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Analysis of edible fruits against glycolytic enzymes and glycation: *In vitro* approaches with *in silico* validation

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Diabetes is the most quotidian endocrine disorder and one of the fastest growing non-communicable diseases around the globe.Commonly consumed six fruits were investigated to determine their therapeutic potential to inhibitoxidation, key glycolytic enzymes and glycation activity which has relevance in the management of hyperglycemia and type II diabetes. The *in vitro* analysis revealed that amla fruit showed maximum total phenols and total antioxidant capacity among all the six fruits. Amla fruit exhibited potent inhibition for both alpha amylase and alpha glucosidase enzyme activity than the positive control acarbose. The IC_{50}value of alpha amylase inhibition and alpha glucosidase inhibition in amla was found to be high among all the fruits. Further, amla fruit phenolic compound (gallic acid) confirmed better *in silico* enzyme inhibitory action with alpha glucosidase with a binding energy of -6.21kcal/mol than alpha amylase. In antiglycation activity amla and mango fruits showed potent inhibition. Pearson correlation results showed a strong correlation ($p \leq 0.01$) between total phenol with flavonoids, total antioxidant capacity and antiglycation activities.The results obtained in this study showed that amla and mango had potent potential for the management of hyperglycemia, diabetes and the related condition of oxidative stress. Hence, these fruits can be prescribed to treat diabetes in safest way by incorporating them in natural medications.

Key Words : Fruits, Alpha amylase, Alpha glucosidase, Antiglycation, Antioxidant, *In silico*

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INTRODUCTION

Diabetes is one of the most ubiquitous chronic metabolic diseases around the globe. This disease had influenced about 387 million people globally in 2014 and the statistics are projected to proliferate by another 205 million people by 2035 (Guariguata *et al*., 2014). The major classification of diabetes include type I and type II diabetes which is characterized by chronic hyperglycemia resulting from absolute or relative deficiencies in insulin secretion and activity (Guariguata *et al*., 2014). Perpetuated hyperglycaemic state in diabetes patients urged non enzymatic glycation reaction that leads to production of profusion of heterogeneous end products which are termed as advanced glycation end products (AGEs). These AGEs form adducts on proteins, inducing cellular dysfunctions leading to lifelong diabetes secondary complications such as retinopathy, neuropathy,

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nephropathy and cardio-vascular diseases (Brings *et al*., 2017). Therefore, protein glycation is expedited under a hyperglycaemic condition, analogous with diabetes and under oxidative stress, causing irreversible functional and structural damage to contrived molecules of proteins (Perera and Wijetunge, 2015). So, therefore, there is necessitate for compounds with antiglycation and antioxidant potential in controlling and retarding the inception of complication of diabetes.

There are varied types of medications for type II diabetes, including sulfonylureas, biguanides, AGEs inhibitors, alpha amylase inhibitors and alpha glucosidase inhibitors; each works differently (Rang, 2003). The curative approach for controlling postprandial hyperglycaemia is to decelerate absorption of glucose by the inhibition of glycolytic enzymes in the digestive system (Ku *et al.,* 2009). Disintegration of dietary carbohydrates like starch is the major source of glucose in the blood. Pancreatic alpha amylase and intestinal alpha glucosidase are the major enzymes in the digestive system that catalyse the first step in the digestion of starch by hydrolyzing the alpha-1,4-glucoside linkages. The inhibition of these enzymes potentially decreases the digestion and uptake of carbohydrates, thereby curtailing the postprandial blood glucose level in the type II diabetes patients (Fred-Jaiyesimi *et al.,* 2009). Therapeutic medications, such as aminoguanidine and acarbose in current medical treatment are used as AGEs, alpha amylase and alpha glucosidase inhibitors, respectively. The main pitfall of these medications is their undesirable effects such as bloating, abdominal distension, flatulence and possibly diarrhoea (Chakrabarti and Rajagopalan, 2002 and Kimmel and Inzucchi, 2005). In present time there are multitudinous plant materials been reported as potent sources of unique phytochemical compounds such as polyphenols and flavonoids. Varied researchers have investigated potential plant materials for the development of newer therapeutics for biologically active antioxidants, antiglycants and antihyperglycemic agents from natural resources without any adverse effects (Sompong *et al.,* 2016).

Molecular docking is a faux approach that predicts molecules that bind appropriately with targets including enzymes and receptor proteins. The approach in virtual screening is known as hierarchical method which is powerful tool on computing and screening of protein structure to discover new ligand (Kitchen *et al.,* 2004). This ligand should have high affinity with proteins to determine the binding site of one compound for a given receptor as well as the property of interaction and leverage based on a scoring entity (Gilson and Zhou, 2007). For instance, the activity of specific protein in human beings may be antagonized by the inhibitor discovery and protein ligand interactions can be mainly utilized as a potential medication development that is called pharmacophore model, can be used to perform and identify small molecules in silico protein structure and functional prediction. Hence, the demand of improved pharmacophore modelling is important for richly lowering the medication cost and new pathways determined most likely bind to target protein (Yang, 2010 and Jhong *et al.,* 2015).

Fruits are well known to contain high amount of fibre, antioxidants and a diet high in these foods helps to prevent oxidative stress and slows down the diabetic complications (Parengkuan *et al*., 2013). It is better to prefer fresh fruits rather than dry fruits for maximum utilization of food intake. Apple, pear, amla, orange, pomegranate and plum are fruits favourable for diabetic patients. These fruits are rich with high protein concentration than sugar concentration. Consumption ofthese fruits promotes insulin production there by managing diabetes (Roy *et al*., 2011).

In the current research, the rationale of this study was to analyse the selected edible fruits for their associated potential in managing type II diabetes such as abnormally hyperglycemia related against glycolytic enzymes and glycation activity utilizing *in vitro* models with validating it's *in silico* interactions.

METHODOLOGY

Chemicals:

DPPH (2,2- Diphenyl-l-picrylhydrazyl) (D 9132), Trolox (6-Hydroxy-2,5,7,8- tetra methylchromane-2-carboxylic acid) (238813), Gallic acid (G 7384), Rutin hydrate (R 5143), ABTS (2,2-Azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt) (A 1888), PPA (porcine pancreatic α-amylase type VI-B) (A 3176), Acarbose (A 8980), pNPG (4-p-nitrophenyl-α- D-glucopyranoside) (N 1377), α-glucosidase (Type I from baker's yeast) (G 5003), AG (Aminoguanidine Hydrochloride) (396494) were purchased from Sigma Aldrich Company (St. Louis, MO,USA).

Plant materials and extraction:

Six fresh fruits *i.e.* papaya (*Carica papaya*), bael (*Aeglemarmelos*), guava (*Psidium guajava*), mango (*Mangifera indica*), pomegranate (*Punica granatum*) and amla (*Phyllanthus emblica*) were studied. All the fruits were procured from Anand Agriculture University, Anand, Gujarat, India. Two purchases of each sample were made in order to perform the experiment in duplicates. The harvested fruits were thoroughly washed under running tap water, cleaned, peeled and the fresh pulps of the fruits were collected. Subsequently, the fresh pulps were ground (Philips mixer grinder, India) and made into a fine paste prior to extraction. From that fine paste, extract of each fruits were made using 80 per cent methanol (pH 2) and stored at -20^{0}C for the determination of total phenols, total antioxidant capacity (TAC), alpha amylase inhibition, alpha glucosidase inhibition and anti-glycation activity.

Determination of total phenols:

Total phenolic content (TPC) of varied fruits were determined using Folin–Ciocalteu reagent (Singleton and Rossi, 1965) and the results were expressed as per cent of gallic acid equivalents of fresh weight. Flavonoids content was determined using aluminium chloride colorimetric technique (Zhishen *et al.*, 1999) and the results were expressed as per cent of rutin equivalents of fresh weight.

Determination of total antioxidant capacity (TAC):

Ferric reducing antioxidant power assay (FRAP) method was assayed (Benzie and Strain, 1996) as a measure of antioxidant power and the results were determined as per cent of trolox equivalents of fresh weight. Free radical scavenging activity ability by the use of stable DPPH radical (DPPH RSA) was determined (Brand Williams *et al*., 1995) as the ability of methanolic extracts of these fruits to scavenge 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical. The results were demonstrated as per cent of trolox equivalents of fresh weight. Free radical scavenging activity ability by the use of stable ABTS radical (ABTS RSA) activity was determined by ABTS radical cation decolourization assay (Re *et al*., 1999). The results were expressed as per cent of trolox equivalents of fresh weight.

Alpha-amylase inhibition assay:

This was assessed using the slightly modified starch-iodine colour change method (Picot *et al.*, 2014). Briefly, 300 µl of alpha-amylase solution from porcine origin (10 U in 0.1 M sodium acetate buffer pH 7.2) was added to 1 ml soluble starch solution and 100 µl of sodium acetate buffer. The reaction mixture was incubated for 37°C for 1 h. Then, 200 µl from the reaction mixture was discharged into 3ml of distilled water and 100 µl of iodine solution. After mixing, the absorbance of the starch-iodine solution was measured at 565nm using visible-spectrophotometer (Systronics,166 India Ltd.). For assessing the potential inhibitory activity of graded concentrations of all the fruits (400–16000 µg/ml) 100-400 µl extract was pre incubated with 300 µl enzyme solution at 37°C for 30 min. Acarbose (5 mg/ml) solution was used as a positive control. Per cent inhibition of Alpha-amylase enzyme activity = $(A_{ex} - A_e) / (A_s - A_e)*100$, where A_{ex} is the absorbance of extract, A_e is the absorbance of the enzyme and A_s is the absorbance of the starch.

Alpha- glucosidase inhibition assay:

This activity was modified and determined using the substrate pNPG, which could be hydrolysed by alpha-glucosidase to release the product p-nitrophenol and then monitored by a yellow colour reagent at 405 nm. Ten to fifty microliter dilutions of the extracts were mixed with 10 µl of alpha glucosidase (1mg/ml) in 0.1 M phosphate buffer (pH 6.8) solution was added. Then the reaction was terminated by addition of 400 µl 0.1M Na_2CO_3 and mixtures were incubated at 37°C for 75 min before reading the absorbance (Wang *et al.* 2015).

$$\text{Inhibition } \% = (A_{control} - A_{sample}) A_{control} \times 100\%$$

***In silico* validation:**

Molecular docking of major phenolic compound present in the fruit which would be ranking high in all the *in vitro* analysis was taken forward with the two glycolytic enzymes alpha amylase and alpha glucosidase was carried out to identify the interaction of the proteins with the ligand. The alpha amylase (PDB ID 1CPU) and alpha glucosidase (PDB ID 3CTT) were retrieved from RCSB the Protein Data Bank (PDB). Using spdbv 4.10, the structures of the receptors were prepared for docking by removing the water molecules. Automated molecular docking studies of the inhibitory ligand at the binding sites were performed with the Arguslab docking software 4.0.

***In vitro* glycation of bovine serum albumin (BSA):**

Albumin glycation was performed according to the previously studied method (Tupe *et al.*, 2015) with fewer modifications. Glycated samples were prepared by incubating BSA (20 mg/ml), glucose (500 mM) in potassium phosphate buffer (0.2 M, pH 7.4 containing 0.02 % sodium azide) along with methanolic extracts of all the fruits (1 ml) and incubated at 37°C for 30 days. The antiglycation potential of methanolic extracts of fruits was determined every week by estimation of three parameters from the glycation system, *i.e.*, Nitro blue tetrazolium reductive assay, Formation of alpha-dicarbonyl compounds and AGEs.

Nitro blue tetrazolium reductive assay (NBT) was modified and used to determine the level of fructosamines (an amadori product) (Baker *et al*., 1985). Formation of alpha-dicarbonyl compounds method was slightly altered and performed using the Girard-T assay (Wang *et al.*, 2011). Briefly, 200 µl of the incubated solution was mixed with 100 µl of deionized water, 100 µl of Girard-T reagent (500 mM) and 1.7ml of 500 mM sodium formate (pH 2.9) in a test tube and was incubated at ambient temperature at 37°C for 1 h. The absorbance of the solution at 290 nm using UV- visible spectrophotometer (Shimadzu Inc., Kyoto, Japan) was then determined. AGEs fluorescence measurement was assessed by determining the production of these fluorescent products of glycated albumin samples, positive control and control at excitation and emission wavelengths at 370 and 440 nm (slit =10 nm), respectively on fluorescence spectrophotometer (Hitachi F-7000, Japan) (Tupe *et al.*, 2015). The results were expressed as per cent inhibition as calculated by the formula:

$$\% \text{ inhibition} = [(F_0-F_1)/F_0]\times 100\}$$

where, F_0 is the fluorescence of the positive control and F_1 is the fluorescence of the glycated albumin samples co-incubated with methanolic extracts of fruits.

Statistical analysis:

The above all experimental results are expressed as mean ± standard deviation (SD) of four measurements of each sample. Then after, the results were statistically subjected to analysis of variance (ANOVA), the significance of mean differences was determined by Duncan's post hoc test considering $p \leq 0.05$ significant level of difference. The pearson's correlation co-efficients (r) were also calculated to establish relationships among data obtained. All the statistical calculations were done using SPSS version 20.0 software.

OBSERVATIONS AND ASSESSMENT

The antioxidant activities of fruits are shown in Table 1. The total phenolic content ranged from 2287.03 to 263.82 mg GAE/100g among the different fruits studied. The estimated total phenolic content was the highest inamlathan other fruits and the current result was found to be high than the other previous reported studies (Saikia *et al.*, 2016 and Laulloo *et al.*, 2018).The flavonoid content ranged from 781.20 to 16.34 mg RE/100 g among the different fruits studied. The highest content was found in the amla and the lower values of the same were observed by different other investigators than the present study (Liu *et al.*, 2008 and Saikia *et al.*, 2016).

The antioxidant capacity of six different fruits was measured using three different methods namely FRAP, DPPH RSA and ABTS RSA. The principles of antioxidant capacity by different methods are different and therefore, the values obtained for antioxidant capacity are different (Zhishen *et al.*, 1999; Benzie and Strain,1996

Table 1: Antioxidant activity of fruits including total phenol, flavonoid, FRAP, DPPH RSA and ABTS RSA

Fruits	Total phenol (mg GAE/100 g)	Flavonoids (mg RE/100 g)	FRAP (mg TE/100 g)	DPPH RSA (mg TE/100 g)	ABTS RSA (mg TE/100 g)
Papaya	638.79±57.99^{b}	16.34±1.85^{a}	385.63±30.42^{a}	800.54±73.73^{a,b}	70.87±4.63^{a}
Bael	638.79±57.99^{b}	46.54±1.85^{a}	1231.40±3.50^{d}	897.34±107.69^{b}	155.28±14.73^{c}
Guava	446.37±38.85^{a}	332.26±5.87^{c}	999.70±58.61^{c}	726.03±23.85^{a}	274.15±28.75^{d}
Mango	263.82±25.69^{a}	32.35±5.82^{a}	633.35±2.32^{b}	898.97±91.14^{b}	116.94±12.19^{b}
Pomegranate	292.17±13.25^{a}	105.46±4.61^{b}	658.27±46.08^{b}	901.14±83.92^{b}	181.48±12.53^{c}
Amla	2287.03±278.65^{c}	781.20±73.04^{d}	6713.75±162.65^{e}	7038.49±47.65^{c}	1762.82±49.07^{e}
F- value	161.88**	395.42**	4335.49**	4359.90**	2750.30**

Values are mean of ± S.D. of four observations. Mean value of different superscripts within a column are significantly different from each other
** ($p \leq 0.01$). GAE-Gallic acid equivalent, RE-Rutin equivalent, TE-Trolox equivalent

and Brand-Williams *et al.*,1995). The mean values of the FRAP was found to be 6713.75 to 385.63 mg TE/ 100g and the highest was found in amla methanolic extract. The descending order for the FRAP was amla>bael> guava > pomegranate > mango > papaya and these results were comparable with the reported findings (Saikia *et al.,* 2016). The DPPH RSA is the most useful method to measure antioxidant capacity of different plant materials as it is easy to perform, saves time and inexpensive too (Brand-Williams *et al.,* 1995). The mean values of the DPPH RSA were found to be 7083.49 to 726.03 mg TE/ 100g. The highest DPPH RSA was found in methanolic extract of amla and this result was found to be predominant than the reported literature (Saikia *et al.,* 2016; Laulloo *et al*., 2018; Liu *et al.*,2008). The highest ABTS RSA was found in amla (1762.82 mg TE/100g) methanolic extract and it ranged from 1762.82 to 70.87 mg TE/100g among other fruits. The previous investigator reported the lower values of the amla than the present study (Laulloo *et al.,* 2018).

The amla had the highest value of total phenol, flavonoid, FRAP, DPPH RSA and ABTS RSA. It is due to the presence of major potent phenolic compounds in amla like tannic acid with maximum containing twenty five (-OH) groups, quercetin possessing five (-OH) groups, ascorbic acid and gallic acid both containing four (-OH) groups (Srinivasan *et al*., 2018; Muthuraman *et al*., 2011) that helps in donating hydrogen atoms which stabilizes the free radicals to inhibit the oxidation strongly.

The mean values of alpha amylase and alpha glucosidase inhibition in percentage as well as the IC_{50} values are presented in Table 2. The mean value of alpha amylase inhibition of acarbose (positive control) was found to be 22.87 per cent and was found to be similar in reported study (Adisakwattana *et al*., 2011). The alpha amylase inhibition of different fruits ranged from 38.07 to 15.07 per cent and the highest was found in amlamethanolic extract. The IC_{50} value (mg/ml) of alpha amylase inhibition was found in order of acarbose >amla> mango > guava > papaya > pomegranate. These results revealed that amla, mango and guava showed the higher alpha amylase inhibition activity. The papaya and pomegranate showed the moderate inhibition while bael showed poor inhibition of alpha amylase activity. Varied investigators (Poongunran *et al.,* 2015) reported higher inhibition of alpha amylase enzyme in amla which was found to be lower than the present study whereas for guava, papaya and pomegranate there were no reported data found till this date. The alpha amylase is inhibited by amla fruit may be due to the effect of hydroxylation galloylation of flavonoids compound mainly present in it (Xiao *et al*., 2013). The amla contains quercetin, a flavonol, where on B ring at fifth carbon position H→OH or quercetin → myricetin and that facilitates the inhibition of alpha amylase enzyme (Tadera *et al.,* 2006). The above mechanism takes place in presence of double bonds on C2–C3 in the ring C, dihydroxyl or three adjacent hydroxyl groups on the ring B and the presence of hydroxyl groups on C-5 and C-7 in the ring A are said to be required for the antioxidant mechanism of flavonoids (Woodman *et al.,* 2005). Therefore, these reasons exhibits that the hydroxyl group plays a very important role in inhibiting alpha amylase inhibition. The mean value of alpha glucosidase inhibition was 20.42 per cent for acrbose and it was found from 66.01 to 10.01 per cent in six different fruits. Inhibition by acarbose was found to

Table 2: Inhibitory effects of different fruits on the alpha amylase and alpha glucosidase activity

Fruits	Alpha amylase inhibition (%)	IC_{50} mg/ml alpha amylase inhibition	Alpha glucosidase inhibition (%)	IC_{50}µg/ml alpha glucosidase inhibition
Papaya	16.94±2.40^{a}	19.10	35.76±4.06^{a,b,c}	25.00
Bael	15.07±1.39^{a}	26.27	56.21±6.67^{c,d}	10.19
Guava	29.70±3.31^{a}	14.21	42.59±5.03^{b,c,d}	37.99
Mango	33.25±5.17^{a}	12.64	58.55±9.40^{c,d}	2.19
Pomegranate	20.05±5.23^{a}	20.63	10.01±3.20^{a}	72.08
Amla	38.07±4.62^{a}	12.27	66.01±7.96^{d}	0.38
Acarbose	22.87±3.42^{a}	0.11	19.17±4.43^{a,b}	2.21
F- Value	0.625		5.62**	

Values are mean of ± S.D. of four observations.Mean value of different superscripts within a column are significantly different from each other** ($p \leq 0.01$)

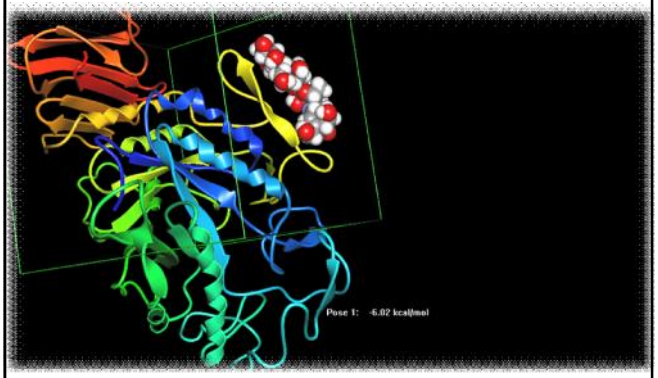
be higher in this present research than the earlier reported once (Rasouli *et al.*,2017 and Salehi *et al*., 2013). The highest value was obtained by the amla methanolic extract. From the results of IC_{50} the following order was found *i.e.* amla> mango > acarbose >bael> papaya >guava > pomegranate. The amla, mango and bael showed the highest alpha glucosidase inhibition activity whereas papaya and guava showed moderate alpha glucosidase inhibition activity among the six studied fruits. The phenol and flavonoids structures and the position and the numbers of hydroxyl groups are pre-determining factors for alpha glucosidase inhibition. Here in A-ring at carbon number position of 5th, 6th and 7th the presence of hydroxyl groups increases the inhibition of enzyme (Gao *et al.*, 2004; Gao and Kawabata, 2005 and Babu *et al.*, 2008). In amla the presence of quercetin contains five (–OH) groups at all the carbon numbers where as in pomegranate the caffeic acid contains only three (–OH) groups and at 5th carbon number position the –OH group is lacking, hence, alpha glucosidase inhibition is found more in amla than in pomegranate in the present study (Gu *et al.*, 2015).

The *in silico* docked analysis of different ligands with alpha amylase protein and alpha glucosidase protein with their binding energies having the most suitable pose are enlisted in Table 3. With alpha amylase 1CPU protein andalpha glucosidase 3CTT protein the gallic acid (major phenolic compound present in amla) and acarbose ligands were docked in the catalytic site and interacted with amino acids with binding energies as shown in Fig. 1 and 2 demonstrating positive binding force and potential enzymatic inhibition. The docked energy of gallic acid with alpha amylase protein was -6.06 Kcal/mol and alpha glucosidase protein was -6.21 Kcal/mol accordingly. These protein and ligand interactions were compared with the interaction of positive control *i.e.* acarbose with alpha amylase inhibition and alpha glucosidase whose molecular docking energies were -6.02 Kcal/mol and -5.68 Kcal/mol Fig. 1 and 2 and Table 3, respectively. It is observable that, gallic acid enzymatic inhibition energies are analogous to positive inhibitor named acarbose compound. It is important to note that, several *in vitro* and *in silico* investigations have been carried out for gallic acid phenolic compound from various sources (Smruthi *et al*., 2016 and Jhong *et al.*, 2015) and showed lower binding energies for both alpha amylase and alpha glucosidase proteins than the present study. Binding strength of

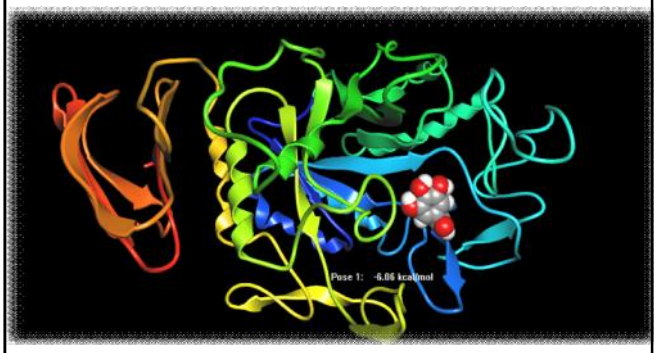
Table 3: Docking analysis of different ligands with alpha amylase and alpha glucosidase proteins

Compounds	Docked energy (Kcal/mol)	No. of hydrogen bonding	Amino acids	Bond length (Å)
		Alpha amylase protein interactions		
Acarbose	-6.02	04	ILE 372	3.60
			GLU 369	3.87
			THR 317	4.79
			ASN 380	5.15
Gallic acid	-6.06	06	GLU 18	10.04
			THR 71	15.81
			ARG 72	15.91
			VAL 22	19.14
			TRP 21	20.81
			ASN 75	22.68
		Alpha glucosidase protein interactions		
Acarbose	-5.68	03	GLY 397	2.2
			LYS 492	2.9
			ASP 396	3.0
Gallic acid	-6.21	05	VAL 310	3.64
			ARG 298	4.59
			ASP 609	5.44
			ARG 298	5.65
			GLY 602	9.30

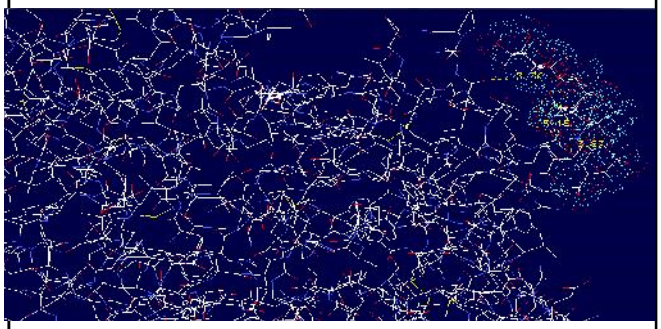
(General overview)
Acarbose with alpha amylase



Gallic acid with alpha amylase



(Detailed interaction)
Acarbose with alpha amylase



Gallic acid with alpha amylase

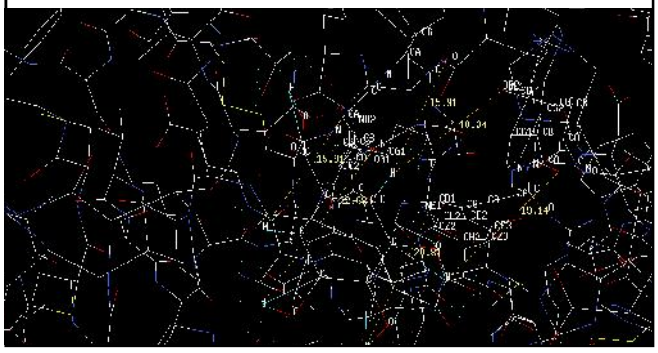
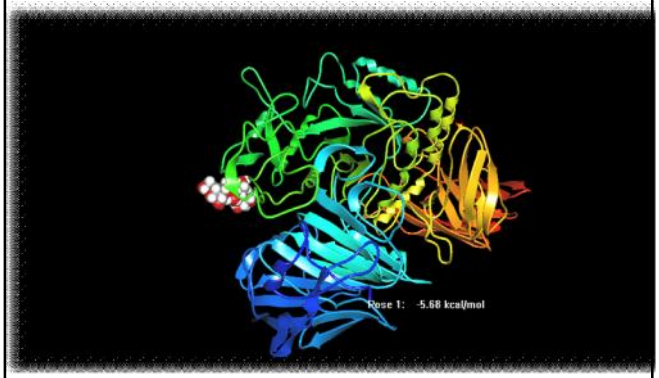
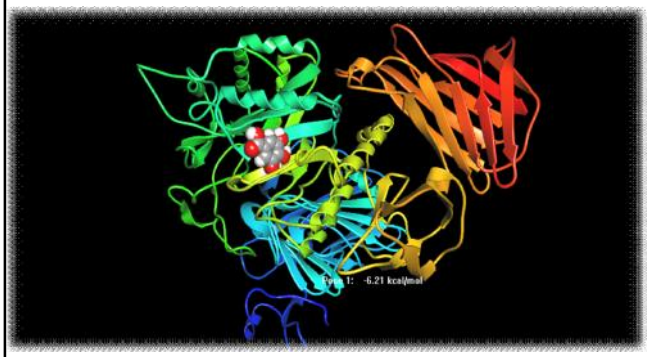


Fig. 1: Acarbose and gallic acid compounds docked with alpha amylase: Molecular docking analysis

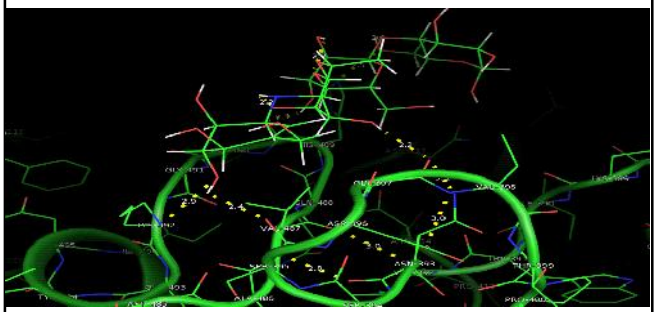
(General overview)
Acarbose with alpha glucosidase



Gallic acid with alpha glucosidase



(Detailed interaction)
Acarbose with alpha glucosidase



Gallic acid with alpha glucosidase

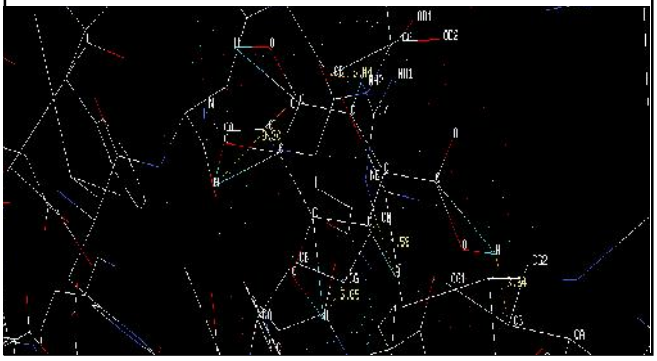


Fig. 2: Acarbose and gallic acid compounds docked with alpha glucosidase: Molecular docking analysis

acarbose was also found to be higher in the present study than the earlier investigated studies (Nivetha *et al.*, 2016; Choudhary and Mishra, 2017 and Ganogpichayagrai *et al*., 2017). Therefore, these *in silico* results validates that phenolic compound mainly gallic acid present in amla may be responsible for potent glycolytic enzymatic inhibition.

The spontaneous post-translational modification of proteins through reducing sugars is called the Maillard reaction or non-enzymatic glycation and the products resulting after the exposure to reducing sugars are called as AGEs. The production of alpha dicarbonyl compounds during the Maillard reaction is an important step in the production of AGEs, among which methylglyoxal (MG) is one of the most highly reactive carbonyl species (RCS) in the human body. The accumulation of RCS, such as MG, in organisms and the associated metabolic imbalance will result in the development of many diabetic secondary complications in humans (Kaewnarin *et al.*, 2014). The polyphenols present in the fruits may possess the ability to inhibit the biosynthesis of AGEs through their antioxidant properties, metal-chelating ability, protein interaction, MG trapping, and/or blocking the receptor for advanced glycation end products (RAGE). Hence, fruits through above blocking mechanism can serve as antiglycation agents and delay or reduce the consequences of AGEs (Yeh *et al.*, 2017). The mean values of reduction of NBT (amadori products), alpha dicarbonyl compounds and AGEs in percentage are displayed in Table 3. In the initial phase of the AGEs reaction, reducing sugars will act on the terminal amino groups of protein to form unstable Schiff bases, which will further become more stable keto-amines, also called Amadori products, after rearrangement (Li *et al.*, 2014). The positive control AG showed 12.91 per cent reduction of NBT and it was ranged from 66.05 per cent to 24.41 per cent among all

Table 4: Inhibition of NBT, alpha dicarbonyl compound and AGEs of methanolic extracts of different fruits

Fruits	Reduction of NBT (%)	Alpha dicarbonyl compound inhibition (%)	AGEs inhibition (%)
Papaya	24.41±2.00^{b}	7.33±0.28^{a}	33.36±1.80^{b}
Bael	36.47±0.74^{d}	33.53±0.70^{c}	55.73±2.77^{e}
Guava	46.98±1.82^{e}	37.78±0.66^{d}	46.63±0.16^{c}
Mango	54.01±0.77^{f}	53.12±0.50^{e,f}	11.42±0.40^{a}
Pomegranate	30.80±0.28^{c}	26.78±0.40^{b}	50.17±0.17^{d}
Amla	66.05±0.52^{g}	51.97±0.37^{e}	71.29±0.02^{f}
Amino guanidine	12.97±1.55^{a}	56.17±6.10^{f}	71.92±1.68^{f}
F- value	1179.89**	224.51**	1232.93**

Values are mean of ± S.D. of four observations.Mean value of different superscripts within a column are significantly different from each other **($p \leq 0.01$)

Table 5: Correlation between antioxidant, antidiabetic and antiglycation properties of fruits

	Total phenol	Flavonoid	FRAP	DPPH RSA	ABTS RSA	Alpha amylase inhibition	Alpha glucosidase inhibition	NBT	Alpha dicaronyl compounds	Age
Total phenol										
Flavonoid	.885**									
FRAP	.964**	.927**								
DPPH RSA	.963**	.908**	.991**							
ABTS RSA	.955**	.947**	.995**	.991**						
Alpha amylase inhibition	.273	.305	.245	.235	.262					
Alpha glucosidase inhibition	.450*	.330	.408*	.372	.373	.683**				
NBT	.657**	.781**	.776**	.756**	.778**	.377	.560**			
Alpha dicaronyl compounds	.355	.517**	.525**	.490*	.514*	.318	.486*	.908**		
Age	.681**	.686**	.685**	.630**	.670**	.025	.064	.241	.060	

* and ** indicate significance of values at P=0.05 and 0.01, respectively

the six methanolic extract of different fruits. The highest NBT reduction was found in amla and mango, whereas the minimum was found in papaya fruit. It is worth mentioning here that polyphenols present in amla like quercetin and gallic acid inhibits AGE production at very initial stage. At the early stage of glycation, these polyphenolstraps the methylglyoxal and free radicals that terminate the glycation process (Li *et al*., 2014). During the process of Amadori rearrangement, the oxidation function induced by the catalytic function of metal ions or oxygen will produce many dicarbonyl compounds, including MG, glyoxal, and 3-deoxyglucosone. This stage is the intermediate phase of the AGEs (Kaewnarin *et al*., 2014). The inhibition of alpha dicarbonyl compounds of AG was 56.17 per cent. Mango showed the highest (53.12%) inhibition for alpha dicarbonyl compounds among six different fruits extract. Mango is rich in chlorogenic acid, vanillic acid and gallic acid that may be able to trap the reactive dicarbonyl species MGO and GO, therefore, can inhibit the formation of AGEs (Yeh *et al.,* 2017). The advanced phase is the last phase of the Maillard reaction. In the advanced phase, dicarbonyl compounds form isomers with the arginine and lysine residues of proteins, called AGEs. In this study AGEs inhibition was studied for methanolic extracts of various fruits and compared with AG. The AGEs inhibition of AG was 71.92 per cent at 20mM after four weeks of incubation at 37^0C. Among the fruits extract amla, bael and pomegranate showed high AGE inhibition (more than 50%). Guava and papaya fruits showed moderate AGE inhibition whereas mango exhibited poor AGE inhibition. In all the parameters of antiglycation activities 40 mg/ml of methanolic extract was used in the antiglycation system *in vitro* model. Amla is rich in tannic acid, quercetin, ascorbic acid and gallic acid could inhibit AGE production and the subsequent crosslinking of proteins (Wu *et al*., 2010). The basic structure of phenolic acid has many hydroxyl groups, therefore, it may have excellent antiglycative function (Lo *et al*., 2011).

Pearson's correlation co-efficient (r) of various parameters was studied in Table 4. The total phenolic content had a strong positive correlation with the flavonoid ($r = 0.885$, $p \leq 0.01$), FRAP ($r = 0.964$, $p \leq 0.01$), DPPH RSA ($r = 0.963$, $p \leq 0.01$) and ABTS RSA ($r = 0.955$, $p \leq 0.01$). The total phenol also had a moderate and significant correlation with alpha glucosidase inhibition ($r = 0.450$, $p \leq 0.05$),NBT reduction ($r = 0.657$, $p \leq 0.01$) and with AGE inhibition ($r = 0.681$, $p \leq 0.01$). The flavonoid content of all the fruits had a potent positive correlation with the FRAP ($r = 0.927$, $p \leq 0.01$), DPPH RSA ($r = 0.908$, $p \leq 0.01$), ABTS RSA ($r = 0.947$, $p \leq 0.01$), NBT reduction ($r = 0.781$, $p \leq 0.01$) and moderate correlation with alpha dicarbonyl inhibition ($r = 0.517$, $p \leq 0.01$) andAGE inhibition ($r = 0.686$, $p \leq 0.01$). Varied methods of examining antioxidant capacities of fruits have been studied here. The major phenolic compounds and flavonoids present in all fruits are gallic acid, quercetin, catechin, ferulic acid, ascorbic acid, vanillic acid, tannic acid and chlorogenic acid. Their chemical structure has at least minimum one or maximum twenty five hydroxyl (-OH) groups that acts upon oxidant groups and serves as potent antioxidant compounds by donating hydrogen atoms (Srinivasan *et al.,* 2018 and Muthuraman *et al.,* 2011). The basic structure of all phenolic acid has hydroxyl groups so they all have excellent antiglycation and MG trapping functions. So all of the fruits are enriched with phenolic acids and flavonoids as they possess three or more (–OH) groups that has the ability to trap MG and act as potent antioxidant and antiglycating agents (Muthuraman *et al*., 2014).

Conclusion:

The results of the present study revealed that mainly amla, mango and bael fruits had the better ability to ameliorate diabetes. It might also reduce secondary complications of diabetes as they also exhibited better antiglycation activity compared to other fruits. Therefore, amla, mango and bael could be used as a potential nutraceutical for diabetes.

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LITERATURE CITED

Adisakwattana, S., Lerdsuwankij, O., Poputtachai, U., Minipun, A. and Suparpprom, C. (2011). Inhibitory activity of cinnamon bark species and their combination effect with acarbose against intestinal α-glucosidase and pancreatic α-amylase. *Plant Foods for Human Nutr.*, **66** (2) : 143-148.

Babu, T. H., Rao, V. R. S., Tiwari, A. K., Babu, K. S., Srinivas, P. V., Ali, A. Z. and Rao, J.M. (2008). Synthesis and biological evaluation of novel 8-aminomethylated oroxylinA analogues as α-glucosidase inhibitors.

Bioorganic & Medicinal Chem. Letters, **18**(5): 1659-1662.

Baker, J. R., Metcalf, P. A., Johnson, R., Newman, D. and Rietz, P. (1985). Use of protein-based standards in automated colorimetric determinations of fructosamine in serum. *Clinical Chemistry*, **31**(9) : 1550-1554.

Benzie, I. F. and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochem.*, **239**(1): 70-76.

Brand-Williams, W., Cuvelier, M. E. and Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science & Technol.*, **28**(1): 25 - 30.

Brings, S., Fleming, T., Freichel, M., Muckenthaler, M., Herzig, S. and Nawroth, P. (2017). Dicarbonyls and advanced glycation end-products in the development of diabetic complications and targets for intervention. *International J. Molecular Sci.*, **18**(5): 984.

Chakrabarti, R. and Rajagopalan, R. (2002). Diabetes and insulin resistance associated disorders: disease and the therapy. *Current Science*, **83** (12): 1533-1538.

Choudhary, D. K. and Mishra, A. (2017). *In vitro* and *in silico* interaction of porcine α-amylase with *Viciafaba crude* seed extract and evaluation of antidiabetic activity. *Bioengineered*, **8** (4) : 393-403.

Fred-Jaiyesimi, A., Kio, A. and Richard, W. (2009). α-Amylase inhibitory effect of 3 β-olean-12-en-3-yl (9Z)-hexadec-9-enoate isolated from Spondiasmombin leaf. *Food Chemistry*, **116**(1), 285-288.

Ganogpichayagrai, A., Palanuvej, C. and Ruangrungsi, N. (2017). Antidiabetic and anticancer activities of *Mangifera indica* cv. OKRONG leaves. *J.Adv. Pharmaceut. Technol. & Res.*, **8** (1) : 19.

Gao, H., Nishioka, T., Kawabata, J. and Kasai, T. (2004). Structure-activity relationships for α-glucosidase inhibition of baicalein, 5, 6, 7-trihydroxyflavone: the effect of A-ring substitution. *Bioscience, Biotechnology & Biochemistry*, **68** (2) : 369-375.

Gao, H. and Kawabata, J. (2005). α - glucosidase inhibition of 6- hydroxyflavones. Part 3: Synthesis and evaluation of 2, 3, 4-trihydroxybenzoyl-containing flavonoid analogs and 6-aminoflavones as α-glucosidase inhibitors. *Bioorganic & Medicinal Chemistry*, **13**(5) : 1661-1671.

Gilson, M. K. and Zhou, H. X. (2007). Calculation of protein-ligand binding affinities. *Annu. Rev. Biophys. Biomol. Struct.*, **36** : 21-42.

Guariguata, L., Whiting, D. R., Hambleton, I., Beagley, J., Linnenkamp, U. and Shaw, J. E. (2014). Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res. & Clinical Practice*, **103**(2) : 137-149.

Gu, Jenny, Strauss, Clara, Bond, Rod and Cavanagh, Kate (2015). How do mindfulness-based cognitive therapy and mindfulness-based stress reduction improve mental health and wellbeing? A systematic review and meta-analysis of mediation studies. *Clinical Psychology Review,* **37**: 1–12.

Jhong, C. H., Riyaphan, J., Lin, S. H., Chia, Y. C. and Weng, C. F. (2015). Screening alpha glucosidase and alpha amylase inhibitors from natural compounds by molecular docking in silico. *Biofactors*, **41**(4) : 242-251.

Kaewnarin, K., Niamsup, H., Shank, L. and Rakariyatham, N. (2014). Antioxidant and antiglycation activities of some edible and medicinal plants. *Chiang Mai. J. Sci.*, **41**(1) : 105-116.

Kimmel, B. and Inzucchi, S. E. (2005). Oral agents for type 2 diabetes: an update. *Clinical Diabetes*, **23**(2) : 64-76.

Kitchen, D. B., Decornez, H., Furr, J. R. and Bajorath, J. (2004). Docking and scoring in virtual screening for drug discovery: methods and applications. *Nature Rev. Drug Discovery*, **3**(11) : 935.

Ku, S., You, H. J. and Ji, G. E. (2009). Enhancement of anti-tumorigenic polysaccharide production, adhesion and branch formation of *Bifidobacteriumbifidum* BGN4 by phytic acid. *Food Sci.& Biotechnol.*, **18**(3) : 749-754.

Laulloo, S.J., Bhowon, M.G., Chua, L.S. and Gaungoo, H. (2018). Phytochemical screening and antioxidant properties of phyllanthusemblica from mauritius. *Chemistry of Natural Compounds*, **54** (1) : 50-55.

Liu, X., Zhao, M., Wang, J., Yang, B. and Jiang, Y. (2008). Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthusemblica* L.) from six regions in China. *J. Food Composition & Analysis*, **21**(3) : 219-228.

Li, X., Zheng, T., Sang, S. and Lv, L. (2014). Quercetin inhibits advanced glycation end product formation by trapping methylglyoxal and glyoxal. *J. Agric. & Food Chem.*, **62** (50) : 12152-12158.

Lo, C. Y., Hsiao, W. T. and Chen, X. Y. (2011). Efficiency of trapping methylglyoxal by phenols and phenolic acids. *J. Food Sci.*, **76**(3) : 90-96.

Muthuraman, A., Sood, S. and Singla, S. K. (2011). The antiinflammatory potential of phenolic compounds from *Emblicaofficinalis* L. in rat. *Inflammopharmacology*, **19** (6) : 327-334.

Muthuraman, M., Hellriegel, H., Hoogenboom, N., Anwar, A.R., Mideksa, K.G., Krause, H., Schnitzler, A., Deuschl, G.

and Raethjen, J. (2014). Beamformer source analysis and connectivity on concurrent EEG and MEG Data during voluntary movements. PLoS One, **9** (3): e91441, 10.1371/journal.pone.0091441.

Ng, K., Gu, C., Zhang, H. and Putri, C. Y. (2015). Evaluation of α-amylase and α-glucosidase inhibitory activity of flavonoids. *Internat. J. Food & Nutr. Sci.*, **2**(2): 174-179.

Nivetha, G., Vishnupriya, V. and Gayathri, R. (2016). Comparative evaluation of anti-diabetic activity of lemon grass oil and *Tulasi* Oil. *Int. J. Pharm. Sci. Rev. Res*., **39** (1) : 221-225.

Parengkuan, L., Yagi, M., Matsushima, M., Ogura, M., Hamada, U. and Yonei, Y. (2013). Anti-glycation activity of various fruits. *Anti-Aging Med.*, **10** : 70 -76.

Perera, H. K. I. and Wijetunge, D. C. R. (2015). Strong protein glycation inhibitory potential of clove and coriander. *British J. Pharmaceutical Res.*, **6** (5) : 306-312.

Picot, C., Subratty, A. H. and Mahomoodally, M. F. (2014). Inhibitory potential of five traditionally used native antidiabetic medicinal plants on α-amylase, α-glucosidase, glucose entrapment and amylolysis kinetics *in vitro*. *Adv. Pharmacological Sci.*, http://dx.doi.org/10.1155/2014/739834.

Poongunran, J., Perera, H. K.I., Fernando, W. I. T., Jayasinghe, L. and Sivakanesan, R. (2015). Alpha-glucosidase and alpha-amylase inhibitory activities of nine Sri Lankan antidiabetic plants. *British J. Pharmaceutical Res.*, **7**(5) : 365-374.

Rang, H. P. (2003). *Anthelmintic drugs*. In: Rang, H.P., Dale, M.M., Ritter, J.M. and Moore, P.K., *Book Review: Pharmacology*, 5th Ed., Churchill Livingstone, Edinburgh.

Rasouli, H., Hosseini-Ghazvini, S.M.B., Adibi, H. and Khodarahmi, R. (2017). Differential α-amylase/α-glucosidase inhibitory activities of plant-derived phenolic compounds: a virtual screening perspective for the treatment of obesity and diabetes. *Food & Function*, **8** (5) : 1942-1954.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cationdecolorization assay. *Free Radical Biology & Medicine*, **26**(9-10) : 1231-1237.

Roy, A., Geetha, R. V., Lakshmi, T. and Nallanayagam, M. (2011). Edible fruits—nature's gift for diabetic patients a comprehensive review. *Internat. J. Pharmaceutical Sci. Rev. & Res.*, **9**(2) : 170-180.

Saikia, S., Mahnot, N.K. and Mahanta, C.L. (2016). Phytochemical content and antioxidant activities of thirteen fruits of Assam, India. *Food Bioscience*, **13** : 15-20.

Salehi, P., Asghari, B., Esmaeili, M. A., Dehghan, H. and Ghazi, I. (2013). α-glucosidase and α-amylase inhibitory effect and antioxidant activity of ten plant extracts traditionally used in Iran for diabetes. *J. Medicinal Plants Res.*, **7**(6): 257-266.

Singleton, V. L. and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American J. Enol. & Viticult.*, **16**(3): 144-158.

Smruthi, G., Mahadevan, V., Vadivel, V. and Brindha, P. (2016). Docking studies on antidiabetic molecular targets of phytochemical compounds of [*Syzygium cumini* (L.) Skeels]. *Asian J. Pharmaceutical & Clinical Research*, **9** (3) : 287-293.

Sompong, W., Muangngam, N., Kongpatpharnich, A., Manacharoenlarp, C., Amorworasin, C., Suantawee, T. and Adisakwattana, S. (2016). The inhibitory activity of herbal medicines on the keys enzymes and steps related to carbohydrate and lipid digestion.*BMC Complementary & Alternative Medicine*, **16**(1) : 439.

Srinivasan, P., Vijayakumar, S., Kothandaraman, S. and Palani, M. (2018). Anti-diabetic activity of quercetin extracted from *Phyllanthusemblica* L. fruit: *In silico* and *in vivo* approaches. *J. Pharmaceutical Analysis*, **8** (2) : 109-118.

Tadera, K., Minami, Y., Takamatsu, K. and Matsuoka, T. (2006). Inhibition of α-glucosidase and α-amylase by flavonoids. *J. Nutr. Sci. Vitaminol.*, **52**(2) : 149-153.

Tupe, R. S., Sankhe, N. M., Shaikh, S. A., Phatak, D. V., Parikh, J. U., Khaire, A. A. and Kemse, N. G. (2015). Aqueous extract of some indigenous medicinal plants inhibits glycation at multiple stages and protects erythrocytes from oxidative damage–an *in vitro* study. *J. Food Sci. & Technol*,, **52**(4) : 1911-1923.

Wang, S. H., Chang, J. C., Pokkaew, R., Lee, J. F. and Chiou, R. Y. Y. (2011). Modified fast procedure for the detection and screening of antiglycative phytochemicals. *J. Agric. & Food Chem.*, **59**(13) : 6906-6912.

Wang, T., Li, X., Zhou, B., Li, H., Zeng, J. and Gao, W. (2015). Anti-diabetic activity in type 2 diabetic mice and α-glucosidase inhibitory, antioxidant and anti-inflammatory potential of chemically profiled pear peel and pulp extracts (*Pyrus* spp.). *J. Functional Foods*, **13** : 276-288.

Woodman, O.L., Meeker, W.F. and Boujaoude, M. (2005). Vasorelaxant and antioxidant activity of flavonols and flavones: structure-activity relationships. *J. Cardio-vascular Pharmacol.*, **46**(3) : 302-309.

Wu, C. H., Yeh, C. T., Shih, P. H. and Yen, G. C. (2010). Dietary phenolic acids attenuate multiple stages of protein glycation and high glucose stimulated proinflammatory IL -1β activation by interfering with chromatin remodeling and transcription in monocytes. *Molecular Nutr. & Food Res.*, **54**(S2) : S127-S140.

Xiao, J., Ni, X., Kai, G. and Chen, X. (2013). A review on structure–activity relationship of dietary polyphenols inhibiting α-amylase. *Critical Rev. Food Sci. & Nutr.*, **53**(5) : 497-506.

Yang, S. Y. (2010). Pharmacophore modeling and applications in drug discovery: challenges and recent advances. *Drug Discovery Today*, **15**(11-12) : 444-450.

Yeh, W. J., Hsia, S. M., Lee, W. H. and Wu, C. H. (2017). Polyphenols with antiglycation activity and mechanisms of action: A review of recent findings. *J. Food & Drug Analy.*, **25**(1) : 84-92.

Zhishen, J., Mengcheng, T. and Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.*, **64** (4) : 555-559.

■ WEBLIOGRAPHY

www.pdb.org/pdb.

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