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EVALUATION OF ALPHA AMYLASE, ALPHA GLUCOSIDASE AND PROTEIN GLYCATION INHIBITORY ACTIVITIES OF DIFFERENT ELEMENTS OF MANGO (*Mangiferaindica*)

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ABSTRACT

The current study was conducted to analyse in vitro alpha amylase, alpha glucosidase and protein glycation inhibitory activities of different elements of mango (pulp, peel and leaves). The results represent that total phenolic content (TPC), flavonoids and total antioxidant capacity (TAC) by FRAP, DPPH-RSA and ABTS-RSA exhibited marked variation ranging from 1360.40 to 3394.46 mg GAE/100 gm, 420.55 to 2331.06 mg RE/100gm, 772.90 to 7303.25 mg TE/100gm, 300.76 to 4375.98 mg TE/100gm and 267.44 to 481.95 mg TE/100gm, respectively. The inhibitory effect of alpha-amylase and alpha-glucosidase assay ranged from 5.97% to 40.26% and 12.78 % to 73.42% among all the elements of mango, respectively. Mango pulp exhibited significantly ($p<0.05$) elevated inhibitory effect on AGE's formation in the BSA glycation system among all the elements of mango. Hence mango pulp may be used for controlling blood glucose level and in the prevention of the development of secondary complications in type 2 diabetes.

Keywords: mango elements, alpha amylase, alpha glucosidase, antioxidant, anti-glycation.

INTRODUCTION

Diabetes mellitus (DM) is a vital persistent disease caused by the improper balance of glucose homeostasis. The disease is characterized by hyperglycemia, hyperlipidemia and inadequacy of secretion or action of endogenous insulin. Increasing evidence in variedreported studies suggests that there is a substantial decrease in innate antioxidant status in diabetic patients while the simultaneous increase of oxidative stress plays a major role in the pathogenesis [1]. Free radicals are formed disproportionately in diabetes by glucose oxidation, glycation of proteins (albumin), and the subsequent oxidative degradation of glycated proteins. These lead to impairment of cellular organelles and enzymes, increased lipid peroxidation, β-cell dysfunction, impaired glucose tolerance and development of insulin resistance [2].The aftermath of the formation of advanced glycation end products (AGEs) in the pathogenesis of secondary diabetic complications are very life-threatening and have long lasting pernicious effects on multiple functional systems in the body like excretory system, nervous system, endothelial dysfunction, etc. [3,4]. Therefore there is a surge of demand for compounds with antioxidant and anti-glycation activities as they may offer therapeutic potential in delaying, controlling or preventing the onset of diabetic complications [5].

Diabetes is multifactorial in origin and one therapeutic approach to treat diabetes is to retard the absorption of glucose via inhibition of enzymes, such as alpha- amylase and glucosidase. Hydrolysis of dietary carbohydrates such as starch by alpha-amylase and intestinal alphaglucosidases is the major source of glucose in the blood. Inhibiting of these intestinal enzymes retards the elevation of blood sugar following a carbohydrate meal. So this has been a dietary supplement target for reducing the postprandial glucose load in the body [6].Medicinal plants are reported to be a good source of potent, safe and affordable alpha-glucosidase and amylase inhibitors and enhancers of glucose uptake and recently it is seen that there is a high demand for the same in treating diabetes [7,8].

Mango (*Mangifera indica*), also called "the king of fruits", is one of the most popular fruits in tropical regions [9]. This fruit is available plenty in season and it is a good and cheap source for the exploration of the development of nutraceuticals for diabetes [5]. It is considered as a good source of dietary compounds, such as ascorbic acid, phenolic compounds and carotenoids, which are beneficial to health due to their antioxidant capacity [9]. But no detailed studies are available on the effects of mango fruit pulp, peel and leaves on various targets relevant to diabetes to explore the antidiabetic properties of this edible fruit except for afew reported studies [10, 11].Current therapeutics for type 2

diabetes is often associated with undesirable side effects. Type 2 diabetes is mainly due to insulin resistance induced complications, the scope for the development of therapeutics is plenty [5].

Hence, the present investigation was designed to evaluate the carbohydrate digesting intestinal enzyme inhibition(alpha-amylase, alpha glucosidase) and antiglycation potential of mango fruit elements (pulp, peel and leaves).

MATERIALS AND METHODS

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), TPTZ (2,4,6-Tris (2-pyridyl)-s-triazine) (T 1253), 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),NBT (Nitro blue tetrozolium) (N 5514), Girard's T stock (G 900) and Sodium formate (71539)were purchased from Sigma Aldrich Company (St. Louis, MO,USA). Soluble starch (1949150) was purchased from SRL Pvt. Ltd, Mumbai, India. All other chemicals procured were from local manufacturer and were of AR grade.

Plant Materials and Extraction: Elements of'*desi'* mango (pulp, peel and leaves) were procured from Anand Agricultural University, Anand. All the elements of mango were further identified and authenticated by the Department of Horticulture, Anand Agricultural University, Anand, Gujarat. The harvested mango elements were thoroughly washed underrunning tap water and air-dried until constant weight was obtained. Subsequently, the dried samples were ground (PHILIPS mixer grinder, India) and sieved to fine powder and then stored in a cool dry place prior to extraction. From this fine powder, duplicate extracts of each elements of mango was made using 80% methanol (pH 2) and stored at - 20^0C 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 elements of mango was determined using Folin–Ciocalteu reagent [12] and the results were expressed as % of gallic acid equivalentsof dry weight.
Flavonoid content was determined using the aluminium chloride colorimetric technique [13] and the results were expressed as % of rutin equivalents of dry weight.

Determination of Total Antioxidant Capacity:
Ferric Reducing Antioxidant Power Assay (FRAP): FRAP was assayed by Benzie and Strain (1996) [14] as a measure of antioxidant power and the results were determined as % of trolox equivalents of dry weight.
Free radical scavenging activity ability by the use of stable DPPH radical: The Antioxidant activity was determined by Brand Willams et al., 1995 [15] as the ability of methanolic extracts of these elements of mango to scavenge 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical. The results were demonstrated as % of trolox equivalents on dry weight basis.

Free radical scavenging activity ability by the use of stable ABTS radical: The free radical-scavenging activity was determined by ABTS radical cation decolorization assay described by Re et al., 1999 [16]. The results were expressed as % of trolox equivalents on dry weight basis.

Alpha-amylase Inhibition capacity:
***Alpha-amylase Inhibition by colorimetric assay*:**Alpha-Amylase activity was assessed using the slightly modified starch-iodine colour change method [17]. Briefly, 300 μl of alpha-amylase solution from porcine origin was added to 1 ml soluble starch solution and100 μlof sodium acetate buffer (0.1 M, pH 7.2).The reaction mixture was incubated for 37◦C for 1 h. Then, 100 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 a sprectophotometer (Systronics,166 India ltd.).For assessing the potential inhibitory activity of graded concentrations of elements of mango extracts (400–16000 μg/mL) 100-400 μL extract was preincubated with 300 μL enzyme solution at 37◦C for 30 min. Acarbose

solution was used as a positive control. The assay was then conducted as described above. Only starch (no enzyme), only enzyme (no starch) and substrate (enzyme and starch both) were carried out under similar assay conditions.The results were expressed as % inhibition of enzyme activity and calculated according to the following equation:
% 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-amylase Inhibition by disc assay*:**The inhibitory capacity of elements of mango (pulp, peel and leaves) methanolic extracts against a-amylase by the bore method was evaluated according to the methods described by Roberta et al., 2004 [18].

Alpha-glucosidase Inhibition capacity:
The alpha-glucosidase inhibitory activity was modified and determined using the substrate p-nitrophenyl- α-D-glucopyranoside (pNPG), which could be hydrolyzed by alpha-glucosidase to release the product p-nitrophenol, and then monitored by a yellow colour development at 405 nm [19].The alpha-glucosidase inhibitory activity was expressed as percentage inhibition,
Inhibition % = $(A_{control}– A_{sample}) A_{control} \times 100\%$.

Measurement of antiglycation potential of plant extracts
In vitro glycation of BSA: Albumin glycation was performed according to the method of Tupe et al., 2013 [20]with some modifications. Glycated samples were prepared by incubating 10 ml of BSA (20 mg/ml), 5 ml of glucose (500 mM) in 10 ml of potassium phosphate buffer (0.2 M, pH 7.4 containing 0.02 % sodium azide) along with methanolicextracts of elements of mango (1 ml) and incubated at 37 °C for 30 days. Positive control (BSA+ AG + glucose) and control (BSA + glucose–plant extract) was maintained under similar conditions and all the incubations were performed in duplicates. Before incubation, all the solutions were placed in sterile plastic-capped vials to maintain sterility and strict asepsis was maintained during the entire process. After the incubation period, it was ensured that all the samples were free of microbiological contamination. The antiglycation potential of methanolic extracts of elements of mango was determined every week by estimation of three parameters from the glycation reaction mixture, i.e.–i) Nitro blue tetrazolium reductive assay, ii) Formation of alpha-dicarbonyl compounds and iii) AGEs.

Nitro blue tetrazolium reductive assay: Nitroblue tetrazolium (NBT) assay was modified and used to determine the level offructosamines (an amadori product) [21].

Formation of alpha-dicarbonyl compounds: The determination of alpha-dicarbonyl compounds was slightly altered and performed using the Girard-T assay [22]. Briefly, 200µL of the incubated solution was mixed with 100µL of deionized water, 100 µL of Girard-T reagent (500 mMin 20 mM sodium phosphate buffer), and 1.7ml of500 mM sodium formate (pH 2.9) in a test tube and incubated at ambient temperature (37 °C) for 1 h. The absorbance of thesolution at 290 nm using UV visible spectrophotometer (Shimadzu Inc., Kyoto, Japan) was then determined.

AGEs fluorescence measurement:The formation of total advanced glycation end products (AGEs)were assessed by determining the production of these fluorescent products of glycated albumin samples, positive control and control at excitation and emission wavelengths of 370 and 440 nm (slit = 10 nm) respectively on Hitachi F-7000 fluorescence spectrophotometer [20]. The results were expressed aspercent inhibition as calculated by the formula:$\%Inhibition=[(F_0–F_1)/F_0]\times100$, 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 elements of mango [20].

Statistical analysis: All the experimental results are expressed as mean ± standard deviation (SD) of four measurements of each sample. The results were also subjected to Analysis of Variance (ANOVA), while the significance of mean differences was determined by Duncan's post hoc test considering $p\leq0.05$ as the significant level of difference. Pearson's correlation coefficients (r) were also calculated to establish relationships among the data obtained. All the

statistical calculations were done using SPSS version 20.

RESULTS AND DISCUSSION

The total phenol, flavonoid content and FRAP, DPPH, ABTS free radical scavenging activity of the elements of mango i.e. pulp, peel and leaves are presented in **Table 1**.

The total phenol content of pulp, peel and leaves of mango was found to be 1360.40, 1953.27 and 3394.46 mg/100gm respectively. Previous studies reported lower total phenol in pulp (2.01mg/g) [23], peel (83.0 mg/g) [24] and leaves (30.73g/100gm) [25] than the present study. The flavonoid content of the element of mango was found to be highest in mango leaves 2331.06 mg RE/100 gm than the other elements of mango. Various researchers showed lower values of flavonoids for the elements of mango i.e. (151 µg QE/g) in pulp [26], (24.95 mg/g) in peel [23] and 37.57g QE/ 100gm in leaves [25] than the present findings. Radical scavenging activity by FRAP exhibited 772.90, 1066.01 and 7303 mg TE/100 gm in the pulp, peel and leaves of the mango.

The earlier reported studies were found to have decreased radical scavenging activity by FRAP in pulp (118.66-211.27 µg/ml) [27] and in peel (5.56mg/g) [24] than the present study. Hence, our results showed that mango leaves had significantly the highest ($p \leq 0.05$) total phenol, flavonoid and FRAPcontent than the other elements of mango in the present study andsimilar consistent results were found in the previous study also [27]. The antioxidant activity of phenolics is due to the reactivity of phenol moiety (OH group on aromatic ring). They have the ability to scavenge free radicals via hydrogen donation or electron donation [30]. Other radical scavenging activities of the present study by DPPH showed the highest activity in mango peel 4375 mg TE/100gm and in ABTS it was found to be high in mango pulp 481.95 mg TE/100 gm.The present study had the highest DPPH activity among all the elements of mango than values reported by various researchers [23, 25, 26]. The total antioxidant activity of these elements of mango have been reported to be modulated by the level of maturity, cultivar type, agronomic practices, climatic conditions, ripeness at harvest, and the postharvest storage conditions of the fruit [28].

The enzymatic inhibitory effects of elements of mango methanolic extracts on alpha amylase and alpha glucosidase activity are depicted in **Table 2**.

Diet containing high content of alpha-amylase and alpha-glucosidase inhibitors is one of the key target areas to control postprandial glucose levels. These intestinal enzyme inhibitors decrease the rate of glucose absorption through delayed carbohydrate digestion and prolong digestion time. As synthetic inhibitors exhibit side effects, inhibitors from plant sources offer an attractive therapeutic approach [24].

Table 1: Total Phenol, Flavonoid, FRAP, DPPH and ABTS of the elements of mango.

ELEMENTS OF MANGO	TOTAL PHENOL (mg GAE/100 gm)	FLAVONOIDS (mg RE/100 gm)	FRAP (mg TE/100 gm)	DPPH (mg TE/100 gm)	ABTS (mg TE/100 gm)
Pulp	1360.40±154.29^{a}	420.55±19.66^{a}	772.90±19.28^{a}	1468.75±107.17^{b}	481.95±20.88^{c}
Peel	1953.27±154.20^{b}	445.28±44.50^{a}	1066.01±28.49^{a}	4375.98±71.71^{c}	267.44±10.08^{a}
Leaves	3394.46±46.46^{c}	2331.06±7.41^{b}	7303.25±675.15^{b}	300.76±49.05^{a}	396.79±16.18^{b}
F-Value	264.02*	5952.53*	357.25*	2776.64*	175.16*

Values are mean of ± S.D. of four observations. Mean value of different superscripts within a column are significantly different from each other ($p \leq 0.05$). GAE-Gallic acid equivalent, RE-Rutin equivalent, TE-Trolox equivalent.

Therefore, *in vitro* antidiabetic property of the methanolic extract was examined by determining the inhibition of alpha- amylase and alpha-glucosidase activities by different elements of mango. The percentage inhibition of alpha amylase enzyme was found to be 40.26% in pulp, 7.06% in peel and 5.97% in leaves respectively. The percentage inhibition of alpha glucosidase enzyme was found to be the highest in pulp 73.42%, lower in peel 28.66% and the lowest in leaves 12.78% respectively. Mango pulp showed significantly ($p<0.05$) the highest inhibition among both alpha amylase and alpha glucosidase *in vitro* enzymatic activity in **Table 2**. Methanolic extracts of the elements

of mango showed alpha amylase IC 50 value of 11.65 mg/ml in pulp, 109.30 mg/ml in peel and 71.83 mg /ml in leaves respectively. The alpha glucosidase IC 50 value of 54.15 µg/ml in pulp, 381.11 µg/ml in peel and 1552.47 µg/ml in leaves.These IC50 values obtained in the present study, for both alpha amylase and alpha glucosidase inhibition with all the elements of mango extract are much lower than some of the reported values [24, 29]. The highest alpha amylase and alpha glucosidase inhibition value for mango pulp found in the present study may be possibly due to the presence of mangiferin and other phenols, flavonoids and carotenoids like gallic, eallagic, protocatecuic acids, quercitin, rutin and ß-carotene as reported by Ajila et al., 2010 [31].
The anti-amylase activity (%) by disc assay as depicted in **Plate 1** was found to be 100% in all the elements of mango (pulp, peel and leaves) at 24mg concentration where all the methanolic extracts have completely inhibited alpha amylase and have shown no clear zone compared to acarbose (alpha- amylase inhibitor) which has shown 12.46% inhibition at 120 µgconcentration.

The early stage glycation products by NBT assay i.e. amadori products were found to be inhibited by different elements of mango

extracts ranging from 35 to 45 %, where mango pulp exhibited maximum inhibition **(Figure 2)**, while the protein alpha dicarbony

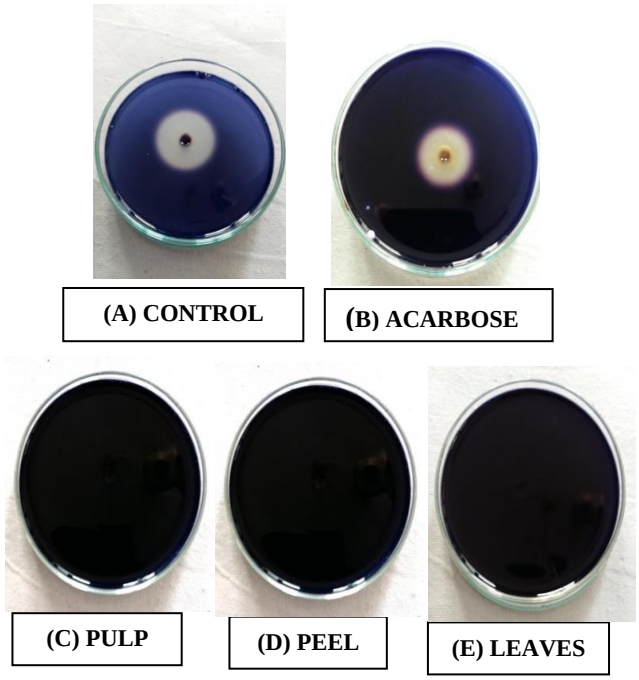

Plate 1: Alpha amylase inhibition by Disc Assay. The clear zone around the centre disc in (A) represents the area where starch has been degraded by a-amylase activity. (B) The clear zone around the centre disc represents area where starch has been degraded by Acarbose (120 µg) (C) Mango pulp, (D) Mango peel and (E) Mango leaves at 24 mgconcentration have inhibited amylase completely and show no clear zone.

Table 2: Elements of the Mango extracts with inhibitory effects on alpha amylase and alpha glucosidase activity

ELEMENTS OF MANGO	% alpha amylase inhibition	IC50 mg/ml alpha amylase inhibition	% alpha glucosidase inhibition	IC 50 µg/ml alpha glucosidase inhibition
Pulp	40.26±4.83^{b}	11.65	73.42±8.85^{b}	54.15
Peel	7.06±4.37^{a}	109.30	28.66±3.43^{a}	381.11
Leaves	5.97±1.72^{a}	71.83	12.78±3.00^{a}	1552.47
F-Value	4.83*		14.21*	

Values are mean of ± S.D. of four observations. Mean value of different superscripts within a column are significantly different from each other ($p \leq 0.05$)

compounds by Girad T assay, i.e. secondary stage markers was found to be inhibited upto 21–34 % and among the three elements in mango pulp extract it was maximally reduced. Fluorescence analysis of the later stage of glycation moieties-namely advanced glycationend products (AGEs) is also shown in Figure 2, suggested that all the elements of mango can inhibit these in the range of47 to 75 %. The values obtained in the present study, for AGE's inhibition with all the elements of mango extract are much lower than the reported values carried out by varied researchers [26].

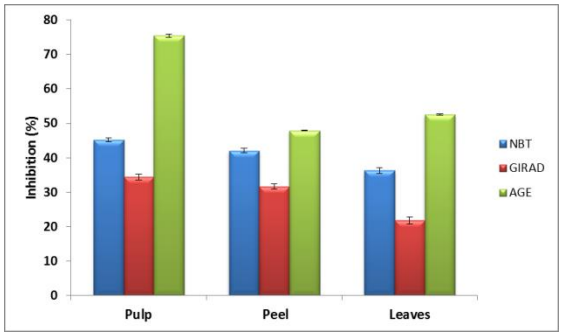

Figure 2: The effect of elements of mango methanolic extract on albumin glycation modification in terms of NBT assay, GIRAD T Assay and AGE's inhibition activity of pulp, peel and leaves of mango extract

Pearson's Correlation Coefficient (r) of various parameters was studied.The total phenolic content had a strong positive correlation with the flavonoid content (r =0.954, p≤0.01) and FRAP (r =0.955, p≤0.01). Total phenol content also had a negative and significant correlation with alpha glucosidase inhibition (r =-0.700, p≤0.05) and AGE's inhibition (r =-0.600, p≤0.05). The flavonoid content of all the elements of mango had a potent positive correlation withFRAP (r =0.993, p≤0.01) and a strong negative correlation with DPPH RSA (r =-0.713, p≤0.05). Flavonoid content also showed a negative and significant correlation with alpha glucosidase inhibition (r = - 0.622, p≤0.05).

Mangiferin and its derivatives i.e. phenolic acids, alkaloids, flavonoids, gallic acid, eallagic acid, rutin and other bioactive components are present in all the elements of mango. The most prominent one is the phenolic compoundmangiferin. Maniferin is being a potent phenolic compound maximally present in all the different elements of mango in varying amounts depending upon its different genotypes. It contains four (OH) groups at different positions and acts as a potential inhibitor for oxidants, glycation, alpha amylase and alpha glucosidase [32]. In the present study the total phenol and flavonoid content showed a negative relation with alpha amylase inhibition, alpha glucosidase inhibition and anti glycation activities. These results suggested that the high alpha amylase inhibition, alpha glucosidase inhibition and anti glycation activities of the different elements of mango could be due to non-phenolic bioactive compounds.

The obtained results are noteworthy not only because the phenolic and flavonoid of the extracts showed a positive relationship with the antioxidant activity, but not with the alpha amylaseinhibition assay, alpha glucosidase inhibition assay and antiglycation property.Many published studies have suggested that the phenolic and flavonoid compounds in plant extracts are responsible for the alpha amylase inhibition assay, alpha glucosidase inhibition assay and antiglycation property [26].

CONCLUSION

The results of the present study revealed that the pulp of mango had a better ability to ameliorate diabetes. It might also reduce secondary complications of diabetes as it also showed better antiglycation activity compared to the other two elements of mango. Hence, mango pulp could be used as a potential nutraceutical for diabetes.

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