JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2022/26819
RESEARCH ARTICLE

In-Vitro and *In-Silico* Alpha Amylase and Alpha Glucosidase Inhibitory Activity of Baicaelin

A. Dinesh, Srikanth Logesh Kumar, T. Geethanjali, S. C. Dhanalakshmi, V. Iyyappan, P. Aravind, V. Hemapriya, D. Arulmozhi and J. Srikanth*

Department of Pharmacology, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (Deemed to be university), Porur, Chennai – 600116, Tamil Nadu, India; srikanth.j@sriramachandra.edu.in

Abstract

Diabetes mellitus is a metabolic disorder characterized by high blood glucose level in the body. It occurs due to the inadequate amount of insulin secreted in the body or resistance of insulin receptors. In the present study, *Baicalein* a flavone glycoside was evaluated for its effect on alpha-amylase and alpha-glucosidase enzymes using *in-vitro* assays by extracting respective enzymes from whole wheat and barley in combination with *in-silico* analysis. *In-vitro* alpha amylase inhibitory activity and *in-vitro* alpha glucosidase inhibitory activity was performed using acarbose as a standard drug. The molecular docking study was performed using Schrodinger (Maestro V 11.5) software. The parameters glide score, Lipinski rule for drug likeliness, bioactive scoring and ADME properties were assessed in the docking study. Further, the antioxidant activity of *baicalein* was performed using DPPH assay, Nitric oxide scavenging activity. The cytotoxicity of baicalein was evaluated using the Brine shrimp lethality assay. The alpha-amylase assay performed showed IC_{50} value of 48.40 µg/ml for baicalein whereas alpha-glucosidase assay showed an IC_{50} value of 16.03 µg/ml. *Baicalein* shows the glide score of-5.565 with 5EOF and glide score of -5.339 with 5NN8 in the molecular docking study. The highest percentage of DPPH radical scavenging activity and nitrous oxide scavenging activity were found to be 27% at 160 µg/ml and 50.02% at 160 µg/ml respectively. Based on further *in vivo* and clinical trials, *baicalein* may be used for the management of hyperglycaemia.

Keywords: Alpha-amylase, Alpha-glucosidase, *Baicalein, Insilico*, Diabetes Mellitus

1. Introduction

There are many research works carried out on diabetes mellitus, since it is a lifestyle modification disorder in current population. The resistance of insulin receptors present in the beta (β) cells of the pancreas cause type 2 diabetes mellitus which may produce serious effects in humans such as diabetic nephropathy, neuropathy, retinopathy, cardiovascular and cerebrovascular disease[1,2]. It has been judged that about 592 million adults will enhance to diabetes by the year 2035 due to increase in population size, ageing and to the extent of calories rich fatty fast foods[3]. The diagnostic approach to treat diabetes is to reduce postprandial diabetes. This can be procured by inhibiting the α - amylase and α - glucosidase carbohydrate hydrolysing enzymes. These two enzymes play a major role in the digestion of carbohydrates. The breakdown of large insoluble starch to absorbable molecule is performed by an enzyme called alpha-amylase, whereas the hydrolysing enzyme catalysing the digestion of starch and disaccharides at the end-stage is called as alpha-glucosidase[4]. Therefore, inhibiting these enzymes delays the absorption or production of glucose in the blood. Thus the inhibition plays a main role to decrease postprandial hyperglycaemia. Due to some gastrointestinal side effects of acarbose and voglibose, a synthetic inhibitor of this enzyme occurs here preferred over natural sources like flavonoids which have no side effects. Hence, in the present study a synthetic drug *baicalein* is

*Author for correspondence

Article Received on: 25.01.2021 Revised on: 17.09.2021 Accepted on: 07.10.2021

used to evaluate the hyperglycaemic activity against the specified target enzyme by *in-vitro* & *in-silico* analysis[5].

The root of *Scutellaria baicalensis* is the most studied source of *baicalein* (5.41%). In addition, *baicalein* has also been found in the seed, fruit, root bark and leaf parts of *Oroxylum indicum*. *Scutellaria baicalensis* is a Chinese herbal medicine used in the avoidance of diabetic mellitus issue. *Baicalein* consists of three hydroxyl group at the position of (C-5,6,7). The dried root of *Scutellaria baicalensis* contains thirty types of flavonoids, out of which three flavonoids namely baicalin, baicalein and wogonin are the major components[6]. *Baicalein* possesses several health beneficial properties such as antioxidant, anti-HIV, anticancer, antiviral, anti-inflammatory and free radical scavenging effects. Many literature reviews possess that dietary consumption of baicalein enhances glucose tolerance in obese mice. Previous study by Yu Fu *et al,* identified that dietary consumption of baicalein enhances the blood insulin level and hyperglycaemia in obese diabetic mice[7]. Another study by Zhang *et al* identified that anti-diabetic effect of baicalein is associated with the modulation of gut microbiota in animal models[8].

The present study is carried out to evaluate the antidiabetic activity of *baicalein* against the target enzyme alpha-amylase and alpha-glucosidase using *in vitro* & *in-silico* analysis.

2. Materials and Methods

2.1 Materials

Baicalein was purchased from Sigma Aldrich (Product ID: 465119). All the chemicals used in the study were obtained from Merck and Co chemicals Pvt Ltd. The absorbance of the *in vitro* assays was performed using Shimadzu UV spectrophotometer UV 1800 Model. The brine shrimp lethality assay was evaluated in a 24 well plate and the lethality was observed using Magnus MLX-B Plus (SP) Inclined Binocular Microscope. Aquatic Remedies Artemia Bhrime Shrimps was purchased from Amazon sold by Sagar aquarium.

2.2 *In-vitro* Alpha Amylase Inhibitory Activity

The extraction of wheat alpha amylase was performed by the method as described by Nair *et al*. The assay mixture containing 200 µl of 0.02 M sodium phosphate buffer, 20 µl of enzyme and the plant extracts in a concentration ranging from 5–160 µg/ml were incubated for 10 min at room temperature followed by addition of 200 µl of starch in all test tubes. 400 µl DNS reagent was added and placed in boiling water bath for 5 min, cooled and diluted with 15 ml of distilled water and the absorbance was measured at 540 nm.

The % inhibition was calculated according to the formula:

$$\%\ inhibition = \frac{Abs\ (control) - Abs\ (sample)}{\text{Abs (Control)}} \times 100$$

The IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha-amylase inhibitor. All tests were performed in triplicate[9].

2.3 *In-vitro* Alpha Glucosidase Inhibitory Activity

The yeast alpha glucosidase was dissolved in 100 mM phosphate buffer pH 6.8 and was used as the enzyme extract. P-Nitrophenyl-α-D-glucopyranoside was used as the substrate. Baicalein were used in the concentration ranging from 20-100 µg/ml. Different concentrations of *baicalein* were mixed with 320 µl of 100 mM phosphate buffer pH 6.8 at 30 °C for 5 minutes. 3 ml of 50 mM sodium hydroxide was added to the mixture and the absorbance was read at 410 nm. The control samples were prepared without *baicalein*. The % inhibition was calculated according to the formula. The IC_{50} Values were determined from the percent inhibition of alpha-glucosidase. Acarbose is used as the reference drug[9]. The % inhibition was calculated according to the formula:

$$\%inhibition = \frac{Abs\ (control) - Abs\ (sample)}{\text{Abs (Control)}} \times 100$$

The IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha-glucosidase inhibitor. All tests were performed in triplicate[10].

2.4 Antioxidant Assay

2.4.1 DPPH Free Radical Scavenging Activity

The DPPH assay is utilized to anticipate antioxidant activity by determining the scavenging potential of DPPH radical. The method relays on the scavenging of DPPH by an antioxidant, which upon reduction decolourize the DPPH ethanol solution. The extent of discolouration depicts the scavenging of the antioxidant compound in terms of hydrogen donating ability. The DPPH assay was performed by adding 25µl DPPH (1mm) dissolved in ethanol to 10 µl of each sample (0.2-1 µl) in DMSO (Dimethyl Sulfoxide). Then it was vortexed for about 30 minutes in the dark and maintained at room temperature. Finally, the DPPH assay was analysed by measuring the absorbance of the vortexed mixture at 490 nm by using UV Spectrophotometer and it was compared with standard drug[11].

Scavenging ratio DPPH assay was calculated as follows:

$$\%\ DPPHradicalscavengingactivity = \frac{Abs\ (control) - Abs\ (sample)}{\text{Abs (Control)}} \times 100$$

2.4.2 Nitric Oxide Scavenging Activity

Nitric oxide scavenging assay was done by adding a different concentration of test sample to different test tubes. Then, add 1.5 ml of sodium nitroprusside (5mM) in phosphate buffer to each test tubes and incubated for 30 min at 25°C. Finally, 1.5 ml of Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 3% phosphoric acid) was added to each test tubes. Then, the absorbance of coloured solution was measured at 546 nm using UV Spectrophotometer and it was compared with standard drug[11].

$$\%\ Nitricoxidescavengingactivity = \frac{Abs\ (control) - Abs\ (sample)}{\text{Abs (Control)}} \times 100$$

3. Brine Shrimp Lethality Assay

Artemia salina lethality assay was carried out to assess the cytotoxicity of baicalein. *Artemia salina* (1g) cysts were incubated for hatching in a conical container (separating funnel) filled with sea water. 0.06% yeast solution was added to the hatching chamber to feed the larvae after 24 hours, filled with sea water under constant aeration for 48 hours. After 48 hours, active nauplii free from egg shells were collected from the hatching chamber and used for the assay.

From the hatching chamber, 10–15 nauplii were drawn using a Pasteur pipette and introduced into the petri dish containing sea water along with a drop of yeast solution. Different concentrations (0.1, 1, 10,100 and 1000 µg/mL) of baicalein and positive control (potassium dichromate) were prepared. 0.5 ml was added to 4.5 ml of sea water and maintained at room temperature for 24 hours, to remain in contact with the active nauplii in the petri dish. The number of surviving nauplii in each petri dish was counted after 24 hours. The percentage death was calculated by comparing the mean surviving larvae of the test substance baicalein and control system. LC_{50} values were obtained from the best-fit line plotted concentration versus percentage lethality[12,13].

$$\%\ Death = \frac{\text{Number of dead napulii}}{(\text{No of dead nauplii} + \text{No of live nauplii})} \times 100$$

The number of survivors was counted and the vials were inspected by a magnifying glass after 24 h of treatment with the sample. For each dilution, the percent (%) mortality was projected.

4. Molecular Docking

Generally, docking is a process where the ligand and receptor molecules are combined together to form a stable complex structure. Here the molecular docking study was performed by using Schrodinger (Maestro V 11.5) software which shows a great recognition among researchers and the pharmaceutical field[14]. The maestro software tool relays on flexible visualization, 3D realism of a compound, Quantitative structural

analysis, molecular properties such as vibrational modes, molecular orbits or electron density are easily visualized in Maestro.

4.1 Software Used

Ligand preparation, Protein preparation, Molecular docking - Maestro 11.8 (Schrodinger 2018-4 package).

4.2 Protein Preparation

The 3D structure of alpha-amylase (PDB ID: 5U3A) and alpha-glucosidase (PDB ID: 5NN8) were retrieved from the RCSB protein data bank. The selected protein was imported via the protein preparation wizard. Then the proteins are further pre-processed, refined, optimized and then minimized.

4.3 Ligand Preparation

The 3D structure of the ligand compounds is retrieved from Pubchem database (i.e., baicalein, acarbose, voglibose in .sdf format. Then the ligands were prepared using LigPrep wizard in maestro 2018[15].

4.4 Receptor Grid Generation

The receptor grids were enumerated for the developed protein such that individual ligand binds with the existed active site during docking. In Glide grids were initiated keeping the default parameters of van der Waals scaling factor 1.00 and charge cut off 0.25 expose to OPLS 2005 force field. The cubic box of certain proportions focus around the centroid of the active site was produced for the receptor[16].

4.5 Ligand Docking

The ligand docking was performed. The final score was obtained based on the energy minimizing poses and reveal as a glide score. The best-docked pose with the least glide score value was recorded for the respective ligands.

Table 1. *In-vitro* Alpha Amylase inhibitory activity of Baicalein

Concentration µg/ml	**Sample Absorbance**	**% Inhibition**	**Standard Absorbance**	**Inhibition %**
5	0.0096	4	0.6887	15.6
10	0.0125	25	0.5078	17.3
20	0.034	33	0.8299	39.3
40	0.0513	41.3	0.8899	49.3
80	0.0616	51.6	0.9468	58.9
160	0.1023	92.3	1.176	97.4

All values are expressed as % inhibition calculated using the formula. IC_{50} values are calculated by non-linear regression analysis of log dose *vs* % inhibition as represented in Figure 1. All experiments were performed in triplicate (n=3) for a specific concentration.

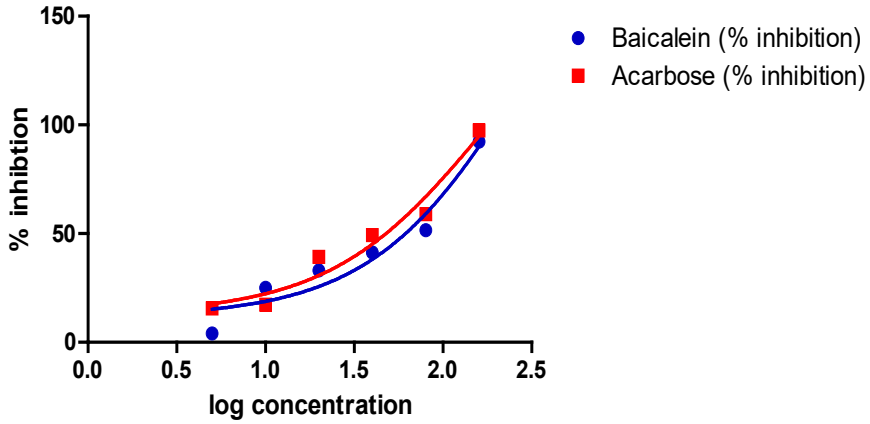

Figure 1. *In-vitro* Alpha Amylase inhibitory activity of Baicalein & Acarbose.

5. Results and Discussions

5.1 *In-vitro* Alpha Amylase Inhibitory Activity

The alpha-amylase assay performed shows 92.3% of inhibition. The inhibitory concentration of baicalein on alpha-amylase was found to be (IC_{50} = 46.78 µg/ml) and the reference standard Acarbose was found to be (IC_{50} = 36.36 µg/ml) as represented in Table 1 and Figure 1.

5.2 *In-vitro* Alpha Glucosidase Inhibitory Activity

The alpha-glucosidase assay performed shows 33.8% inhibition. The inhibitory concentration of baicalein on alpha-glucosidase was found to be (IC_{50}= 584.1 µg/ml) and the reference standard Acarbose was found to be (IC_{50} = 67.00 µg/ml) as represented in Table 2 and Figure 2. The key role of the alpha-glucosidase enzyme is to catalyse dietary carbohydrate and starch hydrolysis to generate glucose for intestinal absorption. Consequently, inhibiting the work of these enzymes can delay glucose production following dietary digestion, which in turn reduces hyperglycaemia[17].

5.3 Antioxidant Assay

In this study, antioxidant assay was performed in which the percentage inhibition of DPPH radical scavenging activity was found to be 21.86%, 37.82%, 54.46%, 62.26%, 72.12%, 89.76% at the concentrations of 5,10,20,40,80,160 µg/ml respectively as represented in Table 3 and Figure 3; nitric oxide scavenging shows an inhibition of 19.9%, 35.46%, 41.4%, 48.24%, 48.2%, 50.02% at the concentrations of 5,10,20,40,80,160 µg/ml respectively as represented in Table 4 and Figure 4. The IC_{50} value of baicalein for DPPH and Nitric oxide scavenging activity was found to be 19.41 *µg/ml* &

Table 2. *In-vitro* Alpha Glucosidase inhibitory activity of Baicalein & Acarbose

Concentration (µg/ml)	Sample Absorbance	% Inhibition	Standard Absorbance	Inhibition%
5	0.498	18.3	0.6887	12.4
10	0.9151	25.3	0.5078	15.6
20	0.9646	26.1	0.8299	31.4
40	1.419	27.8	0.8899	42.6
80	1.064	33.8	0.9468	54.6
160	1.4204	42.4	1.176	62.8

All values are expressed as % inhibition calculated using the formula. IC_{50} values are calculated by non-linear regression analysis of log dose *vs* % inhibition as represented in Figure 2. All experiments were performed in triplicate (n=3) for a specific concentration.

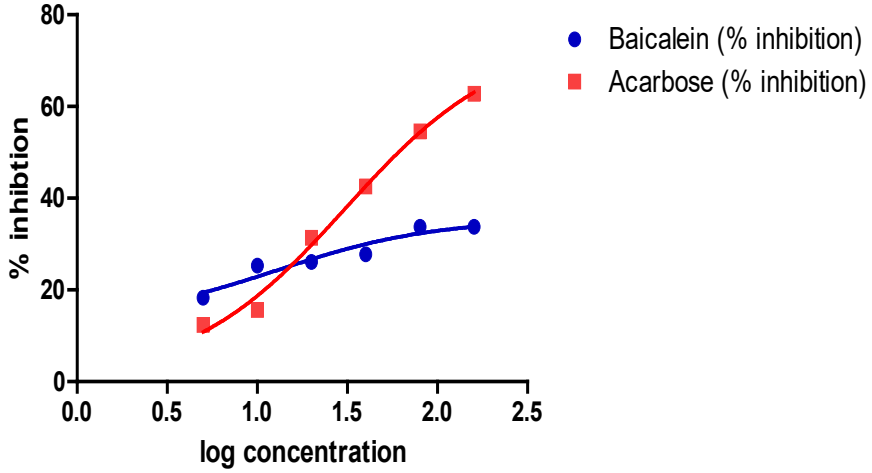

Figure 2. *In-vitro* Alpha Glucosidase inhibitory activity of Bacteria & Acarbose.

Table 3. DPPH free radical scavenging activity of Baicalein

Concentration (µg/ml)	Absorbance sample	% Inhibition of sample	Absorbance standard	% Inhibition of standard
5	0.05	21.86	1.688	8.14
10	0.22	37.82	1.647	16.48
20	0.224	54.46	1.63	37.92
40	0.297	62.26	1.539	56.72
80	0.533	72.12	1.591	75.48
160	1.068	89.76	1.176	95.98

All values are expressed as % inhibition calculated using the formula. IC_{50} values are calculated by non-linear regression analysis of log dose *vs* % inhibition as represented in Figure 3. All experiments were performed in triplicate (n=3) for a specific concentration.

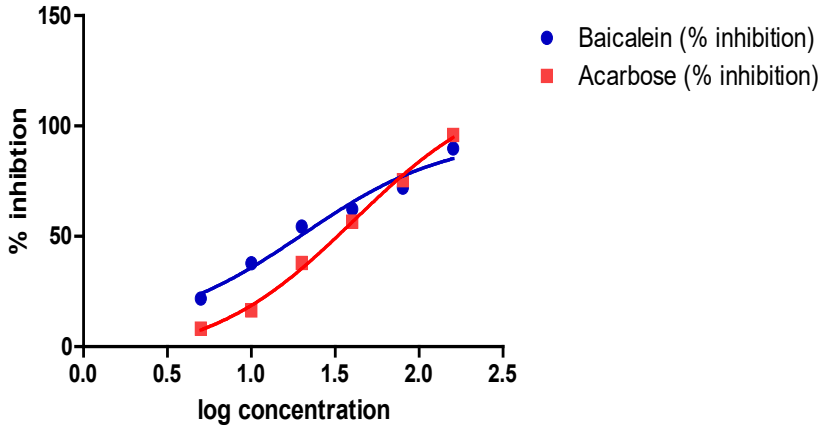

Figure 3. DPPH free radical scavenging activity of Baicalein & Acarbose.

Table 4. Nitric oxide scavenging activity of Baicalein

Concentration (µg/ml)	Absorbance sample	% Inhibition of sample	Absorbance of standard	% Inhibition of standard
5	0.1843	19.9	0.2042	27.2
10	0.1909	35.46	0.2213	32.9
20	0.1908	41.4	0.2328	33
40	0.2161	48.2	0.2468	36.8
80	0.238	48.24	0.2471	39.9
160	0.2954	50.02	0.2682	44.6

All values are expressed as % inhibition calculated using the formula. IC_{50} values are calculated by non-linear regression analysis of log dose vs % inhibition as represented in Figure 4. All experiments were performed in triplicate (n=3) for a specific concentration.

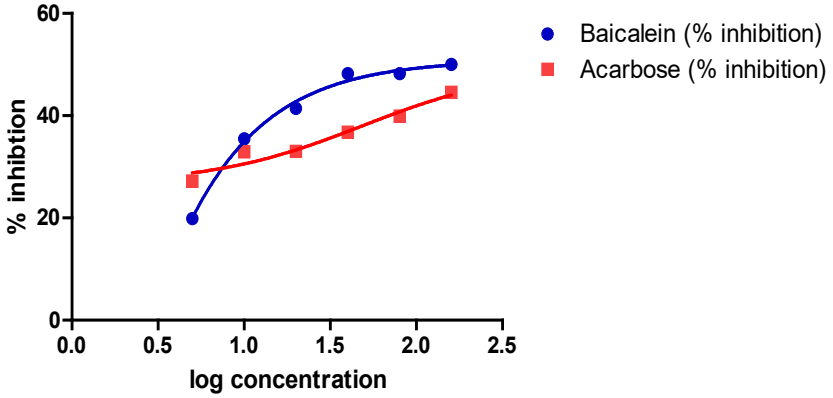

Figure 4. Nitric oxide scavenging activity of Baicalein & Acarbose.

92.30 µg/ml respectively as compared to the standard drug acarbose which showed an IC_{50} value of 31.06 *µg/ml* & 48.42 µg/ml respectively for DPPH and Nitric oxide scavenging activity.

5.4 Brine Shrimp Lethality Assay

The brine shrimp assay was carried out for Baicalein and the lethality dose was calculated by using the standard potassium dichromate solution. The lethality dose was calculated at 24 hours. The brine shrimps were taken in 24 well plate and observed under microscope after 24 hours of exposure as represented in Figure 5a and b respectively. The values obtained were entered in GraphPad software and the probit analysis was observed for both potassium dichromate and MCFE and the results were tabulated in Table 5.

5.5 In Silico Studies

The association of baicalein, acarbose & voglibose with alpha-amylase target was comparatively analysed. Comparative analysis of docking scores, glide scores, H-Bonding and Hydrophobic pockets are seen in Table 6 and the docked view of the compound was represented in Figure 6a, b and c respectively for baicalein, acarbose & voglibose against PDB ID: 5U3A.

The association of baicalein, acarbose & voglibose with alpha-glucosidase target was comparatively analysed. Comparative analysis of docking scores, glide scores, H-Bonding and Hydrophobic pockets are seen in Table 7 and the docked view of the compound was represented in Figure 7a, b and c respectively for baicalein, acarbose and voglibose against PDB ID: 5NN8.

The aim of this work was to evaluate the inhibitory activities of the compound baicalein on wheat alpha-amylase and Barley alpha-glucosidase at varying concentrations. The enzyme alpha-amylase and alpha-glucosidase inhibitors are used to achieve greater control over hyperglycaemia in type 2 diabetes mellitus. A research to determine the hypoglycaemic effect of baicalein (insulin sensitivity and glucose uptake) was performed by Singh *et al* and concluded that 50% aqueous ethanolic *Oroxylum indicum* stem bark containing baicalein demonstrated anti-hyperglycaemic effects through inhibition of alpha-glucosidase activity in the 3T3-L1 adipocyte cell line and diabetic rat models. The significant effect of baicalein to suppress bovine serum albumin glycation, as stated by Singh *et al.* (2013), may be helpful in the management of anti -hyperglycaemia by maintaining beta cells and enhancing insulin sensitivity.[17]

The present study intends to screen novel alpha-amylase and alpha-glucosidase inhibitors from natural sources like flavonoids in order to minimize the side effects of the inhibitors currently used to control hyperglycaemia.

The *in-silico* analysis was carried out for the ligand interaction and binding affinity of a compound. In alpha-amylase, acarbose possesses the highest glide score of -6.390 with 5EOF having 3 hydrogen bond with THR 163, HIS 299, GLU 233 amino acid and with 3 water molecules. Voglibose shows the glide score of -6.052 with 5EOF having 3 hydrogen bond with ASP 197, ARG 195, HIP amino acids and with two water molecules. *Baicalein* has the glide score of-5.565 with 5EOF having 3 hydrogen bond with one HIS 299,

Table 5. Brine shrimp lethality assay of Baicalein

Concentration (µg/ ml)	Log Concentration	% death of nauplii after 24 hours of exposure
0.10	-1	18.42+2.12
1.00	0	29.38+1.08
10.00	1	48.72+2.72
100.00	2	68.82+1.86
1000.00	3	92.36+1.78
Assays were performed in triplicate. Each value represents Mean ± S.E.M.		

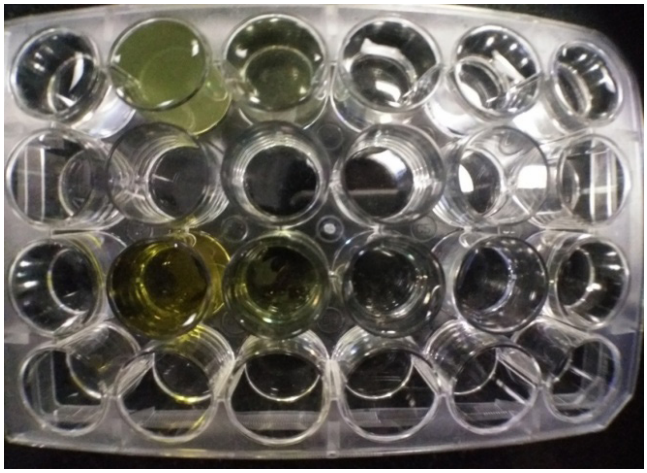

Figure 5a. 24 well plate exposure of 10 brine shrimps for a period of 24 hours.

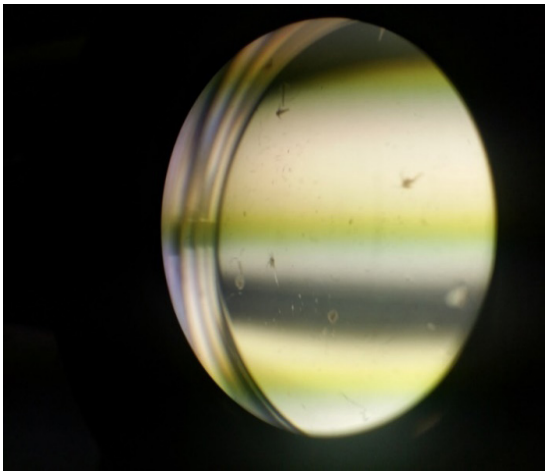

Figure 5b. Observations were made under a microscope showing images of brine shrimp.

Table 6. Molecular docking study of Baicalein, Acarbose and voglibose on alpha-amylase (PDB ID: 5U3A)

S.No	Protein	Ligand	Docking Score	Glide Score	H Bond	Aminoacid Interaction Site
1.	**α -** amylase	Baicalein	-5.513	-5.565	3	His 299, Asp 197
		Acarbose	-6.602	-6.390	6	Trp 59, Thr 163, Arg 195, His 299, Glu 233
		Voglibose	-6.047	-6.052	5	Hip 305, Arg 195, Asp 197

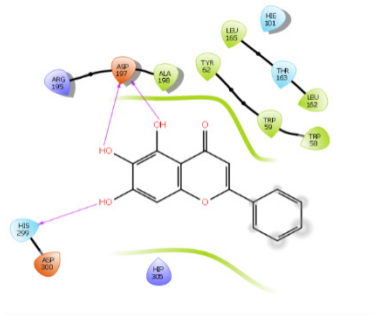

Figure 6a. Docked view of Baicalein on alpha-amylase (PDB ID: 5U3A).

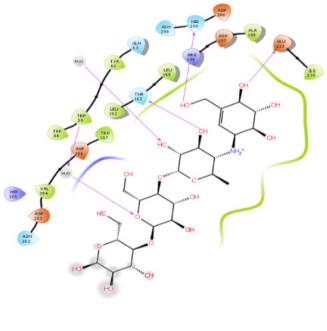

Figure 6b. Docked view of Acarbose on alpha-amylase (PDB ID: 5U3A).

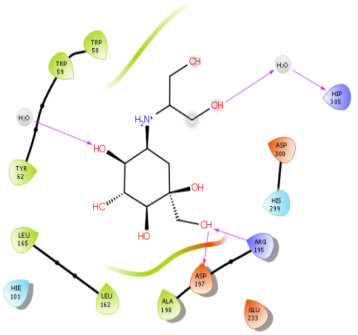

Figure 6c. Docked view of Voglibose on alpha-amylase (PDB ID: 5U3A).

Table 7. Molecular docking study of Baicalein, Acarbose and voglibose on alpha-glucosidase (PDB ID: 5NN8)

S.No	Protein	Ligand	Docking Score	Glide Score	H bond	Aminoacid Interaction Site
1.	α – glucosidase (PDB ID: 5NN8)	Baicalein	-5.287	-5.339	4	Asp 404, Phe 649, Trp 481, Arg 600, Asp 518
		Acarbose	-5.590	-5.918	8	Tyr 110, Gly 123, Trp 126, Asp 91, Ala 93, Arg 275, Arg 331
		Voglibose	-6.396	-6.401	7	Asp 91, Lys 96, Cys 127, Trp 126, Gly 123, Arg 331,

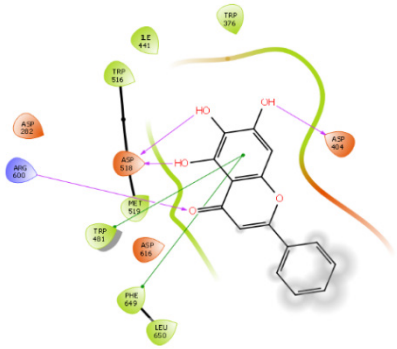

Figure 7a. Docked view of Baicalein on alpha-glucosidase (PDB ID: 5NN8).

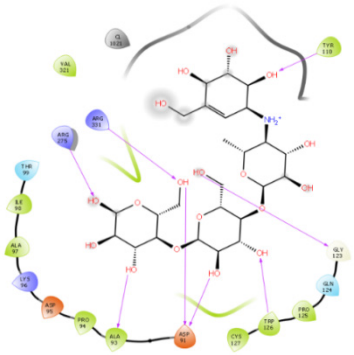

Figure 7b. Docked view of Acarbose on alpha-glucosidase (PDB ID: 5NN8)

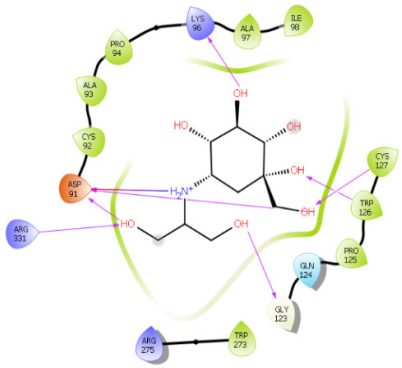

Figure 7c. Docked view 0f Voglibose on alpha-glucosidase (PDB ID: 5NN8)

and with two ASP 197 amino acids, here the water molecules are absent.

In alpha-glucosidase, voglibose possesses the highest glide score of -6.401with 5NN8 having 7 hydrogen bond with LYS 96, TRP 126, CYS 127, ARG 331, GLY 123 and with two ASP 91 amino acids. Acarbose has the glide score of -5.918 with 5NN8 having 8 hydrogen bond with one TYR 110, ARG 331, ARG 275, ALA 93, TRP 126, GLY 123, and with two ASP 91. Baicalein has the glide score of -5.339 with 5NN8 having 4 hydrogen bond with one ARG 600, ASP 404, TRY 481, PHE 649 and with two ASP 518 amino acids. Here totally the water molecules are absent

6. Conclusion

Finally, the results of *in-silco* and *in-vitro* study of baicalein against alpha-amylase and alpha-glucosidase states that baicalein has an antidiabetic activity based on the glide score and the binding affinity. The results of the work therefore clearly indicate the potential of baicalein to manage hyperglycaemia. The hypothesis of this study is the product of the *in-vitro, in-silico* prediction and further *in- vivo* experiments are required to validate the prediction.

7. Acknowledgement

The authors would like to thank the management of Sri Ramachandra Institute of Higher Education and Research (DU) for providing the software and chemicals for the successful completion of this research work.

8. References

1. Wade DM, Hankins M, Smyth DA, Rhone EE, Mythen MG, Howell DC, Weinman JA. Detecting acute distress and risk of future psychological morbidity in critically ill patients: Validation of the intensive care psychological assessment tool. 2014; Crit. Care; 18(5):519. https://doi.org/10.1186/s13054-014-0519-8. PMid:25248614. PMCid:PMC4207352

2. Laoufi H, Benariba N, Adjdir S, Djaziri R. In vitro α-amylase and α-glucosidase inhibitory activity of Ononis angustissima extracts. J. Appli. Pharm. Sci. 2017; 7(2):191–8.
3. Vinayagam R, Xu B. Antidiabetic properties of dietary flavonoids: a cellular mechanism review. Nutr. Metab. 2015; 12(1):60. https://doi.org/10.1186/s12986-015-0057-7 PMid:26705405 PMCid:PMC4690284
4. Sama K, Murugesan K, Sivaraj R. In vitro alpha amylase and alpha glucosidase inhibition activity of crude ethanol extract of *Cissus arnottiana*. Asian Journal of Plant Science and Research. 2012; 2(4):550–53.
5. Proença C, Freitas M, Ribeiro D, Oliveira EF, Sousa JL, Tomé SM. α-Glucosidase Inhibition by Flavonoids; 2017. p. 1216–28. https://doi.org/10.1080/14756366.2017.1368503. PMid:28933564. PMCid:PMC6009965
6. Fu Y, Luo J, Jia Z, Zhen W, Zhou K, Gilbert E, Liu D. Baicalein protects against type 2 diabetes via promoting islet β-cell function in obese diabetic mice. International journal of Endocrinology. 2014. https://doi.org/10.1155/2014/846742. PMid:25147566. PMCid:PMC4132321
7. Waisundara VY, Hsu A, Huang D, Tan BKH. S*cutellaria baicalensis* enhances the anti-diabetic activity of metformin in streptozotocin-induced diabetic Wistar rats. *The* Am. J. Chin. Med. 2008; 36(03):517–40. https://doi.org/10.1142/S0192415X08005953. Mid:18543386
8. Zhang B, Sun W, Yu N, Sun J, Yu X, Li X, Dong Y. Anti-diabetic effect of baicalein is associated with the modulation of gut microbiota in streptozotocin and high-fat-diet induced diabetic rats. J. Funct. Foods. 2018; 46:256–67.
9. Nair SS, Kavrekar V, Mishra A. *In vitro* studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. Eur. J. Exp. Biol. 2013; 3(1):128–32.
10. Stark JR, Yin XS. Evidence for the presence of maltase and α-glucosidase isoenzymes in barley. J. Inst. Brew. 1987; 93(2):108–12. https://doi.org/10.1002/j.2050-0416.1987.tb04485.x
11. Mahrous R, Ghareeb DA, Fathy HM, EL-Khair RMA, Omar AA. The protective effect of Egyptian *Withania somnifera* against Alzheimer's. Med. Aromat. Plants. 2017; 6(285). https://doi.org/10.4172/2167-0412.1000285
12. McLaughlin JL. Brine shrimp: A convenient general bioassay for active constituents. Planta Med. 1982; 45:31–2. https://doi.org/10.1055/s-2007-971236
13. Sarah QS, Anny FC, Mir M. Brine shrimp lethality assay. Bangladesh Journal of Pharmacology. 2017; 12(2):186–9. https://doi.org/10.3329/bjp.v12i2.32796
14. Nachammai V, Srikanth J, Lavanya R, Reddy C, Maheswara U. *In-vitro* and *in Silico* Alpha Amylase and Alpha-Glucosidase Inhibitory Activity of *Emblica officinalis*. Res. J. Pharm. Biol. Chem. Sci. 2018; 9(5):1784–94.
15. Barua J, Sany MA, Barua L, Hossen M, Absar N, Hossain Z, Akther S. Insilico molecular docking of some isolated selected compounds of *Phoenix sylvestris* (L.) against Diabetes. Biomed J Sci Tech Res. 2019; 16(2):11883–6. https://doi.org/10.26717/BJSTR.2019.16.002819
16. Jackie B, Sagar S, Alamgir H, Razowanul MF, Sumaiya F, Emon D, *et al*. In silico Molecular Docking and ADME/T Analysis of Some Selected Isolated Compounds of *Phyllanthus emblica* against Type 2 Diabetics. Am. J. Ethnomed. 2018; 5(2):9.
17. Singh J, Kakkar P. Modulation of liver function, antioxidant responses, insulin resistance and glucose transport by *Oroxylum indicum* stem bark in STZ induced diabetic rats. Food Chem. Toxicol. 2013; 62:722–31. https://doi.org/10.1016/j.fct.2013.09.035. PMid:24140466