

ORIGINAL ARTICLE

Formulation, Development and Quality Evaluation of a Fortified Biscuit with Antidiabetic Potential

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ABSTRACT

Background: Medicinal plants and herbs contain a plethora of phytochemicals that have demonstrated promising therapeutic potential in the management of hyperglycemia. Therefore, this study was undertaken to develop a fortified nutraceutical biscuit with antidiabetic potential.

Methods: Fortified biscuits were prepared by adding medicinal plants. Their chemical composition (moisture, protein, fat, ash, and carbohydrate), total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity and antidiabetic potential were evaluated. Data were compared using one-way analysis of variance (ANOVA) followed by Fisher's LSD test at a 5% level of significance.

Results: Substitution of medicinal plants with wheat flour increased the moisture content from 8.24 to 9.86% and protein content from 1.28 to 1.86%. A decreasing trend was observed for ash (1.45-1.21%), fat (11.81-11.45%) and carbohydrate (77.22-75.62%). The TPC of biscuits varied from 7.11 to 9.69 (mg GAE/100 g), where sample C showed a significantly higher ($p<0.05$) TPC than others. The TFC of biscuit samples ranged from 17.95 to 32.07 (mg QE/100 g). Gradual increasing of medicinal plants to plain biscuits manifested an enhancement of the overall antioxidant activity (8.12 to 9.14%). Furthermore, sample C showed the highest inhibitory activity against the dominant digestive enzymes such as α -amylase (66.9%) and α -glucosidase (51.4%), which were accountable for the prompt digestion and absorption of carbohydrates.

Conclusion: The findings of this study showed that fortification of biscuits with medicinal plants would improve the therapeutic properties of the biscuits with increased antioxidant and antidiabetic potency.

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Introduction

Diabetes mellitus is a non-communicable inherited metabolic disorder characterized by hyperglycemia and abnormal carbohydrate metabolism. It is the leading cause of morbidity and mortality, which

has affected almost 9% of the adult population worldwide (1). It is associated with other diseases like cardiovascular diseases, kidney diseases, etc. which is either caused by diminished insulin secretion by the β cell of pancreatic Langerhans

or by the lowering of insulin resistance owing to undue absorption of glucose (2). Synthetic oral hypoglycemic drugs and insulin is the mainstay of treatment of diabetes. Since these drugs have prominent side effects and are unable to reverse the course of their complications (3). Hence, the focus has been shifted towards more intensified research regarding complementary antidiabetic agents. Notwithstanding the availability of antidiabetic drugs in the modern age, the treatment of diabetes with medicinal plants has been extensively used in many parts of the world (4).

However, in comparison to conventional drugs, medicinal plants and herbs are rich sources of phytochemicals and possess no or fewer side effects, and are widely available and are relatively inexpensive (5, 6). Previously researchers have reviewed a number of medicinal plants that have been reported to possess antidiabetic activity. Concerning diabetes and medicinal plants, the anti-diabetic effects of ghrithkumari (*Aloe vera*) have already been established and have been ascertained to decrease blood sugar levels, which can be beneficial for type 2 diabetic patients (7). Therapeutic usage of moringa (*Moringa oleifera*) leaves has also been assessed in diabetes owing to their potential to reduce blood glucose concentrations after ingestion as they contain certain polyphenols such as quercetin-3-glycoside, rutin, kaempferol, and glycosides (8).

Moreover, insulin plant (*Costus igneus*), bitter gourd (*Momordica charantia*), carrot (*Daucus carota*), menthe (*Mentha spicata*), thankuni (*Hydrocotyle asiatica*), and basil (*Ocimum basilicum*) contain a variety of bioactive substances with anti-diabetic properties and has been exploited in the treatment and management of diabetes (9-12). These substances either work individually or together to help reducing blood sugar levels (13). Although numerous studies have investigated the role of medicinal plants containing bioactive compounds in the management of diabetes, there are very few studies on the development of food products by incorporating those medicinal plants in the mixture form (14).

Furthermore, food industries are currently confronted with the challenge of producing foods high in bioactive ingredients in order to meet the current demand for nutraceutical and functional foods. Considering the nutritional and functional requirements, protein, and fiber enriched flour together with medicinal plants might be rated as highly benefactions (14). Therefore, the present study was planned to develop a plant-based fortified biscuit by incorporating medicinal plants and evaluating its chemical composition, antioxidant capacity as well

as *in vitro* antidiabetic properties.

Materials and Methods

Raw ingredients required for biscuit formulation such as wheat flour, baking powder, milk, etc. were purchased from the local supermarkets. Indigenous medicinal plants and herbs; moringa (*Moringa oleifera*) leaves, ghrithkumari (*Aloe vera*), basil (*Ocimum basilicum*), carrot (*Daucus carota*), bitter gourd (*Momordica charantia*), menthe (*Mentha spicata*), thankuni (*hydrocotyle asiatica*) leaves and insulin (*Costus igneus*) plants were also collected from different locations of Bangladesh. Raw plant samples were spread on trays and dried in cabinet dryer at 60°C for 12 hours. After that, dried plants were ground separately in a mechanical grinder to form powder. An equal amount of powder from each of the plant materials were stored in food grade airtight bags at 4°C in a refrigerator. The fortified biscuits were prepared according to the method described by Han *et al.* (2010) with slight modification (15). Biscuits were obtained from the blends of wheat flour, baking ingredients and different proportions of plant powder as described in Table 1.

However, the biscuits (Figure 1) were prepared using standardized recipes described by Chauhan *et al.* (2016) with the fortification of plant powder in different proportions (16). Butter and milk were creamed in a mixer. Then flour, plant powder, baking powder and water were mixed thoroughly and subsequently added to the cream and mixed for about 5 min to obtain a hard-extensive dough texture. The dough was then allowed to ferment for 3 h. After that, it was kneaded to a thickness of 1 mm and molded using a rectangular shaped biscuit cutter. The baking was carried out at 150-200°C for 15 min in a baking oven. The biscuits were cooled to room temperature before being packed in heat-sealed high density polyethylene (HDPE) bags and kept in the cold room for further analysis.

The chemical compositions of the fortified biscuits were determined by the methods described in the Association of Official Analytical Chemists (AOAC, 2016) (17). Samples were ground prior to analysis. All the analyses were performed in triplicates after two days of biscuit production. The moisture was measured by oven drying at 105°C up to getting a constant weight (17). 5 g of each biscuit sample was taken to a previously weighted dried empty crucible. Then, the crucible was placed in a hot air oven and dried at a temperature of 105°C for 3 h. After drying, the crucible was transferred to the desiccator for cooling down and its dried sample was reweighed. The processes were continued until

Table 1: Formulation of fortified biscuits.

Ingredients	Control	Sample A (1% substitution)	Sample B (2% substitution)	Sample C (3% substitution)
Flour	200 g	198 g	196 g	194 g
Carrot	-	0.25 g	0.50 g	0.75 g
Bitter gourd	-	0.25 g	0.50 g	0.75 g
Menthe	-	0.25 g	0.50 g	0.75 g
Thankuni leaves	-	0.25 g	0.50 g	0.75 g
Insulin plant	-	0.25 g	0.50 g	0.75 g
Moringa Leaves	-	0.25 g	0.50 g	0.75 g
Ghritkumari	-	0.25 g	0.50 g	0.75 g
Basil	-	0.25 g	0.50 g	0.75 g
Milk	10 mL	10 mL	10 mL	10 mL
Butter	6.25 g	6.25 g	6.25 g	6.25 g
Egg	2 no.	2 no.	2 no.	2 no.
Salt	1.5 g	1.5 g	1.5 g	1.5 g
Baking Powder	2 g	2 g	2 g	2 g
Water	40 mL	40 mL	40 mL	40 mL

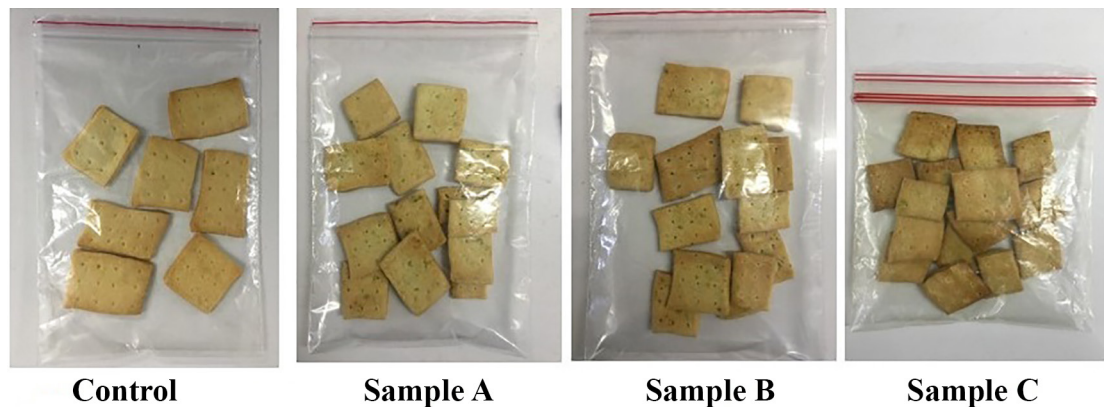


Figure 1: Developed biscuits from wheat flour and mixed medicinal plant powder. *Control=Biscuits made with wheat flour (100%), A=Biscuits made with wheat flour + mixed plant powder (1%), B=Biscuits made with wheat flour + mixed plant powder (2%), C=Biscuits made with wheat flour + mixed plant powder (3%).

two consecutive weighing got the same value. The percentage of moisture present in biscuit samples was calculated by the difference between fresh and dry weights.

The crude protein content was measured by the Kjeldahl procedure ($6.25 \times N$). Two grams of each biscuit sample, 3 g of digestion mixture and 25 mL of H_2SO_4 were added and homogeneously mixed in a dried Kjeldahl digestion flask. It was then heated for 4 h in the Kjeldahl digestion and distillation unit. Appearance of pale yellow color denoted the end of digestion. Then digested samples along with 10 mL of 2% boric acid and 2-3 drops of mixed indicator were transferred to the Kjeldahl flask for distillation. Then 100 mL of water, 100 mL of 40% NaOH and glass blitz were added to the mixture. Distillation continued till the collection of about 100 mL of distillate in the receiving flask. Collected distillate (ammonia) was then titrated with 0.1 N HCl solutions and the titer value was recorded. Percentages of protein in the biscuit sample were

calculated thereafter using the conversion factor of 6.25 to convert total nitrogen to crude protein.

Total fat/lipid content of the biscuit sample was determined by using the Soxhlet apparatus (17). Two grams of each dried biscuit sample recovered after moisture determination was placed in a thimble and plugged the top of the thimble with fat-free cotton. The thimble was then transferred to the Soxhlet flask with a condenser attached at the top. A total of 75 mL of diethyl ether was added to the flask. The sample was extracted for 6 h. After completion of the extraction period, the thimble was removed from the apparatus. Evaporation of diethyl ether was carried out on a rotary evaporator at low heat. The extracted materials left after the solvent had evaporated were weighed, and the fat content was calculated.

Ash content of biscuit samples was measured gravimetrically in a furnace by heating at $550^\circ C$ up to getting constant weight (17). Five grams of each biscuit sample was transferred to a muffle furnace and heated at $550^\circ C$ for 4 h after charging over an

electric heater. The percentage of ash was calculated by subtracting the weight of ash from the initial weight. Total carbohydrates were calculated by the difference method described by Asimi *et al.* (2018) with slight modifications as [Carbohydrate (%) = 100 - (% moisture + % ash + % crude protein + % fat)] (18).

Two grams of each biscuit sample was refluxed with 20 mL of methanol containing 1% HCl for 2 h at 60±2°C (19). The mixtures were then centrifuged at 5000 rpm for 20 min and the supernatants were filtered and used for the analysis of total phenolic and total flavonoid contents as well as antioxidant activity. The total phenol content (TPC) was determined according to the method described by Singleton *et al.* (1999) with slight modifications (20). Briefly, 0.1 mL of each acidified methanolic extract and 5 mL of distilled water were taken in a 50 mL volumetric flask. Then 2.5 mL of 10% Folin-Ciocalteu's reagent (v/v) and 7.5 mL of 15% sodium carbonate solution were also added and mixed thoroughly. Total volume was made up to 50 mL and left for 30 min to allow the reaction. Finally, the absorbance of the samples was measured at 765 nm in the UV Visible spectrophotometer (UV-2600, Shimadzu Corporation, USA). The absorbances of the samples were compared against the standard curve of gallic acid. TPC was calculated and expressed as mg of gallic acid equivalent (GAE) per 100 gram of the sample.

The total flavonoid content (TFC) of the sample extracts were determined using the method reported by Meda *et al.* (2005) with slight modifications (21). Briefly, 0.5 mL of appropriately diluted each sample was mixed with 0.5 mL of methanol, 50 µL of 10% Aluminium chloride (AlCl₃), 50 µL of 1M potassium acetate, and 1.4 mL of water, and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was subsequently measured at 415 nm by using a UV Visible spectrophotometer (UV-2600, Shimadzu Corporation, USA). TFC was calculated using quercetin as standard and expressed as mg of quercetin equivalent (QE) per 100 gram of the sample.

The method described by De Ancos *et al.* (2002) was used to determine the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of developed biscuits (22). In brief, 10 µL aliquot of the acidified methanolic extract was mixed with 90 µL distilled water and 3.9 mL of methanolic 0.1 M DPPH solution. The mixture was thoroughly mixed in vortex equipment and kept in dark places for 30 min. The absorbance was then measured at 515 nm using a UV Visible spectrophotometer (UV-2600, Shimadzu Corporation, USA). The result was expressed as percentage (%) inhibition of the DPPH radical.

The α-amylase inhibitory activity was determined according to an assay from the Worthington Enzyme Manual with slight modifications (23). Briefly, 100 µL of sample extract, 100 µL of 0.02 M sodium phosphate buffer (pH: 6.9) containing α-amylase solution (1 mg/mL), and 0.006 M NaCl were thoroughly mixed and incubated at 25°C for 10 min. Subsequently, 100 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH: 6.9, containing 0.006 M NaCl) was added to the reaction mixture, which was incubated at 25°C for 10 min. The reaction was terminated by adding 200 µL of dinitrosalicylic acid reagent, followed by incubation in a boiling water bath for 5 min. After cooling to room temperature, 3 mL of distilled water was added. The absorbance of the sample for control 1 and control 2 was measured at 540 nm. Here, control 1 denoted a mixture of starch solution and sample without the addition of enzyme, and control 2 denoted a mixture of starch solution and enzyme without the addition of sample. Acarbose, a potent antidiabetic drug was used as the positive control in this inhibition assay. The α-amylase inhibitory activity was expressed as percent inhibition and calculated as the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Abs (control2)} - [\text{Abs (sample)} - \text{Abs (control1)}]}{\text{Abs (control2)}} \times 100$$

× 100

A modified version of the assay described by Worthington Enzyme Manual and Mccue *et al.* (2005) was followed to determine the α-glucosidase inhibitory activity of the developed biscuits (23, 24). Rat intestinal acetone powder (300 mg), used as crude enzyme extract was suspended in 9 mL of 0.9% NaCl solution and centrifuged at 10,000 rpm for 30 min (4°C). The supernatant was then filtered and collected. In brief, 50 µL of each sample extract and 100 µL of 0.1 M phosphate buffer (pH: 6.9) containing crude-glucosidase solution (25 mg/mL) was incubated in 96-well clear plates at 25°C for 10 min. Following pre-incubation, 100 µL of 5 mM p-nitrophenyl--D-glucopyranoside solution in 0.1 M phosphate buffer (pH: 6.9) was added to each well at consequent intervals. After that, the reaction mixtures were incubated at 37°C for 30 min. Before and after incubation, absorbance readings were recorded at 405 nm and compared to control having 50 µL of buffer solution in place of the extract. Acarbose, a potent antidiabetic drug was used as the positive control in this inhibition assay. The α-glucosidase inhibitory activity was expressed as percentage inhibition and was calculated as follows:

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

Data were subjected to one-way analysis of variance (ANOVA) using R Statistical Software (Version 3.4.1; R Foundation for Statistical Computing, Vienna, Austria) followed by Fisher's LSD test to determine statistical differences among them to test the level of significance ($p < 0.05$). Results were presented as mean value \pm standard deviation (SD).

Results

The results of the chemical composition of the fortified biscuits were displayed in Table 2. The findings demonstrated that the addition of medicinal plant powder increased the moisture and protein content of the fortified biscuits. On the contrary, the content of fat, ash and carbohydrate decreased as the substitution progresses. Results regarding physicochemical properties, all the samples have shown significant differences ($p < 0.05$) among all the parameters evaluated in this study.

The total phenolic content (TPC) of biscuit

samples ranged from 7.11 to 9.69 mg GAE/100 g with significant differences ($p < 0.05$) between the control, Sample A, B and C (Table 3). The highest phenolic content was reported in Sample C (9.69 mg GAE/100 g) and the least was reported in the control (7.11 mg GAE/100 g) biscuit. Table 3 also illustrated that the addition of medicinal plant materials contributed to the increased flavonoid content (TFC) in the biscuits compared to control ($p < 0.05$). Sample C had the highest total flavonoid content (32.07 mg QE/100 g) followed by sample B (28.18 mg QE/100 g), sample A (22.36 mg QE/100 g) and control (17.95 mg QE/100 g). Inhibition of 2, 2-diphenyl-2-picrylhydrazyl (DPPH) of biscuit samples varied from 8.12 to 9.14% and significant differences ($p < 0.05$) were observed among the control and medicinal plant based biscuit samples (Table 3). The lowest DPPH was observed in control (8.12%) and the highest DPPH was obtained in sample C (9.14%). The results also revealed a positive relationship among the TPC, TFC, and

Table 2: Chemical properties of fortified biscuits.

Variables	Control	Sample A	Sample B	Sample C
Moisture (%)	8.24 \pm 0.15 ^d	8.74 \pm 0.22 ^c	9.08 \pm 0.19 ^b	9.86 \pm 0.11 ^a
Protein (%)	1.28 \pm 0.03 ^d	1.42 \pm 0.02 ^c	1.68 \pm 0.01 ^b	1.86 \pm 0.02 ^a
Fat (%)	11.81 \pm 0.02 ^a	11.65 \pm 0.02 ^b	11.56 \pm 0.03 ^c	11.45 \pm 0.03 ^d
Carbohydrate (%)	77.22 \pm 1.78 ^a	76.86 \pm 0.98 ^c	76.45 \pm 1.33 ^b	75.62 \pm 1.48 ^d
Ash (%)	1.45 \pm 0.02 ^a	1.33 \pm 0.06 ^b	1.23 \pm 0.01 ^c	1.21 \pm 0.02 ^d

*Data in the same row with different letters are significantly different ($p < 0.05$). Control: Biscuits made with wheat flour (100%), A: Biscuits made with wheat flour+mixed plant powder (1%), B: Biscuits made with wheat flour+mixed plant powder (2%), C: Biscuits made with wheat flour+mixed plant powder (3%).

Table 3: Total phenolic and flavonoid contents and antioxidant capacity of fortified biscuits.

Variables	Control	Sample A	Sample B	Sample C
TPC (mg GAE/100gm)	7.11 \pm 0.05 ^d	7.92 \pm 0.02 ^c	8.81 \pm 0.07 ^b	9.69 \pm 0.05 ^a
TFC (mg QE/100gm)	17.95 \pm 0.04 ^d	22.36 \pm 0.12 ^c	28.18 \pm 0.02 ^b	32.07 \pm 0.12 ^a
DPPH (%)	8.12 \pm 0.08 ^d	8.86 \pm 0.04 ^c	9.08 \pm 0.01 ^b	9.14 \pm 0.02 ^a

*Data in the same row with different letters were significantly different ($p < 0.05$). Control: Biscuits made with wheat flour (100%), A: Biscuits made with wheat flour+mixed plant powder (1%), B: Biscuits made with wheat flour+mixed plant powder (2%), C: Biscuits made with wheat flour+mixed plant powder (3%).

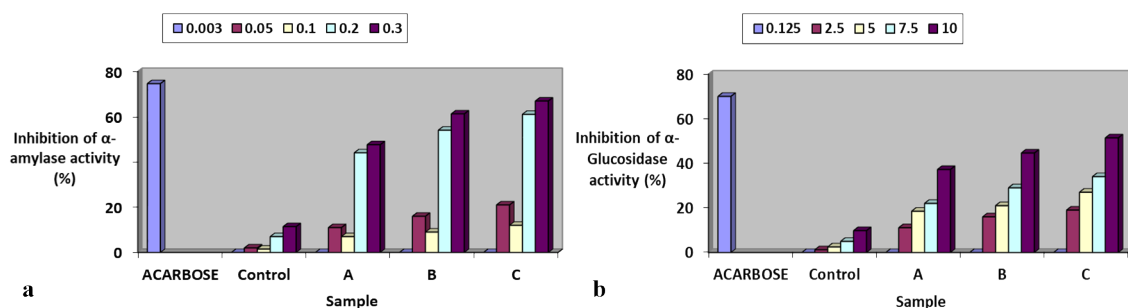


Figure 2: a. Percentage α -amylase inhibition of formulated biscuits at different concentrations (0.003, 0.05, 0.1, 0.2 and 0.3 mg/mL). Acarbose: Positive control; Control: Biscuits made with wheat flour (100%); A: Biscuits made with wheat flour+mixed plant powder (1%); B: Biscuits made with wheat flour+mixed plant powder (2%); C: Biscuits made with wheat flour+mixed plant powder (3%). b. Percentage α -glucosidase inhibition of formulated biscuits at different concentrations (0.125, 2.5, 5.0, 7.5 and 10.0 mg/mL). Acarbose: Positive control; Control: Biscuits made with wheat flour (100%); A: Biscuits made with wheat flour+mixed plant powder (1%); B: Biscuits made with wheat flour+mixed plant powder (2%); C: Biscuits made with wheat flour+mixed plant powder (3%).

DPPH radical scavenging activity of the control and medicinal plant based biscuit samples.

Figure 2a illustrates the percentage inhibition of α -amylase of control, mixed medicinal plant substituted biscuits (sample A, B and C) and positive control (Acarbose). Control had the least α -amylase inhibitory activity (11.4%), while sample C exhibited the highest α -amylase inhibitory activity (66.9%) followed by sample B (61.2%) and sample A (47.6%). The percentage of α -glucosidase inhibition of control and developed medicinal plant based biscuits was presented in Figure 2b. The findings illustrated that percentage inhibition was concentration-dependent as inhibition increased with the increased concentration. However, a higher concentration (10 mg/mL) was required to achieve the significant inhibitory activity. Like α -amylase inhibition, control showed the least α -glucosidase inhibitory activity (9.81%), whereas sample A (37.22%), sample B (44.6%), and sample C (51.4%) showed significant α -glucosidase inhibition.

Discussion

Moisture plays a crucial role in terms of quality, acceptability, and shelf-life of baked products (25, 26). The highest moisture content was reported in sample C (9.86%) followed by sample B (9.08 %), sample A (%) and control (8.24%). Incorporation of mixed plants powder into biscuits preparation has increased the moisture content ($p < 0.05$). However, similar result was also reported by Gadallah and Ashoush, (2016) on the increased moisture content of biscuits by the addition of Desert Truffle Powder (DTP) (27).

The protein contents of the biscuit samples ranged from 1.28 to 1.86%. Sample C with 3% mixed plant powder had the highest protein content (1.86%), while the control sample had the lowest protein content (1.28%). The result depicted that protein content increased as the substitution of mixed plant powder increased (0 to 3%) among all the biscuit samples ($p < 0.05$). However, this progression is attributed to the significant quantity of protein in medicinal plants (28). A similar trend of increased protein (10.98 to 12.43%) in wheat-pumpkin powder based cookies was reported by Anitha *et al.* (2020) (29). Alam *et al.* (2014) also reported an increase in the protein content with a subsequent increase in the proportion of plant material supplementation in biscuits (30).

Fat is an important factor that serves as the lubricating agent to improve the texture, rheology, and overall quality of the food product. Table 2 depicts that the fat content of the biscuit samples ranged from 11.45 to 11.81%. The control sample had the highest fat content (11.81%), while sample C of

3% mixed plant powder had the least value (11.45%), even though an equal quantity of oil was used for biscuit preparation. However, the fat contents of the biscuit samples were within the standard limit ($\leq 25\%$) for food products as reported by Ikuomola *et al.* (2017); which may alleviate oxidative rancidity and unpleasant odors (31).

Ash content of the biscuits ranged from 1.21 to 1.45% (Table 2). The ash content of the biscuit samples decreased at each substitution level up to 3%. The lowered ash contents in biscuits could be due to the prolonged milling and soaking of plant materials (31). However, slight decrease (1.42 to 1.39%) in ash content was also noted by Mhiko (2012) during prolonged storage (32). The Carbohydrate content of biscuits ranged between 75.62 to 77.22% (Table 2). Biscuit sample C had the lowest carbohydrate content (75.62%), while the reference control sample had the highest content (77.22%). The increase in the level of medicinal plant materials brought a decrease in the carbohydrate content of the biscuits. Similarly, Alam *et al.* (2014) have found decreased carbohydrate contents (56.6 to 54.6%) of biscuit samples made with plants portions (30). However, low carbohydrate foods are linked to health protective effects. Thus, the developed biscuit might be useful to the consumers to tackle the challenges of consuming low carbohydrate foods.

The increasing trend of TPC and TFC in this study is consistent with the previous literature as medicinal plants are known to be the rich sources of polyphenols and these are being extensively used to improve the functional properties of certain food products (33). Similar findings were also reported by Elhassaneen *et al.* (2016), where incorporation (5%) of prickly pear peel and potato peel powders improved the total phenolic content (TPC) of the biscuits compared to control (110.23 to 192.79 mg/100 g of sample) (34). While for TFC, a similar result was also reported by Pasqualone *et al.* (2015) where incorporation of plant by-products along with semolina flour increased the flavonoid in biscuits (35). However, decreased TFC in control biscuit may be attributed to the occurrence of Maillard reaction during the baking process (36). Further, crucial processing step baking might influence the increasing trend of the antioxidant activity of biscuit (37). However, the result is inconsistent with the findings of Ajibola *et al.* (2015), where antioxidant properties of biscuits increased with the increasing quantity of *Moringa oleifera* leaves and cocoa powder (38). Jan *et al.* (2015) also reported that buckwheat flour with wheat flour improved the inhibition of DPPH by 55.53 to 61.65% (39).

Results from *in vitro* α -Amylase and

α -glucosidase inhibitory activities of fortified biscuits depicted that percentage of inhibition was concentration-dependent and it showed a progressive increase with the increased concentration. However, obtained results were somewhat lower than that of positive control, acarbose (74.56% at 0.003 mg/mL). But the present study showed a promising α -amylase inhibition by the medicinal plant substituted biscuits. Some other previous study also ascertained that plant phytochemicals and underutilized medicinal plant inhibited the salivary and pancreatic α -amylase activities (40). The higher percentage of α -amylase inhibition might help to slow down the absorption of carbohydrates after the food intake. The supplementation of the biscuits with mixed medicinal plant powder (proteinaceous α -amylase inhibitors) might be responsible for the inhibition of digestive enzymes, which may pose some antidiabetic properties.

Similar to α -amylase inhibition, control showed the least α -glucosidase inhibitory activity (9.81%), whereas sample A (37.22%), sample B (44.6%), and sample C (51.4%) showed significant α -glucosidase inhibition. Ademiluyi and Obboh (2012) reported a higher α -glucosidase inhibition activity by underutilized plants, which may help to lower postprandial hyperglycemia by partially inhibiting the enzymatic hydrolysis of complex carbohydrates that delays the rapid absorption of glucose (40). The supplementation of the biscuits with medicinal plant powder flour might be responsible for inhibiting the digestive enzymes like α -glucosidase that will slow down the breakdown of disaccharide to monosaccharide, thus it may reduce the amount of glucose absorbed into the bloodstream. As both the enzymes had a synergistic effect on blood glucose and the developed medicinal plant based biscuits possess higher inhibitory activity against these two key digestive enzymes, it might be a potential complementary or supplementary product along with the available medicine for the management of diabetes.

Conclusion

According to the present study, biscuits fortified with mixed medicinal plant materials along with the wheat flour showed increased protein, total phenolic and flavonoids content. This clearly suggests that biscuits prepared with increased levels of medicinal plant material improved the overall antioxidant activities and subsequently their therapeutic potential. However, the addition of mixed plant powder also exhibited promising antidiabetic potential via gradual inhibition of prominent digestive enzymes such as α -amylase and

α -glucosidase which are liable for the breakdown and absorption of carbohydrates. It can be concluded that traditionally known medicinal plants have been successfully utilized to improve the antioxidant and antidiabetic potentials of the fortified nutraceutical biscuits which would benefit overall human health. Further research should focus more on combining advanced food technology methods with cutting-edge analytical techniques to discover more novel functional foods with significant health benefits.

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Conflict of Interest

None declared.

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