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Development of Okra-Based Antidiabetic Nutraceutical Formulation from *Abelmoschus esculentus* (L.) Moench (Ex-maradi Variety)

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ABSTRACT

Diabetes mellitus is a complex metabolic disorder that is considered a worldwide epidemic and is associated with the development of many complications. Okra fruit is rich in fibre and other medicinal phytochemicals hence could be a potential source for the development of antidiabetic nutraceutical. Subsequent to our earlier reports on the antidiabetic effects of various parts of ex-maradi okra fruit, the current study was designed to develop appropriate formulation from the fruit seeds and peels. Various proportions of the powdered seeds and peels samples were thoroughly mixed in a ratio of 90:10; 80:20; 70:30; 60:40, 50:50%, respectively and vice-versa. The proximate composition and some functional properties viz: Antioxidant Capacity (AC), Water Adsorption Capacity (WAC), Oil Adsorption Capacity (OAC), Glucose Adsorption Capacity (GAC), Glucose Dialysis (GD), and Glucose Dialysis Retardation Index (GDRI) of each formulation were evaluated *in-vitro*. The results revealed that 10:90% (Seeds:Peels) formulation exhibited the highest significant ($P < 0.05$) percentage of Crude ash, fibre, and protein content; it also showed highest significant ($P < 0.05$) values for AC, WAC, OAC, GAC and GDRI as well as the lowest value for GD. Conclusively, the study showed that the 10:90% (Seeds:Peels) formulation has a reduction effect the rate of glucose adsorption and diffusion and thus can retard increase in postprandial blood glucose level than the other combinations. Therefore, this combination was found to be the most suitable for the development of okra-based antidiabetic nutraceutical formulation for the management of diabetes mellitus.

Keywords: Diabetes Mellitus, *Abelmoschus esculentus*, Functional properties, Antioxidants, Nutraceutical.

Introduction

Diabetes mellitus (DM) is a widespread endocrine and metabolic disorder that is characterized by persistent hyperglycemia and associated with considerable morbidity and mortality in all population throughout the world.[1,2] The estimated worldwide prevalence of diabetes among adults in 2010 was 285 million adults (6.4 %) and this value is predicted to rise to around 439 million adults (7.7 %) by 2030 where about 90 to 95% of them are of type 2 DM.[1,3] Also, the large increases are predicted to occur mostly in developing countries and in adults especially between 45 and 64 years of age due to changes in lifestyles especially diet and physical inactivity.[4] These predictions indicate a growing burden of diabetes, particularly in developing countries. In 2011, the prevalence of diabetes mellitus was about 2.7% in Nigeria.[5]

The management of diabetes mellitus by insulin therapy and oral hypoglycemic drugs has several drawbacks as they are associated with various side effects.[6] Resistance to such medications has also been reported after a prolonged period of treatment. Not only that, people affected by DM faces regular economic burden because the ailment demands regular therapy.[3] Recently, high-fiber diet is being used as asupplement in controlling DM. Water-soluble dietary fibers have the potentials to reduce glucose absorption, increase the hepatic extraction of insulin and increase insulin sensitivity at the cellular level.[7] Okra due to its high fibre content is fast gaining a reputation as 'superfood' for individuals with or at risk of DM, ulcer and other ailments.[8]

The term "nutraceutical" was coined from "nutrition" and pharmaceutical in 1989 by Stephen Defelice; founder and Chairman of the Foundation for Innovation in Medicine (FIM).[9] Defelice define nutraceutical as "a food or part of food that provide medicinal or health benefit including the prevention and/or treatment of diseases." [9]

Abelmoschus esculentus, (L.) Moench (Ex- maradi variety) is a flowering plant of the Malvaceae family. It is one of the most important and utilized species of vegetables widely known and cultivated throughout the world for its tender fruits.[10] This plant is an economically important vegetable crop that has been used for a long time as a daily food in many countries because of its nourishing components.[10] It is characterized by mucilaginous properties and high fiber content.[11] The fruit of the plant is rich in nutrients like protein, Niacin, Riboflavin, Phosphorus, Zinc, Copper, Potassium, vitamins A, B6, C and K, Thiamine, Folate, Magnesium, Calcium, and Manganese. Okra is rich in phenolic compounds with important biological properties like quercetin and flavonol derivatives, catechin oligomers and hydroxycinnamic derivatives.[12] It is a multipurpose crop due to the various uses of its parts (leaves, buds, flowers, pods, stems and seeds). The immature okra fruits are consumed as vegetables and can be used in salads, soups and stews,

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fresh or dried, fried or boiled as it maintains its mucilaginous consistency after cooking.[13]

The nutritional quality and potential health benefits of okra fruit are not to be overemphasized. It is an important vegetable crop with a diverse array of nutritional quality and potential health benefits.[13] Okra has been called "a perfect villager's vegetable" because of its robust nature, dietary fiber content and distinct seed protein balance of both lysine and tryptophan amino acids comparable to that of soybean.[14] The seeds are also potential source of oils including linoleic acid (a polyunsaturated fatty acid essential for human nutrition) which is up to 47.4% of okra seed oil.[15] Okra fruit has also been referred to as a powerhouse of valuable nutrients with abundant array of carbohydrates, minerals, and vitamins which play vital roles in human diet and health.[15] Also, the fruit is rich in polyphenolic compounds with important biological properties like quercetin and flavonol derivatives, catechin oligomers and hydroxycinnamic derivatives which explain its high antioxidants activity and potentially beneficial effects on some of the important human diseases like cardiovascular diseases, type 2 diabetes, digestive diseases and some cancers.[13]

Ex-maradi Okra fruit is a local type of Okra fruit commonly found in Northern part of Nigeria. This type of okra fruit is locally called "Kubewa Y'ar Marad'i by the Hausa people of Nigeria because it is mostly cultivated and marketed by the Marad'i and Damagaran vegetable growers in Niger Republic. Usually, the fruits of such okra are harvested in their young/immature tender state in large quantity then processed into the dry form for sale in various markets.

Our earlier reports on the antidiabetic effects of various parts (seed, peel and whole fruit) of Ex-maradi okra fruit on Alloxan-induced diabetic Rats showed that both the okra peel and seed have significant antidiabetic effects. However, the okra peel due to its fibrous nature and characteristic viscous principle showed more effect in retarding glucose diffusion and absorption in the intestine thereby postponing postprandial hyperglycemia. The okra seeds showed more significant antioxidant effects whereas the whole okra fruit showed synergistic effects.[16]

Affordable phytomedicines/nutraceuticals such as okra fruit are needed in ameliorating diabetes mellitus because they are more advantageous than allopathic drugs which are not only more expensive but are also associated with many unwanted side effects. The possible use of this economical and relatively non-toxic, non–hazardous natural remedy of plant origin could be easily explored for the management of DM as alternative to synthetic agents. Hence, the current study was designed to develop appropriate formulations of a nutraceutical from the fruit seeds and peels of Ex-maradi Okra fruit. The developed formulations were subjected to some analytical evaluations to select the formula with the best *in-vitro* effectiveness in lowering blood glucose level, improve the oxidative status and also protect against other complications of diabetes mellitus.

Materials and Methods

Chemicals and Reagents

Analytical grade laboratory chemicals and reagents were used for this study.

Okra Sample Collection

Ex-maradi (a commercially available dry-Okra fruits from the vegetable growers/sellers at Maradi, Niger) were obtained from Maggi market at Sokoto State, Nigeria. The sample was identified and authenticated by Mal. A Umar a taxonomist at the Botany unit of the Department of Biological Sciences at Usmanu Danfidiyo University, Sokoto. A voucher specimen number (UDUH/ANS/0066) was assigned to the sample while the specimen sample was deposited in the Herbarium of the same Department.

Okra Sample Preparation

The procured Okra sample was thoroughly sorted to remove debris and 5000 g of the Okra fruit sample was weighed and broken to separate the seeds from the pods. The two portions of the samples (Okra peels and the seeds) were separately grounded to a fine powder. The powdered samples were sieved with a fine mesh and placed in labeled sealed glass containers and stored at normal laboratory conditions until required for further evaluation (Figure 1a-f).

Development of the Okra-based Antidiabetic Nutraceutical Formulation

Powdered seeds and peels samples were accurately weighed and thoroughly mixed to obtain the appropriate percentage ratios (w/w %) then transferred to the respective labeled air-tight containers as follows:

Sample A -	Seed (S)	=	100%
Sample B -	Peel (P)	=	100%
Sample C -	S:P	=	10:90
Sample D -	S:P	=	20:80
Sample E -	S:P	=	30:70
Sample F -	S:P	=	40:60
Sample G -	S:P	=	50:50
Sample H -	S:P	=	90:10
Sample I -	S:P	=	80:20
Sample J -	S:P	=	70:30
Sample K -	S:P	=	60:40

Proximate Analysis

The Moisture Content, Ash content, Crude protein, Crude lipids, and Carbohydrate content of the different proportions were estimated by using the standard procedures of Association of Official Analytical Chemists.[17]

Estimation of Some Functional Properties of the Various Formulations

The Antioxidant Capacity (AC), Water Adsorption Capacity (WAC), Oil Adsorption Capacity (OAC), Glucose Adsorption Capacity (GAC), Glucose Dialysis (GD), and Glucose Dialysis Retardation Index (GDRI) of each formulation were evaluated *in-vitro* using the following methods:

The Antioxidant Capacity (AC)

DPPH Free Radical Scavenging Activity and Ferric Reducing Antioxidant Power Assay (FRAP) were used to estimate the antioxidant potentials of the different formulations as follows:

Evaluation of 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) Free Radical Scavenging Activity: The free radical scavenging activity of the various proportions were estimated by the modified method of McCune and Johns.[18] Briefly, 1 mL of 0.3 mM solution of DPPH in methanol was added to 3 mL of 1.0 mg/mL of the various sample formulations dissolved in methanol. After 30 min of incubation in the dark, the absorbance was measured at 517 nm against a blank. Ascorbic acid was used as the standard control. The Percentage of inhibition was calculated using the formula:

$$\text{Scavenging Activity (\%)} = (A0 - A1 / A0) \times 100$$

where: A0 is the absorbance of control and A1 is the absorbance of sample.

Evaluation of Ferric Reducing Antioxidant Power (FRAP): The ferric reducing antioxidant power assessment of the different proportions was estimated by using the modified method of Athukorala.[19] Briefly, 2 mL of 1.0 mg/mL solution of the various sample formulations were mixed with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of 30 mM potassium ferricyanide ($K_3Fe(CN)_6$. The mixture was incubated at 50°C for 20 min. Thereafter, 2.5 mL of 600 mM Trichloroacetic acid (TCA) was added to the reacting mixture, then centrifuged at 3000 rpm for 10 min. To 2.5 mL of the supernatant, 2.5 mL of distilled water and 0.5 mL of 6 mM ferric chloride ($FeCl_3$) were added. The absorbance was read at 700 nm against a blank using spectrophotometer (SP 300 Optima, Germany). Ascorbic acid was used as positive control.[20] The reducing capacity was calculated as follows:

$$\text{Reducing Power (\%)} = (AC - AM / AC) \times 100$$

where: AC is the absorbance of control mixture, and AM is the absorbance of reacting mixture.

The respective values of the DPPH scavenging activity and that of the ferric reducing antioxidant power (FRAP) were calculated and presented as the antioxidant capacity of the respective formulations.

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Estimation of Water Adsorption Capacity (WAC) and Oil Adsorption Capacity (OAC)

The percentage water or oil adsorption capacities of the various proportions were estimated by the centrifugal method as described by Chau.[21] Briefly, 1 g of the different proportions were weighed and transferred into their respective test tubes containing 20 mL distilled water or oil and stirred thoroughly, followed by centrifugation at 3500 rpm for 15 min. At the end of the adsorption, the respective supernatant was measured and the respective amount of water or oil adsorbed (%) by each proportion was estimated by the following calculations:

Amount of water or oil adsorbed (%) = (IA – FA / IA) × 100

where: IA is the Initial amount of water or oil before adsorption, and FA is the final amount of water or oil after adsorption.

Estimation of Glucose Adsorption Capacity (GAC)

The glucose adsorption capacity in (mmol/g) of the different proportions were estimated according to the method described by Ou,[22] and Chau[23] with slight modification. The principle was based on the ability of the fibre to effectively bind to glucose thereby reducing the amount of available glucose in the system.

Briefly, 1.0 g of the different proportions (samples) was separately mixed with 20 mL of prepared glucose solution (100 mg/mL) in respective test tubes labeled A-K (Figure 1g). The mixtures were stirred, held in a water bath at 37°C for 90 min, followed by centrifugation at 3500 rpm for 15 min. At the end of the adsorption, the glucose concentration in each supernatant was measured using the glucose oxidase/peroxidase method,[24] while the amount of glucose adsorbed (%) on the samples were calculated as follows:

Amount of glucose adsorbed (%) = (IG – FG / IG) × 100

where: IG is the Initial glucose concentration before adsorption, and FG is the final glucose concentration after adsorption.

Estimation of Glucose Diffusion Capacity (GDC)

The *in-vitro* glucose diffusion of the different proportions of the formulations was studied using the *in-vitro* dialysis model as reported by Davis.[25]

The principle is based on the fact that soluble dietary fibres increase the viscosity of the contents in the test tube resulting in the entrapment of glucose molecules and thereby retarding the rate of diffusion of the sugar molecules through the membrane of the inverted test tube into the beaker containing distilled water (Figure 1h-i). Movement in this system is not by true diffusion but is assisted by the convective activity by the stirring of the *in-vitro* models.[26] Briefly, to prepare the *in-vitro* Dialysis Model, 1 g each of the respective formulations was weighed and transferred into respective test tubes. 20 mL of 100 mg/mL glucose solution was added, and the solutions were steered thoroughly. Muslin cloth (ultra-fine membrane filter) was used to cover the test tubes and fastened well. Each test tube was inverted and soaked in 50 mL distilled water contained in glass beakers (Figure 1h-i). A single test tube containing same glucose solution but without fiber (okra) was set up as the control. Then the beakers containing the inverted test tubes were incubated in a water bath at 37°C with mild shaking. After 90 min of the incubation, 1 mL of the solution (dialysate) from each beaker was taken out and transferred to new respectively labeled test tubes for estimation of the amount of glucose diffused by using the glucose oxidase/peroxidase method.[24]

Estimation of Glucose Dialysis Retardation Index (GDRI)

The effect of the dietary fiber (formulations) on glucose diffusion was analyzed by the glucose dialysis retardation index[22] which could be calculated by the relation below based on the glucose concentrations obtained from each dialysis system above:

$$\mathbf{GDRI = 100 - \frac{Total\ glucose\ diffused\ from\ sac\ containing\ fiber\ (test\ sample)}{Total\ glucose\ diffused\ from\ sac\ with\ no\ fibre\ addition}\ X\ 100}$$

Data Analysis:

The data obtained were presented as mean ± standard error of the mean. Results of the Biochemical parameters were analyzed statistically by one-way Analysis of Variance (ANOVA) followed by Post Hoc (Duncan multiple comparison test) using the statistical package for Social Sciences (SPSS) Software, version 20. A P-value < 0.05 was considered statistically significant.

Figure 1a-i: Various components of the experimental set-ups.

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Results and Discussion

The increasing interest in the use of plant-based formulations is leading to a fast-growing market for Ayurvedic, nutraceutical and polyherbal formulations; unfortunately, the quality of a majority of such items remains uncontrolled because of the large number of varied chemical compounds present in the different medicinal plants.[26] Dietary fiber plays an integral role in the management of some degenerative diseases such as diabetes mellitus. *Abelmoschus esculentus* (Okra) fruits with its characteristic viscous nature and high fiber content in addition to its antioxidant properties have been acclaimed to have various health benefits in the treatment of inflammatory disorders, constipation, etc. It has also been reported to have significant antidiabetic effect.[27-30]
In the present study, an attempt was made to formulate different proportion of the seeds and peels of ex-maradi okra fruit to investigate and evaluate their *in-vitro* antioxidant and antidiabetic functional properties for the development of okra-based antidiabetic nutraceutical formulation for the management of diabetes mellitus.

Proximate Composition
The results of the proximate composition of the various formulations from the peels and seeds of ex-maradi okra fruit are presented in Table 1. The result showed that there is a significant ($P < 0.05$) difference in the percentage proximate composition in the various formulations where sample C (10:90) seeds:peels formulation was observed to have the highest percentage of crude ash and crude fibre than the other formulations (Table 1). The results (Table 1) indicated that the variations in the proportions of okra seeds and peels powder of the formulation resulted in significant differences ($P < 0.05$) in the levels of the proximate composition and the functional properties analyzed as compared with the ordinary seeds and peels powder of the formal okra fruit. From the result of the current study, it was observed that the 10:90% seed:peel ratio (Sample C) was found to be the most preferred proportion as it possesses more of the desired properties when compared to other remaining proportions formulations.
Moisture content is the main determinant of food spoilage. It is well established that low moisture content of food samples reduces the activities of microorganisms, and thereby increases the shelf life of food products.[31] The lower moisture content value ($5.36 \pm 0.20\%$) observed in sample C (10:90) implies that the proportion could be stored for a reasonably long period. From the results, the okra peels showed higher fiber content than the okra seed. Generally, the fibre contents in all the various proportions (formulations) of the present study were high compared to the recommended dietary fibre (25-30 g). However, the fibre content of sample C (10:90) proportion was relatively higher when compared with other samples proportions. This observation could be attributed to the effects of higher peel proportion compared to the seeds in the sample C. Nutritional study has established that adequate fiber intake renders some health benefits like preventing coronary heart diseases, constipation and diabetes.[32] Therefore, this formulation could serve as good source of dietary fiber. Ash content is a measure of the total mineral content of a food. The ash contents were found to be relatively high and significantly ($P < 0.05$) equal in all the various sample proportions. This indicates the potentials of high mineral contents in all the formulations. It was observed that the carbohydrate and lipid content of sample C were low, and within the safe values for diabetic patients corroborating with the findings of Ijarotimi.[33]

Functional Properties of the Various Okra-based Antidiabetic Nutraceutical Formulations
The results of the functional properties of the various formulations are presented in Table 2. The result showed that there is a significant difference ($P < 0.05$) in the estimated functional properties among the various proportions. The result showed that the antioxidant capacity (AC), water adsorption capacity (WAC), oil adsorption capacity (OAC), glucose adsorption capacity (GAC) as well as the glucose dialysis retardation index (GDRI) of sample C (10:90) seeds:peels of the formulation were found to be significantly ($P < 0.05$) higher than that of the other formulations. Same sample proportion (10:90) also showed the least value for glucose diffusion (GD) than the rest of the other proportion formulations (Table 2).

Antioxidant Capacity (AC)
Phenols and flavonoids are known natural antioxidants in many plant substances. The antioxidant properties of the formulation might be connected with the presence of phenols, flavonoids and other antioxidants rich compounds (e.g. carotenoids, riboflavin, ascorbic acid, thiamine, nicotinic acid, etc.) present in the okra fruit.[13] This is obvious in the 10:90 proportion of the formulation. It has been reported that agents with antioxidant or free radical scavenging property may inhibit oxidative reactions associated with diabetes hence reducing diabetes complications.[34]

Water Adsorption Capacity (WAC) and Oil Adsorption Capacity (OAC)
Water Adsorption Capacity is a useful parameter as it serves as index for predicting the fecal bulking ability of a fiber material while Oil Adsorption Capacity is an index that predicts the lipophilic behaviour of fiber matter.[35] According to the results, the observed variations in both the WAC and OAC of the various formulations could be due to the differences in the proportion of the fiber and other biological constituents in the formulations. From the results, sample C showed significantly ($P < 0.05$) higher Water and Oil Adsorption Capacity than the other proportions (50.06 ± 0.80) and (27.46 ± 0.40), respectively (Table 2). This could be attributed to the increase in the protein content of the formulation, which is responsible for high hydrogen bonding and high electrostatic repulsion which according to Altschul[36] are conditions that facilitate binding and entrapment of water as well as the ability to bind fat by capillary attraction.[37] Also, Chau[38] have reported that the ability of fiber matter to adsorb water is closely associated with fiber structure, particle size and the number of its water binding sites. The increased WAC and OAC of the formulation could also indicate possible increase in the degree of stabilization of the formulation (shelf life) and suggest its utility as a thickener in liquid and semi-liquid foods. Such properties could be of a beneficial effect with respect to digestion and absorption of glucose in the intestine.

Glucose Adsorption Capacity (GAC)
GAC is another index for predicting the functional ability of fiber mater to adsorb glucose and hence decreasing the concentration of glucose within the surrounding environment, this could be more of the function of the fiber structure than the chemical composition of the fiber.[38] The significantly ($P < 0.05$) higher GAC observed in the 10:90% formulation (17.26 ± 0.10) compared to the other sample formulations (Table 2) could be attributed to increase in water holding capacity and viscosity of the fibers. This indicates that fibre might help to retain glucose *in-vitro.* Same principle/effect could be mimicked *in-vivo,* and it could be beneficial with respect to reducing the amount of glucose absorbed from the small intestine. Yeh[40] have reported that dietary fibre could act as functional dietary supplements for decreasing the rate of glucose adsorption as well as the concentration of serum glucose when it can effectively absorb glucose. The present results are in agreement with the findings of Lopez *et al*.,[41] where they reported that fiber matter may enhance the entrapment of glucose within the fiber matrix as it could minimize mobility of water on the surface and consequently increasing the hydration ability of the fibre particles which may contribute to glucose retention on the fibre surface.

Glucose diffusion capacity (GDC)
GDC capacity is an important parameter to measure the functionality of a fiber matter. The dialysis experiments mimic events occurring along the gastro intestinal tract. In this experiment, it is worthy to note that the movement of glucose across the dialysis membrane in the experimental system is not a true reflection of diffusion that occurs in *in-vivo* models. Movement in this system is assisted by mimicking the convective activity of intestinal contractions by the stirring of the *in-vitro* models during the experiment.[25] The experiment was carried out to assess the relative differences of the different formulation to inhibit glucose movement thereby evaluating their action in lowering postprandial glucose level. The observed significantly ($P < 0.05$) lower glucose diffusion (GD) in the sample C proportion (20.28 ± 1.00) as compared to the other formulation could be due to the fact that okra dietary fiber could bind to glucose and prevent or delay its diffusion from the *in-vitro* dialysis model. This could be beneficial with respect to reducing the amount of accessible glucose that could be absorbed from the small intestine, hence reducing postprandial blood glucose level. This is in agreement with Adiotomre *et al*.[25] who reported that the delay in glucose adsorption in the GIT is mainly linked to the viscosity of polysaccharides.

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Glucose Dialysis Retardation Index (GDRI)

GDRI is a useful *in vitro* method to predict the effect of fiber on the delay of glucose adsorption in the gastrointestinal tract.[25] Generally, diffusion of glucose from the dialysis membrane could be affected by dietary fibres with time and viscosity which results in higher retardation in glucose diffusion as compared to control. By hypothesis of the effect of dietary fibre on diffusion was mainly due to the viscosity.[34] The diffusion rate would decrease as time increased because of the gradual increase in viscosity of the medium. The diffusion rate of glucose could be decreased by insoluble fibres even if they contribute less to viscosity. This is in agreement with the findings of Ou *et al*.[22] where they reported that insoluble fiber derived from wheat bran could adsorb glucose at different concentrations to decrease the concentration of glucose available in small intestine. This is further elucidated by the observed respective increase in GDRI as presented in Table 2. The highest value of GDRI observed in the sample that has the least glucose diffusion (60.72 ± 2.30) as shown in Table 2 may be due to their differences in viscosity. It has been reported that GDRI increases proportionally with increase in viscosity, thus, the sample with higher viscosity could exhibit the highest GDRI. This may be due to the fibre structure particle sizes which are expected to be hydrated rapidly as time increases from 30 min to 90 min due in part to increase in viscosity which in turn delays in glucose diffusion and thus may help in the control of postprandial blood glucose level.

Table 1: Proximate Composition (%) of the Various Okra-based Antidiabetic Nutraceutical Formulation (%)

X	Moisture	Crude Ash	Crude Protein	Crude Lipid	Crude Fiber	Carbohydrate
A	8.23 ± 0.14^{a}	11.16 ± 0.17^{b}	13.04 ± 0.38^{d}	4.07 ± 0.17^{d}	30.10 ± 0.83^{a}	33.40 ± 0.27^{c}
B	9.70 ± 0.52^{b}	9.63 ± 0.47^{a}	12.76 ± 0.41^{b}	1.41 ± 0.01^{b}	42.76 ± 2.02^{b}	23.72 ± 1.76^{c}
C	5.36 ± 0.20^{a}	10.33 ± 0.08^{a}	11.33 ± 0.17^{c}	1.18 ± 0.01^{b}	52.40 ±1.18^{c}	19.40 ± 0.84^{d}
D	11.86 ± 0.38^{b}	9.50 ± 0.57^{a}	11.55 ± 0.12^{c}	1.36 ± 0.01^{b}	52.23 ± 1.15^{c}	13.50 ± 1.21^{d}
E	10.44 ±0.29^{b}	9.20 ± 0.17^{a}	12.68 ± 0.02^{c}	1.60 ± 0.04^{c}	51.43 ± 0.12^{c}	14.65 ± 0.52^{b}
F	11.26 ± 0.13^{b}	9.86 ± 0.18^{a}	12.92 ± 0.15^{d}	1.38 ± 0.01^{b}	51.73 ± 0.84^{c}	12.85 ± 0.63^{b}
G	6.27 ± 0.14^{a}	9.00 ± 0.36^{a}	10.75 ± 0.47^{b}	1.18 ± 0.01^{b}	50.96 ±1.90^{c}	21.84 ± 2.86^{a}
H	13.31 ± 0.16^{c}	9.93 ± 0.12^{a}	8.42 ± 0.26^{a}	2.69 ± 0.03^{c}	50.66 ± 0.27^{c}	14.99 ± 0.73^{c}
I	11.20 ± 0.17^{b}	9.83 ± 0.16^{a}	7.53 ± 0.12^{a}	1.84 ± 0.01^{c}	42.80 ± 0.78^{b}	26.80 ± 1.02^{c}
J	10.13 ± 0.08^{b}	9.03 ± 0.03^{a}	10.83 ± 0.44^{b}	1.05 ± 0.02^{a}	35.55 ±1.99^{a}	33.41 ± 1.65^{a}
K	12.50 ± 0.36^{c}	9.20 ± 0.36^{a}	10.53 ± 0.46^{c}	1.03 ± 0.06^{a}	34.70 ±1.44^{a}	32.04 ± 3.03^{b}

Values are expressed as mean ± S.E.M., Mean values having different superscript letter in the same column are significantly ($P < 0.05$) different.

X: Sample formulation; A: 100% Seed; B: 100% Peel; C: 10:90; D: 20:80; E: 30:70; F: 40:60; G: 50:50; H: 90:10; I: 80:20; J: 70:30 and K: 60:40 (Okra Seed: Okra Peel %) respectively.

Table 2: Functional Properties (%) of the Various Okra-based Antidiabetic Nutraceutical Formulation

X	AC	WAC	OAC	GAC	GD	GDRI
A	63.03 ± 0.50^{e}	15.93 ± 0.10^{a}	18.63 ± 0.20^{a}	11.37 ± 0.30^{a}	41.08 ± 0.60^{e}	20.58 ± 0.30^{a}
B	60.71 ± 0.30^{d}	47.80 ± 0.50^{f}	21.80 ± 0.20^{b}	19.39 ± 0.40^{e}	23.75 ± 0.80^{b}	54.52 ± 1.70^{e}
C	65.48 ± 0.80^{f}	50.06 ± 0.80^{h}	27.46 ± 0.40^{e}	17.26 ± 0.10^{d}	20.28 ± 1.00^{a}	60.72 ± 2.30^{f}
D	61.03 ± 0.50^{d}	47.76 ± 0.70^{f}	25.23 ± 0.30^{d}	15.58 ± 0.20^{c}	27.43 ± 0.30^{c}	47.05 ± 1.10^{d}
E	60.43 ± 0.30^{d}	44.73 ± 0.20^{e}	23.23 ± 0.10^{c}	15.28 ± 0.40^{c}	27.97 ± 0.20^{c}	45.16 ± 0.50^{d}
F	59.70 ± 0.30^{c}	42.60 ± 0.30^{e}	24.10 ± 0.20^{c}	12.16 ± 0.20^{a}	26.83 ± 0.50^{c}	47.93 ± 1.20^{d}
G	60.70 ± 0.30^{d}	31.93 ± 0.50^{d}	20.46 ± 0.20^{b}	12.92 ± 0.50^{a}	32.79 ± 0.20^{d}	35.70 ± 0.50^{c}
H	58.38 ± 0.80^{c}	33.50 ± 0.10^{d}	21.13 ± 0.50^{b}	10.38 ± 0.30^{a}	31.74 ± 0.20^{d}	37.77 ± 0.40^{c}
I	55.68 ± 0.30^{b}	28.36 ± 0.20^{c}	18.30 ± 0.20^{a}	10.95 ± 0.30^{a}	36.13 ± 0.10^{d}	29.16 ± 0.20^{b}
J	52.76 ± 1.10^{a}	26.46 ± 0.30^{c}	18.16 ± 0.20^{a}	12.19 ± 0.30^{a}	37.79 ± 0.50^{d}	25.90 ± 1.00^{b}
K	53.46 ± 0.80^{a}	24.13 ± 0.30^{b}	18.60 ± 0.30^{a}	13.10 ± 0.10^{b}	28.46 ± 0.30^{c}	44.19 ± 0.60^{d}

Values are expressed as mean ± S.E.M., Mean values having different superscript letter in the same column are significantly ($P < 0.05$) different.

X: Sample formulations; A: 100% Seed; B: 100% Peel; C: 10:90; D: 20:80; E: 30:70; F: 40:60; G: 50:50; H: 90:10; I: 80:20; J: 70:30 and K: 60:40 % (Okra Seed : Okra Peel); AC: (Antioxidant Capacity); WAC: (Water Adsorption Capacity); OAC: (Oil Adsorption Capacity); GAC: (Glucose Adsorption Capacity); GD: (Glucose Difussion/Dialysis); GDRI: (Glucose Dialysis Retardation Index).

Conclusion

The results from this study showed that varying the proportion of okra seed and peel in the right proportion could improve its functional antidiabetic properties as significant differences in proximate analysis, antioxidants, water adsorption and glucose absorption capacities as well as in the glucose dialysis retardation index were observed in the various proportions made. This study suggests that the 10:90 % of okra seeds: peels formulation has highest potentials in antidiabetic functional properties and might be the most suitable in the development of antidiabetic nutraceutical for the management of diabetes mellitus.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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