. Author KSR did the assessment of the manuscript. Authors TSR and PCS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Bioinformatics, an essential and integrated wing of advanced Life sciences which manages, analyses and manipulates the crucial mammoth data of Bio molecules where in, Molecular Modelling and Molecular Docking are its crucial tools. Considering the Flavonoids role as popular α - Amylase enzyme inhibitors, which in turn had a therapeutic role in Diabetes regulation, the current objective of the work has been designed to test the *In silico* analysis of the α - Amylase enzyme. Molecular docking was conducted by employing 6 potential flavonoid ligands: (a). Myricetin (b). Quercetin (c). Zinc Luteolin (d). Catechin (e). Cyanidin and (f). Daidzein. Modeller V 9.17 was used for Homology modelling of α - Amylase and iGEMDOCK v 2.1 was used for Molecular docking between α - Amylase enzyme and the ligands. Results suggests that Myricetin found to be the best of the potential flavonoid ligands with binding energy of - 116.234 kcal/mol, where as the binding energy of the remaining ligands in the descending order: Cyanidin: - 98.8208 kcal/mol ; Catechin: - 97.3075 kcal/mol; Zinc Luteolin: - 93.7762 kcal/mol; Quercetin: - 90.1663 kcal/mol and Daidzein: - 85.4134 kcal/mol. From the work, it can be concluded that Myricetin found to be the best of the flavonoid ligands (dry lab

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analysis), further wet lab analysis at the larger scale has to be put forth to tap its therapeutic potentiality against diabetes treatment.

Keywords: Modeller V 9.17; iGEMDOCK v 2.1; flavonoids and myricetin.

1. INTRODUCTION

Current Bioinformatics approaches enables Biochemical and Clinical tools in enumerating probability of wide dimensional functionality of enzymes and proteins pertaining to different biotic strata ranging from virus, bacteria, plants to animals through the generation of supportive output Kaladhar et al. [1]. Bioinformatics, an indispensable and integrated branch of advanced life sciences which manages, analyses and manipulates the mammoth data of varied components of cell like genome and proteome Bertini and Cavallaro [2]; Kaladhar et al. [3]. Molecular Docking is one of the crucial tools of Bioinformatics, which simply means best fit, useful in predicting the probable atomic interaction between a tiny ligand compound and a large protein molecule, needless to say its conducive role in construing of varied basic biochemical processes vividly in an articulated way McConkey et al. [4].

Molecular Docking majorly involves two fundamental processes: 1. Prediction of the ligand's confirmation, orientation and its position within the active site of the target protein molecule. 2. Assessment of the Affinity of binding owing to promising results in the field of novel drug discovery molecular docking emerges as an essential tool Nisha, [5]. α –Amylase, which is an endo α 1,4 glucan 4 glucanohydrolase EC 3.2.1.1 specifically cleaves α - D - (1-4) glycosidic bonds of starch resulting to the generation of shorter compounds of oligosaccharides Kandra, [6]; Tangphatsornruang et al. [7]. Microbes, Plants and Higher organisms are the promising sources of α − Amylase Selvam et al. [8]. Prohibition of α - Amylase enzymatically had a potential role in diabetes regulation therefore, Inhibitors of α - Amylase enzyme may serve as the promising candidates in diabetes treatment Rahimzadeh et al. [9] under this scenario, Flavonoids reported as the potential candidates for α - Amylase enzyme inhibition Kim et al. [10]; Tadera et al. [11]. Flavonoids have multifarious antioxidant and biochemical effects with respect to diseases ranging from Alzheimer's disease, cancer, atherosclerosis and others. Flavonoids are essential constituents for a range of human health applications for instance, pharmaceutical, nutraceutical, cosmetic and medicine. Such a property of flavonoids could be attributed to its anti inflammatory, anti carcinogenic, anti oxidative and anti mutagenic functionalities associated with its cellular enzymes modulatory ability Panche et al. [12].

Different groups of fruits and vegetables are the potential flavonoids sources. Flavonoids are classified into 6 groups i. Flavanol ii. Flavone iii. Flavanone iv. Isoflavon v. Flavan - 3 - ol and vi. Anthocyanidin Havsteen, [13]. Different workers across the globe conducted varied In silico modelling, Molecular Docking and Enzyme Prohibition studies ranging from L - asparaginase In silico analysis, Comparative enzyme inhibition studies of human and rat α - Amylase molecular docking studies of Galangin and Diosmetin against α - Amylase to enzyme inhibitory potentiality of varied flavonoids Kim et al. [10]; Wulan et al. [14]; Arumugam et al. [15]; Reddy et al. [16]. Nisha [5] performed molecular docking of α - Amylase by employing Quercetin and varied phyto chemical compounds of siddha formulation (Pungampoo choornam), results suggests that Quercetin found to be one of the potential α - Amylase enzyme inhibitor. Similarly Tadera et al. [11] conducted the comparative enzyme inhibitory potentiality studies of varied flavonoid groups against α - Amylase and α - Glucosidases of different sources. Considering the overall scenario of the role of α - Amylase enzyme in Diabetes regulation, and the potential role of flavonoid ligands, the present piece of research conducted the holomology modelling of α - Amylase and employed 6 potential Flavonoid ligands to ascertain the best of the potential flavonoid ligands as the same had a promising role in Diabetes treatment.

2. MATERIALS AND METHODS

2.1 Retrieval of α - Amylase Sequence and Templates for Homology Modelling

Sequence of α - Amylase was retrieved from NCBI data base (https://www.ncbi.nlm.nih.gov/), with accession number GenBank: ASN25326.1 with Streptomyces pluripotens as the mother source for α - Amylase sequence (https://www.ncbi.nlm.nih.gov/search/all/?term=ASN 25326.1). 548 Amino acids were the total number of residues Benson et al. [17]. In order to retrieve α - Amylase template BLAST P was run against PDB data base (Fig. 1) Johnson et al. [18].

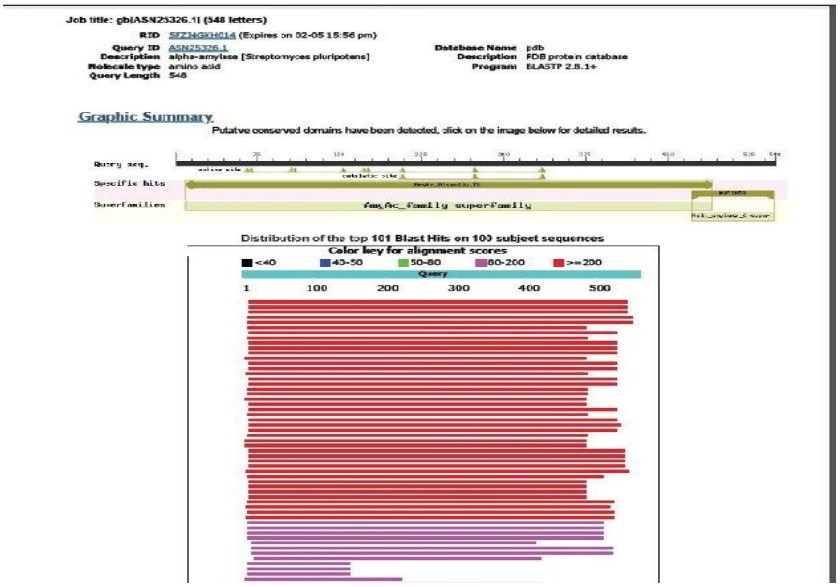


Fig. 1. Retrieval of α - Amylase sequence from NCBI

2.2 Preparation of Ligands and α - Amylase Modelling

For the *In silico* analysis, totally 6 ligands were employed (a). Myricetin (b). Quercetin (c). Zinc Luteolin (d). Catechin (e). Cyanidin and (f). Daidzein. Data base of Drug Bank (https://www.drugbank.com/) was employed for downloading the ligands: Myricetin_DB02375; Quercetin_DB04216; Zinc Luteolin_18185774 and Daidzein_DB13182-1 and Zinc Data base (https://zinc.docking.org/) were employed for downloading the ligands: Catechin_zinc_119983-0 and Cyanidin_zinc_3775158-1 Wishart et al. [19]; Irwin et al. [20]; Pola et al. [21]. For Homology modelling of α - Amylase, Modeller V 9.17 (Python Script) was used where in, three templates of α - Amylase were the raw materials Webb and Sali, [22]; Pola et al. [21]. Ramachandran plot assessment by Rampage was used for the structural validation of α - Amylase Homology model Lovell et al. [23].

2.3 Modeller Software

A Popularly employed modelling software for comparison of protein structure is Modeller Sali and Blundell, [24]; Fiser et al. [25]. The working mechanism of modeller software is by the way of spatial restraints confirmation which comprises of Homology derived restraints which are based on the template structure variables: 1) Distances and Dihedral angels in the target protein sequence Sali and Blundell, [24]. 2) Varied stereo chemical restraints for example; Bond length and Bond Angel Brooks et al. [26]. 3) Dihedral angel's statistical priorities and Non-bonding inter atomic distance Sali and Overington, [27]; Shen and Sali, [28]. 4) Manual Constraints derived from electron microscopy related image reconstruction, NMR Spectroscopy, fluorescence spectroscopy and other related. Of late, Modeller V 9.17 was considered as advanced software was employed for current research Kaufmann et al., [29].

2.4 Molecular Docking

iGemdock V 2.1 Hsu et al. [30]; Reddy et al. [16] was employed for conducting Molecular Docking between 6 ligands - (a). Myricetin (b). Quercetin (c). Zinc Luteolin (d). Catechin (e). Cyanidin and (f). Daidzein and the resulted α - Amylase Homology model and the ligands were performed.

2.5 iGEMDOCK V 2.1

iGEMDOCK V 2.1 was a virtual graphical atmosphere for identifying pharmaceutical and therapeutic interactions and virtual screening applicable for lead compounds analysis and for deciphering mechanism of ligand binding vis - a - vis to pharmacological target under post analysis tools sequential clustering methods and k means were used Hsu et al. [30]. Further, post - interaction profile and post interaction analysis was conducted and the resultant complex molecules were used for sequential simulation analysis Yang and Chen [31]; Dowluru et al. [32]. Pymol V 1.8 (education version) was used for molecular interaction DeLano, [33].

3. RESULTS

3.1 α - Amylase Homology Modelling

Modeller V 9.17 software was employed for α - Amylase Homology modelling by employing three templates as raw materials (Fig. 2 (a), (b), (c)). Pymol V 1.8 software (education version) was used for visualization of resultant α - Amylase homology model (Fig. 3). For both α - Amylase Homology model and Ligands energy minimization was done.

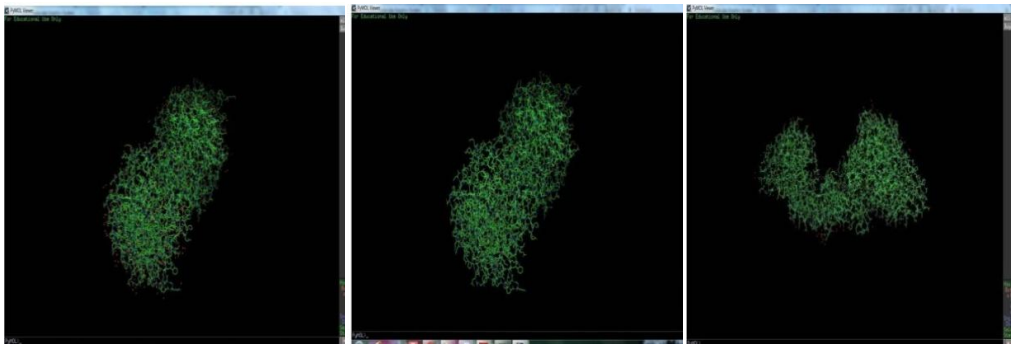
3.2 Ramachandran Plot for Structural Validation

The stereochemistry of the resulted Homology model of α - Amylase was assessed by Ramachandran plot. The assessment suggests that favoured region amino acids stood at 98% where as 2% of residues fall in allowed region (Fig. 4).

3.3 Molecular Docking Analysis

As mentioned software iGEMDOCK V 2.1 was employed for docking of six ligands - (a). Myricetin (b). Quercetin (c). Zinc Luteolin (d). Catechin (e). Cyanidin and (f). Daidzein into the targeted active site of α - Amylase (Query. B99990001) (Fig 5 (a), (b), (c), (d), (e) and (f)). Interaction profile pertaining to molecular docking suggests that Myricetin reported to be the best ligand with binding affinity of - 116.234 kcal/mol against α - Amylase which is higher than other ligands. The descending order of other Ligand's binding affinity against α - Amylase was reported as; Cyanidin: - 98.8208 kcal/mol; Catechin: - 97.3075 kcal/mol; Zinc Luteolin: - 93.7762 kcal/mol; Quercetin: - 90.1663 kcal/mol and Daidzein: - 85.4134 kcal/mol (Table 1(a) and (b)).

Myricetin's Hydrogen bonding pattern with α - Amylase through Interaction analysis shown as ASP - 66; ASP - 208; THR - 230; GLU - 233; TRP - 276; GLN - 301; ARG - 420 and ARG - 423. The Hydrogen Bonding pattern of remaining ligands with that of α - Amylase was shown in the Interaction analysis (Fig. 6 (a) and (b)). Comparative Data analysis of Binding affinity suggests that Myricetin found to be the best of the potential flavonoid ligands which exhibited higher binding energy with that of α - Amylase (Fig. 7).



(a) **(b)** **(c)**

Fig. 2. (a), (b), (c). α-Amylase enzyme templates employed for homology modelling

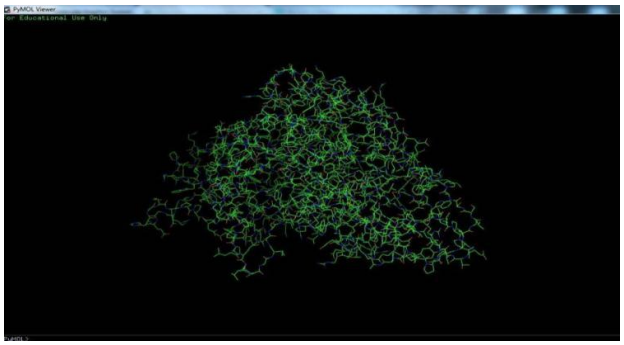


Fig. 3. α-Amylase homology model

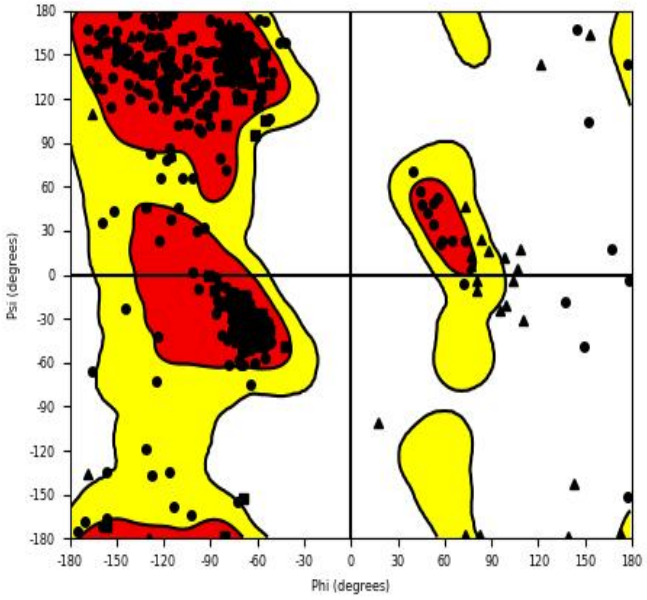


Fig. 4. Ramachandran plot employed for α-Amylase structural validation

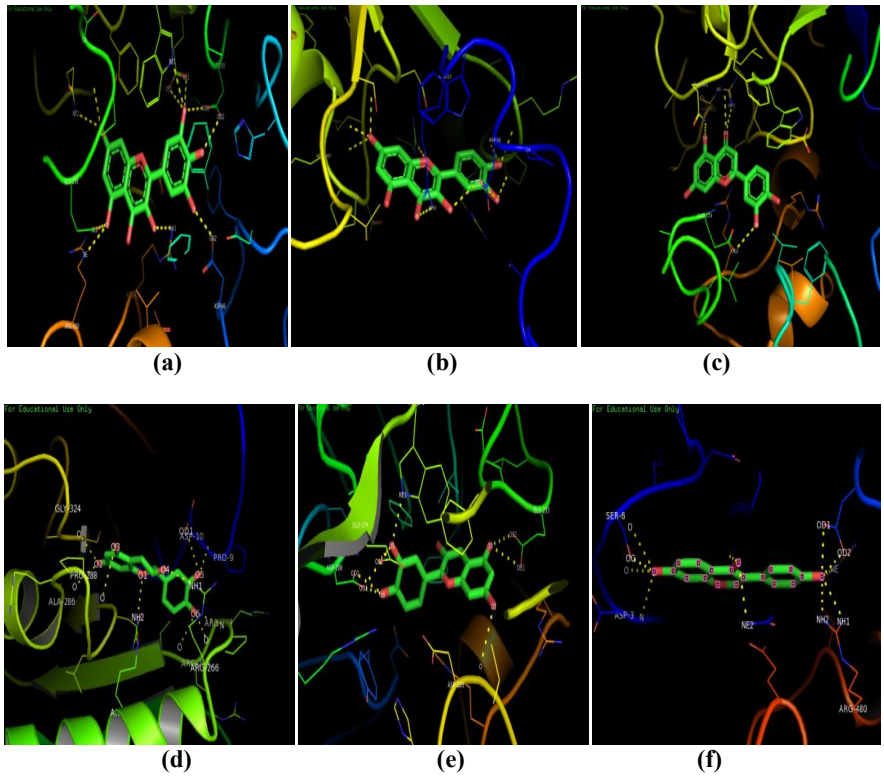


Fig. 5. Molecular docking pose between Ligands and α-Amylase: (a): Docked pose of Myricitin with α-Amylase; (b): Docked pose of Quercetin with α-Amylase; (c): Docked pose of Zinc Luteolin with α-Amylase: (d): Docked pose of Catechin with α-Amylase; (e): Docked pose of Cyanidin with α-Amylase; (f): Docked pose of Daidzein with α-Amylase

Table 1(a). Interaction profile of α-Amylase and ligands (Myricetin; Quercetin and Zinc Luteolin)

Compound	Energy	VDW	H Bond	Elec	Int.ClusterID
query.B99990001-Myricetin_DB02375-1.pdb	-116.234	-76.7208	-39.5132	0	31.7391
query.B99990001-Quercetin_DB04216-0.pdb	-90.1663	-65.6973	-24.469	0	26.7273
query.B99990001-Zinc_Luteolin_18185774-0.pdb	-93.7762	-77.2142	-16.562	0	27.619

Table 1(b). Interaction profile of α-Amylase and ligands (Catechin; Cyanidin and Daidzein)

Compound	Energy	VDW	H Bond	Elec	Aver ConPair
query.B99990001-Catechin_zinc_119983-0.pdb	-97.3075	-68.7976	-28.5099	0	26.9048
query.B99990001-Cyanidin_zinc_3775158-1.pdb	-98.8208	-78.674	-20.1469	0	32.0952
query.B99990001-Daidzein_DB13182-1.pdb	-85.4134	-53.2796	-32.1339	0	23.2105

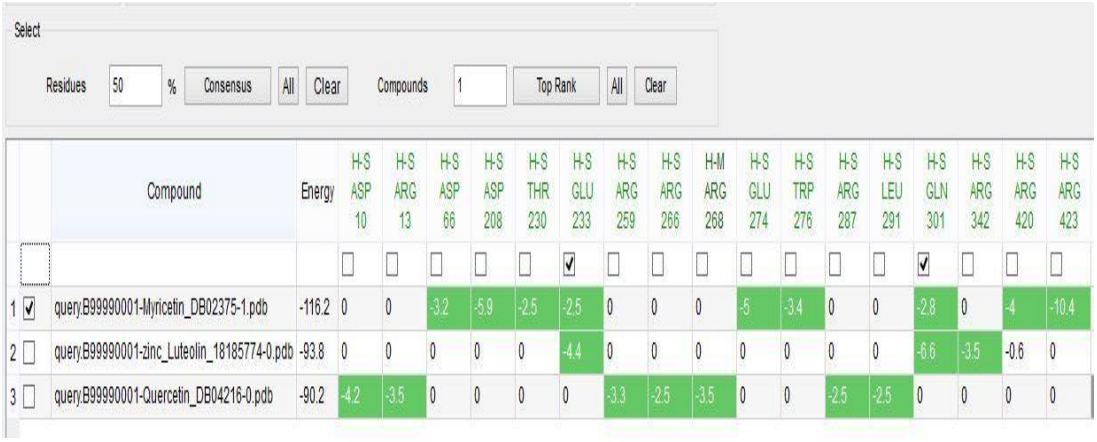


Fig. 6(a). Interaction analysis of ligands and α-Amylase (Myricetin; Zinc Luteolin and Quercetin)

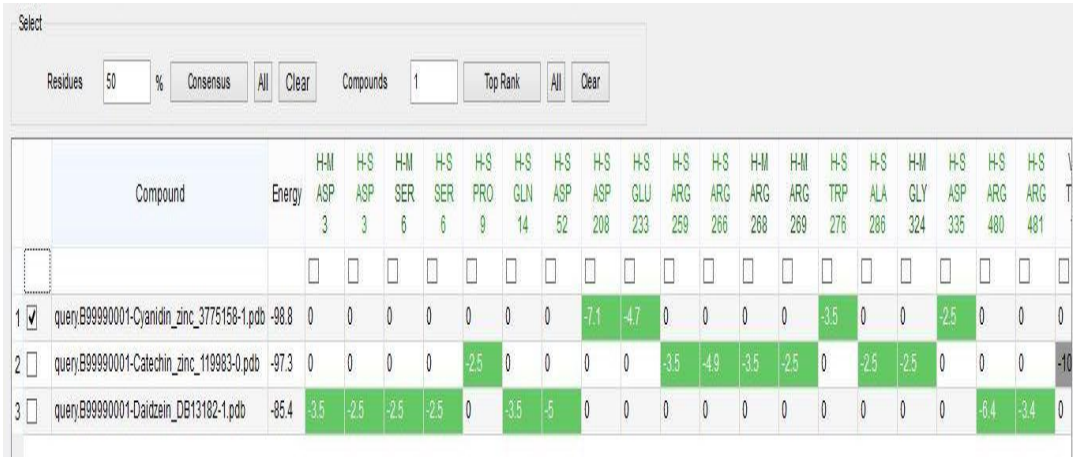


Fig. 6(b). Interaction analysis of ligands and α-Amylase (Cyanidin; Catechin and Daidzein)

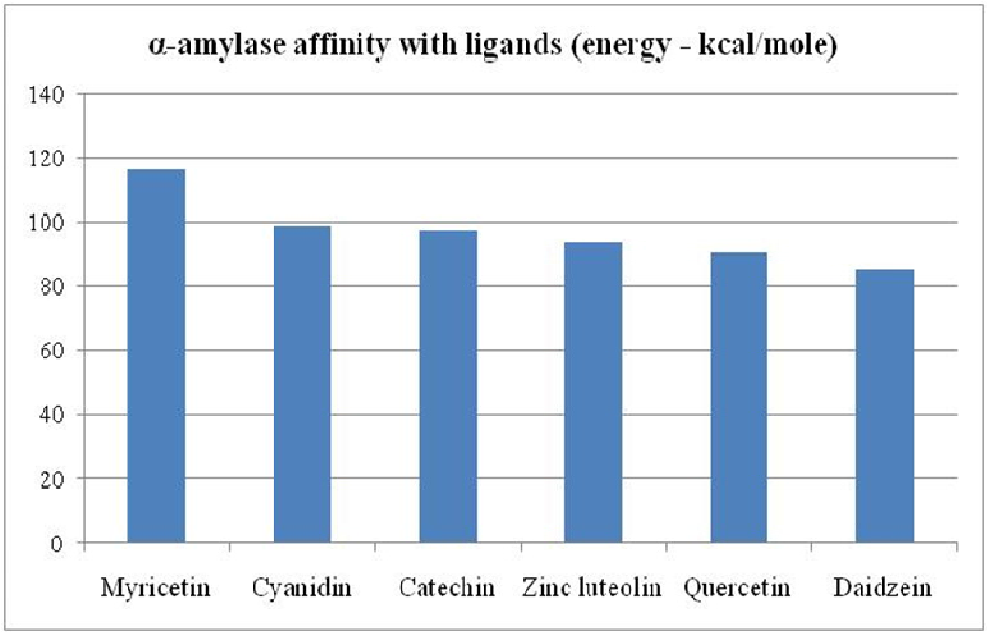


Fig. 7. α-Amylase affinity with different ligands

4. DISCUSSION

Current results of *In silico* analysis and molecular docking were in general consonance with the previous reports though contradicts in terms of quantitative values. Wulan et al. [14] conducted the comparative In silico molecular docking analysis of Human and Rat's α - Amylase of Pancreas by employing different ligands of Ruellia tuberosa L Compounds such as Vanilic acid, Betulin, Luteolin, Flavone and others. Their results suggests that Betulin with binding energy of - 6.66 kcal/mol exhibited against Rat's Pancreatic α - Amylase and - 8.42 kcal/mol with respect to human pancreatic α - Amylase. Where in, Luteolin exhibited binding energies of - 4.79 kcal/mol and - 5.75 kcal/mol against rat's pancreatic α - Amylase and human pancreatic α - Amylase respectively. The said results quantitatively contradict with our results in the way of - 93.7762 kcal/mol of binding energy exhibited by Zinc Luteolin which is lot higher.

Nisha [5] performed the In silico molecular docking analysis by employing different phyto compounds as enzyme inhibitors of Siddha formulation of Pungampoo choornam against α - Amylase enzyme. Compounds employed in the study were Beta sitosterol, Quercetin, Acarbose and others. Results suggests that Acarbose found to be potential enzyme inhibitor of α - Amylase with binding energy - 9.34 kcal/mol whereas, Quercetin exhibited - 6.25 kcal/mol of binding energy which is far exceedingly lesser than our reported results with - 90.1663 kcal/mol of binding energy exhibited by Quercetin. Similarly, Kim et al. [10] suggests that Luteolin is one of the potential flavonoid enzyme inhibitor, under the same line Tadera et al. [11] reported Luteolin, Myricetin and Quercetin were the potential Porcine Pancreatic α - Amylase enzyme inhibitors.

Our results prove that Myricetin was the best of the potential flavonoid enzyme inhibitors. Since In silico analysis (dry lab analysis) prove that Myricetin found to be the potential α - Amylase enzyme inhibitor, the same should be put forward to wet lab for larger scale exploration considering the role of Myricetin in diabetes regulation.

5. CONCLUSION

In silico analysis had a profusing impact on the Pharmacological research as it enables to expand its horizons in the way of molecular docking which may paves the way to novel drug delivery for a wide health complications like Diabetes. Considering the potential role of flavonoid ligands in diabetes regulation our research has employed 6 potential flavonoid ligands, results suggests that Myricetin with binding energy of - 116.234 kcal/mol as the best potential flavonoid ligands against α - Amylase enzyme therefore, the dry lab analysis has to be corroborated with wet lab in such a way that large scale exploration of Myricetin may be done considering its role in diabetes regulation.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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