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Comparative Effects of *Zea Mays* Bran, *Telfairia occidentalis* and *Citrus sinensis* Feeds on Bowel Transit Rate, Postprandial Blood Glucose and Lipids Profile in Male Wistar Rats

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ABSTRACT
Comparative effects of *Zea mays* bran, *Telfairia occidentalis,* and *Citrus sinensis* feeds on bowel transit rate, postprandial blood glucose, and serum lipid profile levels were investigated in 20 male Wistar rats of average weight 160 ± 5 g. Proximate analysis and *in-vitro* total antioxidant capacity (TAC) of the feeds were evaluated. The animals were randomly grouped into four (n=5) and fed with normal rats' chow (control), *Z. mays* bran, *T. occidentalis,* and *C. sinensis* respectively for thirty days. Postprandial blood glucose, plasma lipids profile and bowel transit rate were evaluated. The ash and protein contents were significantly higher ($p<0.05$) in *T. occidentalis,* while *Z. mays* bran, *T. occidentalis,* and *C. sinensis* have high fibre relative to control feed. The TAC was higher in *T. occidentalis* followed by *C. sinensis.* Postprandial blood glucose was significantly reduced ($p<0.05$) in rats fed with *Z. mays* bran and *T. occidentalis*. Plasma total cholesterol, triglyceride and low-density lipoprotein cholesterol levels were significantly reduced ($p<0.05$) in rats fed with *Z. mays* bran, *T. occidentalis,* and *C. sinensis.* Bowel transit rate was significantly increased ($p<0.05$) in the rats fed with *Z. mays* bran, *T. occidentalis,* and *C. sinensis*. The results obtained revealed that *Zea mays* bran may possess laxative activity than other feeds, while the hypoglycemic and hypocholesterolemic activities in the rats fed with the three feeds were similar.
Keywords: *Telfairia occidentalis, Citrus sinensis*, *Zea mays* bran, *in-vitro* antioxidant, Proximate analysis

INTRODUCTION
Plant-based diets are gaining recognition and acceptance as a dietary approach for maintaining healthy living and managing some diseases (Craig, 2009). They have been reported to be used as medical nutrition therapy in the management of metabolic syndrome, including obesity, diabetes and cardiovascular risk (Tonstad *et al*., 2013; Jenkins *et al*., 2014; Le and Sabaté, 2014). Their medicinal values may be ascribed to the presence of high fibre and nutrients (AACC, 2001). In addition, they contain phytochemicals that may be important factors in human health (Liu, 2003). Fibres have been reported to increase bile excretion, reduced serum total cholesterol and low-density lipoprotein cholesterol (Amaral *et al.,* 1992; Story *et al*., 1997). They also regulate energy intake and inflammatory cytokines (Esposito *et al*., 2003).

Zea mays (corn) is one of the major crops in Nigeria with great nutritional value. It is normally used as raw material for manufacturing many industrial products (Afzal *et al.,* 2009; Enyisi *et al*., 2014). *Zea mays* processing for food may require milling that will separate its component parts into endosperm, mesoderm and pericarp (Duensing *et al.,* 2003). Bran is mainly composed of the pericarp (Singh *et al.,* 2000). The main chemical composition of *Zea mays* bran is cellulose and hemicellulose insoluble dietary fibre. It also comprises of starch, lipid, protein, ash, phenolic compounds and other trace phytochemicals (Rose *et al.,* 2009). Generally, *Zea mays* bran is often used to increase dietary fibre content in some foods (Rose *et al.,* 2009).

Telfairia occidentalis (Fluted pumpkin) is natural to West Africa. It is edible and widely planted as a vegetable (Eseyin *et al.,* 2014). The *T.*

occidentalis leaves have been reported to contain high protein, vitamins and minerals when compared with other tropical vegetables (Eseyin *et al.,* 2014). It leaves has been reported to facilitate bowel transit rate (Ofuya *et al.,* 2005; Ojieh, 2012), possess hypoglycemic activity (Salman *et al.,* 2008), anti-diabetic (Nwozo *et al*., 2004; Eseyin *et al.,* 2014), and antioxidant properties (Nwanna and Oboh, 2007; Oboh *et al.,* 2010).

Citrus sinensis (sweet orange) fruit is commonly cultivated throughout the tropics. It is highly nutritious and medicinal (Peterson *et al*., 2006; Tripoli *et al*., 2007). It is rich source of vitamin C, folate, dietary fibre, calcium, magnesium, niacin, thiamine (Angew, 2007). It also contains phytochemicals such as flavonoids, amino acids, triterpenes, phenolic acids and carotenoids (Di Majo *et al*., 2005; Li-ying *et al*., 2008).

This study was designed to compare the *in vitro* antioxidant and proximate composition of *Zea mays* bran, *Telfairia occidentalis* leaves and *Citrus sinensis* fruit feeds and their effects on bowel transit rate postprandial blood glucose and lipids profile in male Wistar rats.

MATERIALS AND METHODS

Preparation of Test Feed

Healthy ripened *C. sinensis* fruits and matured *T. occidentalis* leaves without any signs of pest invasion or diseases were harvested separately in October 2016. They were transferred to the laboratory and processed immediately. *Zea mays* bran was purchased at Okada central market, Edo state. Sliced *C. sinensis* (without pericarp and seeds) and *T. occidentalis* leaves were freeze-dried then pulverised. Ten gram of each pulverised *C. sinensis* fruit, *T. occidentalis* leaves, or *Z. mays* bran was mixed separately with an equal ratio of rat chow and the mixture was pelleted.

Proximate Analysis of Test Feed

The moisture, ash, crude fibre, fat, protein and carbohydrate contents of *C. sinensis* fruit, *T. occidentalis* leaves, and *Z. mays* bran were determined according to the AOAC (2000) method.

Evaluation of *in-vitro* Antioxidant Activity of the Test Feed

Radical scavenging activity using 1, 1-diphenyl-2-picrylhydrazyl hydrate method

The radical scavenging activity of the samples was determined using 1, 1-diphenyl-2-picrylhydrazyl hydrate (DPPH) according to Singh *et al*. (2002). One millilitre of 0.3 mM DPPH in methanol was added to 1.0 mL of various concentrations of samples in a test tube. The mixture was shaken vigorously and incubated in the dark for 30 minutes after which their absorbance was read at 517 nm against a DPPH control containing only 1.0 mL methanol. Radical scavenging activity was expressed as the percentage inhibition.

Evaluation of Total Antioxidant Capacity (TAC)

The total antioxidant capacity of the feeds was evaluated according to the methods of Prieto *et al.* (1999). An aliquot of 0.1 mL of sample was added to 1.0 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The reacting mixture was incubated in a water bath at 95°C for 90 minutes and cooled to room temperature. The absorbance of the mixture was measured at 695 nm against blank. The total antioxidant activities of the samples were expressed as an ascorbic acid equivalent (AAE µmol/g).

Animals

Twenty male Wistar rats weighing between150-170g were used for this study. The rats were obtained in Igbinedion University Okada animals' house, Nigeria and kept in clean well ventilated plastic cages under good laboratory condition. They were fed and had free access to clean water. The national guide for the care and use of laboratory animals was strictly followed. The animals were acclimatised for two weeks before commencement of the experimental procedure.

Animal Grouping and Experimental Procedure
The animals were randomly and equally grouped into four (4), the animals in group 1 were fed with normal rat chow (control) and groups 2 to 4 were fed with formulated *Z. mays* bran, *T. occidentalis* and *C. sinensis* pelleted feeds respectively daily for thirty days.

Determination of Bowel Transit Rate
Bowel transit rate was determined using the method of Obembe *et al.* (2008). All the rats were fasted overnight prior to the experiment but had unhindered access to drinking water. Ten gram (10 g) of each test meal was mixed separately with 2 g of activated charcoal (marker) in 20 mL of distilled water. Two millilitres of the marker mixture was orally given to animals in their respective groups. The animals were anaesthetized after 2 hours and blood sample was collected by cardiac puncture. The abdomen was instantly dissected alone the linea alba to curtail bleeding. The duodenum was cut away from the pyloric sphincter and the ileum was also cut at the ileocecal sphincter. The small intestine was immediately straightened and the location of the marker was identified along the small intestine. A white thread was used to tie the intestine at the point where the indicator stopped. The total length of the small intestine and distance travelled by the marker were measured with measuring tape and recorded. The bowel transit rate was expressed as a percentage travelled by the test meal in the small intestine and calculated as;

$$\text{Bowel transit rate} = \frac{\text{Distance travelled by test meal}}{\text{The total length of small intestine}} \times 100$$

Evaluation of Postprandial Blood Glucose
Postprandial blood glucose was evaluated with glucose oxidase method (glucometer). This method had been shown to correlate excellently with standard biochemical methods (Ajala *et al*., 2003).

Determination of lipid profile
The plasma concentration of total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol were determined by an enzymatic colorimetric method using Roche kits.

Statistical Analysis
Data were analysed using one-way analysis of variance, followed with post hoc test (Least significant difference) with GraphPad Prism (version 7) and presented as mean ± SEM. A p<0.05 was considered significant.

RESULTS

Proximate Composition
Table 1 shows the percentage proximate composition in Z. *mays* bran, *T. occidentalis* and *C. sinensis*. Ash was significantly higher (p<0.05) in *T. occidentalis* compared with control. The percentage crude fibre was significantly higher (p<0.05) in Z. *mays* bran, *T. occidentalis* and *C. sinensis* when compared with control. The protein content in *T. occidentalis* was significantly higher (p<0.05) compared with control, while carbohydrate was significantly lower in *T. occidentalis* compared with control.

Table 1: Percentage Proximate Composition in *Z. mays* bran, *T. occidentalis* and *C. sinensis*

Proximate (%)	Control	*Z. mays* bran	*T. occidentalis*	*C. sinensis*
Moisture	7.1 ± 1.200	6.0 ± 0.970	7.2 ± 1.700	8.7 ± 0.890
Ash	0.8 ± 0.030	1.1 ± 0.020	4.9 ± 0.080*	0.8 ± 0.020
Fat	0.4 ± 0.008	0.3 ± 0.006	0.1 ± 0.001	0.2 ± 0.003
Fibre	5.3 ± 0.740	7.3 ± 1.010*	7.3 ± 0.990*	7.7 ± 1.140*
Protein	5.9 ± 0.920	2.4 ± 0.810	26.0 ± 2.310*	3.3 ± 0.080
Carbohydrate	80.5 ± 6.010	82.9 ± 4.780	54.5 ± 3.780*	79.3 ± 5.130

Values are expressed in mean ± SEM, n = 5, *p<0.05 was considered significant relative to control group

In-vitro Antioxidant

Figure 1 shows that 1, 1-diphenyl-2-picrylhydrazyl hydrate half maximal inhibitory concentration (IC_{50}) was significantly lower ($p<0.05$) in *T. occidentalis* and *C. sinensis* feeds when compared with control. The total antioxidant capacity was significantly high ($p<0.05$) in *T. occidentalis* and *C. sinensis* when compared with control (Figure 2).

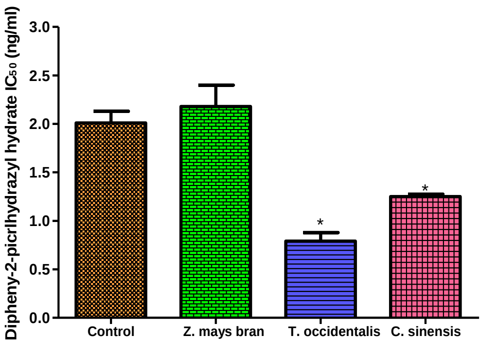

Figure 1: 1, 1-diphenyl-2-picrylhydrazyl hydrate half maximal inhibitory concentration (IC_{50}) of *Z. mays* bran, *T. occidentalis* and *C. sinensis.*

Bars are expressed in mean ± SEM, n = 5, *p<0.05 was considered significant relative to control group

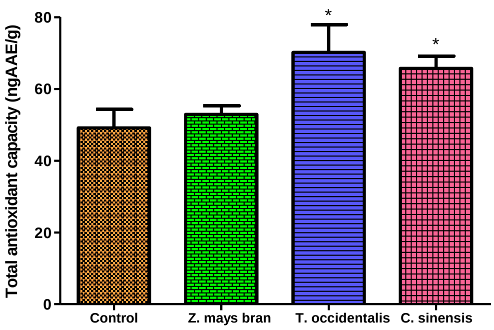

Figure 2: Total antioxidant capacity of *Z. mays* bran, *T. occidentalis* and *C. sinensis.*

Bars are expressed in mean ± SEM, n = 5, *p<0.05 was considered significant relative to control group.

Bowel transit rate

Figure 3 shows that bowel transit rate was significantly increased ($p<0.05$) in rats fed with *Z. mays* bran (95.3%), *T. occidentalis* (84.7%) and *C. sinensis* (88.8%) respectively compared to control (74.9 %).

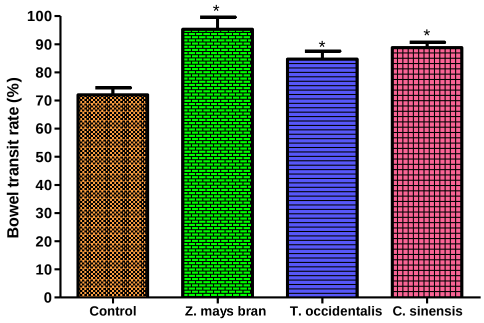

Figure 3: Bowel transit rate in male Wistar rats fed with *Z. mays* bran, *T. occidentalis* and *C. sinensis*.

Bars are expressed in mean ± SEM, n = 5, *p<0.05 was considered significant relative to control group

Postprandial blood glucose and Lipids profile

Figure 4 shows that postprandial blood glucose level was significantly reduced ($p<0.05$) in rats fed with *Z. mays* bran and *T. occidentalis* respectively when compared with the control group.

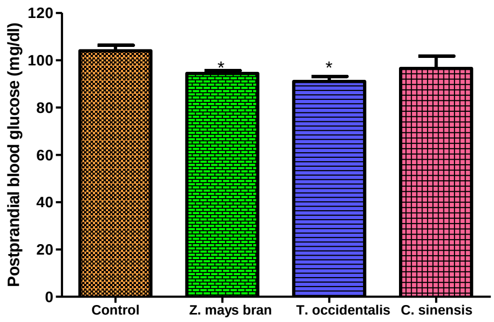

Figure 4: Postprandial blood glucose level in male Wistar rats fed with *Z. mays* bran, *T. occidentalis* and *C. sinensis*.

Bars are expressed in mean ± SEM, n = 5, *p<0.05 was considered significant relative to control group.

Table 2 shows that serum total cholesterol, triglyceride and low-density lipoprotein cholesterol levels were significantly decreased ($p<0.05$) in rats fed with *Z. mays* bran, *T. occidentalis* and *C. sinensis* respectively when compared with the control group. High-density lipoprotein cholesterol was significantly increased ($p<0.05$) in rats fed with *Z. mays* bran, *T. occidentalis* and *C. sinensis* when compared with control group.

DISCUSSION

Food composition and nutrient are the central sources of information for health. The proximate composition of feeds used in this study showed that *T. occidentalis* contains an appropriate proportion of carbohydrate, protein, ash, fibre, lipid and moisture. The percentage carbohydrate composition was higher in *Z. mays* bran and *C. sinensis* feeds. The moisture and fibre contents in the three feeds used in this study were similar. In the current study, it was also observed that ash was more in *T. occidentalis* than the other two feeds. The ash content of the feed is known to be related to mineral elements present it. Also, dietary ash has been proven to buffer acid-alkaline balance of the blood as well as control hyperglycaemia. The hypoglycemic (Salman *et al.,* 2008) and anti-diabetic (Nwozo *et al*., 2004; Eseyin *et al.,* 2014) activities of *T. occidentalis* may be linked to its ash content.

The diphenyl-2-picrylhydrazyl hydrate (DPPH) radical scavenging half maximal concentration (IC_{50}) of the *T. occidentalis* and *C. sinensis* feeds were lower than the control and *Z. mays* bran. The current DPPH results demonstrated that both *T. occidentalis* and *C. sinensis* feeds have good antioxidant activities which may scavenge free radical generate by oxidative stress. Also, the total *in-vitro* antioxidant capacity of *T. occidentalis* and *C. sinensis* feeds was higher than that of the control and *Z. mays* bran. This observation followed the same trend with DPPH result. Although the flavonoids, phenol and vitamin C content were not estimated in this study, they may be responsible for the high *in-vitro* antioxidant observed in *C. sinensis* and *T. occidentalis*. Both *C. sinensis* and *T. occidentalis* have been reported to contain a high content of flavonoids, phenol and vitamin C (Tripoli *et al*., 2007, Nkereuwem *et al.,* 2011; Eseyin *et al*., 2014; Etebu and Nwauzoma, 2014). It may be suggested that *T. occidentalis* and *C. sinensis* feeds may offer a good source of exogenous antioxidant and complement the activity of endogenous antioxidant.

Table 2: Plasma lipid profile in male Wistar rats fed with *Z. mays* bran, *T. occidentalis* and *C. sinensis*

Groups	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	High density lipoprotein (mg/dl)	Low density lipoprotein (mg/dl)
Control	96.2 ± 0.77	76.2 ± 1.77	18.2 ± 0.04	62.8 ± 0.38
Z. mays bran	56.8 ± 0.77*	43.4 ± 0.72*	29.4 ± 0.46*	18.8 ± 0.05*
T. *occidentalis*	51.8 ± 1.16*	43.0 ± 0.89*	25.7 ± 0.35*	17.5 ± 0.63*
C. sinensis	52.2 ± 0.53*	53.4 ± 0.53*	20.9 ± 0.70*	20.6 ± 0.11*

Values are expressed in mean ± SEM, n = 5, *p<0.05 was considered significant relative to control group

The bowel transit rate was increased in all the three feeds used in this study. The observed increase in bowel transit rate was more in *Z. mays* bran, followed by *C. sinensis* and *T. occidentalis.* The facilitating bowel transit rate effect of dietary fibre is well documented (Ofuya *et al*., 2005; Mauro *et al*., 2008; Ojieh, 2012). The mechanism of action of plant-based diets in increasing bowel transit rate has been linked to the presence of high fibre contents in them which increases viscosity, water holding capacity and formation of bulk in the gastrointestinal tract (Bach-Knudsen, 2001). Insoluble fibre usually forms more viscous, high water capacity and bulk which will facilitate gastrointestinal movement. Although the insoluble fibre was not estimated in the current study, it may be inferred that *Z. mays* bran has a high percentage of insoluble fibre that may responsible for the observed increased in bowel transit rate.

The postprandial blood glucose level was reduced in *Z. mays* bran and *T. occidentalis* fed rats in the current study. *Telfairia occidentalis* is more potent in decreasing postprandial blood glucose level than *Z. mays* bran and *C. sinensis* feeds. The observed reduction in postprandial blood glucose level by *T. occidentalis* in this study may be as a result of its hypoglycemic activity reported by Salman *et al.* (2008). Similarly, Ashutosh and Shivesha (2011) reported that plant-based diets lower postprandial blood glucose by inhibiting glucose dialysate and α- amylase activity. Another possible mechanism of action of these feeds in lowering postprandial blood glucose level may be linked to delay in gastric emptying and decreased absorption of macronutrients which can lower insulin levels (Lattimer and Haub, 2010). Furthermore, increase in the viscosity of the small intestinal contents and transit rate by these feeds may modulate the absorption of glucose by the villi in the small intestine (Weickert and Pfeiffer, 2008; Tucker and Thomas, 2009).

The three feeds used in this study significantly reduced plasma total cholesterol, triglyceride and low-density lipoprotein cholesterol levels. The observed reduction in these lipids was more pronounced in *T. occidentalis* group compare to other groups. These observations were in accordance with the previous studies (Eastwood, 1990; Basu *et al*., 1993; Amaral *et al.,* 1992; Story *et al*., 1997). The effect of plant-based diets in lowering plasma lipids profile levels maybe through alteration in lipid absorption, reducing bile acid absorption in the small intestine, altering bile acid absorption in the cecum, or indirectly via short-chain fatty acids, diminishes pancreatic lipase activity (Eastwood and Morris, 1992). Fatty acids and cholesterol have been reported to bind to fibre and prevent the formation of micelles that are needed for fat transport through the unstirred water layers and into the enterocyte Lupton and Kurtz, 1993). The observed increase in plasma high-density lipoprotein cholesterol level of *Z. mays* bran and *T. occidentalis* fed rats may be linked with the high dietary fibre content. Quan *et al.* (2015) reported a direct relationship between dietary fibre intake and high-density lipoprotein cholesterol.

CONCLUSION

Telfairia ocidentalis may be a good source of protein, minerals and antioxidant. *Zea mays* bran may have laxative property more than *Telfairia ocidentalis* and *Citrus sinensis*, while the hypoglycemic and hypocholesterolemic activities in the Wistar rats fed with the three feeds were similar. These data suggested that *Zea mays* bran, *Telfairia ocidentalis* and *Citrus sinensis* may possess therapeutic activity against metabolic syndromes.

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