PAPER

TOTAL PHENOLIC CONTENT, ANTIOXIDATIVE AND ANTIDIABETIC PROPERTIES OF COCONUT (*COCOS NUCIFERA L.*) TESTA AND SELECTED BEAN SEED COATS

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ABSTRACT

Natural alternatives for the treatment of diabetes mellitus have been the interest of many researchers. In this study, the brown testas of mature coconuts were compared to beans seed coats of four varieties in terms of antioxidative and anti-hyperglycaemic properties. The total phenolic and flavonoid contents, the antioxidant potentials and the α-amylase and α-glucosidase inhibitory activities of the crude extracts were studied *in vitro*. The results showed that extracts of coconut testa and red kidney bean seed coat displayed higher α-glucosidase inhibition (IC_{50}=19.90±5.67 and 4.84±1.43 μg/mL) and α-amylase inhibition (IC_{50}=120.5±15.4 and 532.8±68.0 μg/mL) than the other extracts. These two extracts showed higher antioxidant capacities owing to their high phenolic and flavonoid contents. These results suggest that red kidney bean seed coat and tender coconut testa would have higher potential as nutraceuticals and could serve as natural alternative sources of anti-diabetic remedy.

Keywords: anti-diabetic, antioxidants, polyphenolic compounds, coconut testa, bean seed coat

1. INTRODUCTION

Consumption of wholesome food is recognized as an important step to maintain optimal health. In this respect, consumption of whole grains and cereals is recommended as diet to prevent chronic diseases such as diabetes and cancer. As whole grains are rich in vitamins, minerals, and phytochemicals, they are believed to help reduce the risk factors associated with diabetes. Dry beans that include red bean, red kidney bean, white bean and black bean are recognized to have many nutritional attributes. According to XU *et al.* (2007), red bean and red kidney beans are rich in nutrients and dietary fibre and were found to display higher content of phenolics and antioxidant activity. LAYER *et al.*(1985) found that white bean *in vitro* was able to inhibit α-amylase. Recent investigations of TANKO *et al*. (2017) concluded that bran of *Beras merah* and *Beras hitam* rice varieties had high potential to be used in dietary formulations intended for diabetes.

The recent trend advocates exploring the food values of tropical fruits such as coconut for healthy living. The most important part of coconut is kernel or endosperm, which is usually found to possess moisture, fat, protein, carbohydrates, minerals and vitamins. The oil extracted from the kernel is reported to have health benefits owing to the presence of lauric, capric, caproic and caprylic acid (MARIKKAR and MADHRAPPERUMA, 2012). The defatted kernel residue left after extraction of virgin coconut oil is another valuable product. The fibrous component of the defatted kernel residue is of particular interest due to the beneficial health effects (AIN NAJWA *et al*., 2016). As a source of dietary fibre, defatted kernel residue can provide health benefits particularly in relation to chronic illnesses such as cancer and diabetes mellitus. TRINIDAD *et al*. (2003) found that the incorporation of defatted coconut flour in bakery products would lower their glycemic indices. Other than these, there are other reports, which indicated that defatted coconut flour might contribute to reduce the concentration of cholesterol in blood (SENEVIRATNE *et al*., 2009)

The brownish outer covering of coconut kernel serving as a protective layer in the fruit is an interesting by-product, traditionally known as coconut testa (SENEVIRATNE *et al*., 2009). Owing to its dark brown color, testa is usually removed before processing coconut products such as desiccated coconut, coconut cream and milk powder. The testa of coconut constitutes approximately 18% (w/w, wet basis) of the total kernel weight (MARIKKAR and MADHRAPPERUMA, 2012). In an attempt to utilize coconut testa for edible oil production, APPAIAH *et al*. (2014) came to know that the oil was composed of several short and medium chain fatty acids along with phenolics, phytosterols, tocopherols, tocotrienols etc. These studies indicated that the fruit component of coconut such as coconut kernels might have medicinal properties due to these valuable constituents (DEBMANDAL AND MANDAL, 2011). Recently, SALIL *et al*., 2011 found that coconut kernel protein has potent anti-diabetic activity through the reversal of glycogen levels and activities of carbohydrate metabolizing enzymes. To date, no report compared the anti-hyperglycaemic activity of coconut testa with those of common beans namely, red kidney bean, black-eyed pea, red bean and black beans. As the removed testa is largely wasted in processing factories without any value addition, it is timely to undertake further studies on it. The results might have various direct and indirect environmental and economic impacts reducing the disposal costs and increasing the added value of the final products, giving emphasis to explore their health benefits. If the removed testa is crushed in the dry form, it might give flour, which could be used for preparation of wholemeal bread beneficial to those who suffer from type 2 diabetes. Hence, the objective of this study was to compare brown testa of coconut with seed coats of four varieties of beans with respect to total flavonoid and phenolic contents, and antioxidative and anti-hyperglycemic properties.

2. MATERIALS AND METHODS

2.1. Sample collection and preparation

Samples of brown testa from coconut (TCO) were collected in triplicate from the coconut farm located in the university agricultural park, Serdang, Malaysia. Samples of four varieties of *Phaseolus vulgaris* (red kidney bean (RKB), black-eyed pea (BEP), red bean (RB) and black beans (BB) were collected in triplicate from individual farms located at Setiawan, Malaysia. The bean samples were dehulled manually while the testae of coconuts were removed from their shells to be dried and milled into fine particles. Samples were packed in airtight Ziploc plastic bags and stored at 4°C until further analysis.

2.2. Chemicals

Pancreatic α-amylase, α-glucosidase (*Saccharomyces cerevesiae*), 1,1-diphenyl-2-picrylhydrazine (DPPH), hydrochloric acid, p-nitrophenyl- α-d-glucopyranoside were purchased from Sigma-Aldrich. Chemicals, namely, sodium carbonate, sodium acetate buffer (pH 6), sodium potassium tartarate, and sodium hydroxide were purchased from R&M chemicals. Other chemicals such as; dinitrosalicyclic acid (DNS), potassium persulphate were purchased from Acros Organics (Private) Ltd. 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonicacid) diammonium salt (ABTS) from Amresco, Folin Ciocalteu phenol reagent and gallic acid from Cayman. 2,4,6-tripyridyl-s-triazine (TPTZ) was purchased from Affymetrix.

2.3. Preparation of ethanolic extracts of sample

Hundred (100) gram portions of the milled bean seed coats and coconut testa were extracted with 1000 mL of 70% ethanol-water for 48 h at 25 °C. The extracts were centrifuged with eppendorf 8510R centrifuge at 8300 rpm for 10 min and filtered with Whatman no. 1 filter paper. The solvent was evaporated under reduced pressure using a Büchi Model R-205 rotary evaporator (Büchi, Switzerland) and the semi-solid extracts were freeze-dried using a Virtis bench top pro-freeze dryer SJIA-10N (FD-1B-50) (Virtis, New York). They were then re-dissolved in 70% ethanol in the ratio of 10mg/mL of solvent to serve as stock solution for various analyses.

2.4. Total phenolic content (TPC)

TPC was assayed using Folin-Ciocalteu regent in a 96-well microplate according to the method described by SINGLETON *et al*. (1999). About 20 µL of the sample extract was mixed with 110 µL of freshly prepared Folin-Ciocalteu regent. Subsequently, the mixture was added with 70 µL of 20% Na_2CO_3 and allowed to incubate for 30 min at room temperature. After incubating for 30 min at 25±2°C, absorbance was read at 765 nm using a microplate reader (Synergy 2, BioTek instruments, USA). Total phenolic content of each sample was calculated from the mean of triplicates and expressed as milligrams (mg) of gallic acid equivalent (GAE) per 1g of dry sample.

2.5. Total flavonoid content (TFC)

Total flavonoid content of the extracts was determined according to the method described by ZHISHEN *et al.* (1999) with some modifications. Briefly, 25 µL of the ethanolic extract,

125 μL of distilled water and 7.5 μL of 5% $NaNO_2$ were mixed together and the mixture was allowed to stand for 5 min. After adding 15 μL of 10% $AlCl_3$, the mixture was allowed to incubate at 25°C for an additional 5 min. At the 6th min, the mixture was added with 50 μl of 1M NaOH and subsequently diluted by adding 27.5 μl of distilled water. Absorbance of the solution was measured at 515 nm using a microplate reader (Synergy 2, BioTek instruments, USA). Total flavonoid content of each sample was calculated from the mean of triplicates and expressed as milligram (mg) quercetin equivalent (QE) per 1g of dry sample.

2.6. Analysis of flavonoid and phenolic acids by HPLC

Chromatographic analysis was performed on Agilent 1100 series HPLC-DAD system (Agilent Technologies Deutschland GmbH, Germany) using a reverse phase C18 column (150 X 4.5 mm) packed with 5 μm diameter particles which was controlled at a temperature of 30°C. The flow rate was 0.7 mL/min and the injection volume was 50 μL. The mobile phase consisted of 2% acetic acid (Solvent A) and absolute methanol (Solvent B), and the composition gradient was run as follows: 13% of B until 10min and changed to obtain 20%, 30%, 50%, 70%, 20% and 10% B at 20, 30, 40, 50, 60, 70 and 80 min respectively, following the method described by IRONDI *et al*. (2014) with slight modifications. A stock standard solution of individual compound was prepared in methanol at a concentration range of 0.030-0.250 mg/mL for quercetin, kaempferol, catechin, rutin and epicatechin; and 0.050-0.450 mg/mL for caffeic, ferulic, gallic, ellagic and chlorogenic acids. Peaks of chromatograms were confirmed by comparing its retention time with those of reference standards. Gallic acid was detected at 257 nm, catechin and epicatechin at 281 nm, ferulic, chlorogenic, ellagic and caffeic acids at 325 nm and kaempferol, quercetin and rutin at 366 nm. All analyses were carried out in triplicates.

2.7. Antioxidant analyses

2.7.1. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed according to the method described by BENZIE and SZETO (1999) in a 96-well microplate. The working FRAP reagent was prepared by mixing 300 mM of acetate buffer (pH 3.6), 10 mM of TPTZ solution and 20 mM of $FeCl_3.6H_2O$ in a ratio of 10:1:1 and then heating to 37°C. The TPTZ solution was prepared by dissolving 10mM TPTZ in 40 mM HCL. A 20 μL sample solution was added to a mixture of 150 μL of working FRAP reagent and 30 μL of acetate buffer. The whole reaction mixture was allowed to stand for 8 min and the absorbance was recorded at 600 nm. Results were expressed as mmol of $FeSO_4$ per 100 g of the dry sample.

2.7.2. DPPH radical scavenging activity

DPPH scavenging activity was performed according to the method described by BLOIS (1958) in a 96-well microplate. A 150 μL portion of 125 μM DPPH was mixed with 50 μL of sample extract with varying concentrations ranging from 3.12-200 μg/mL in a microplate, the mixture was allowed to incubate at 25±2°C for fifteen minutes in the dark and the absorbance was measured at 517 nm using a Biotek microplate reader.

The percentage of free radical scavenging activity was calculated as follows:

$$\% \text{ DPPH Scavenging effect} = \frac{Ac - As}{Ac} x100$$

Where Ac = absorbance of control, As = absorbance of sample.

2.7.3. $ABTS^{\cdot}$ radical scavenging activity

The $ABTS^{\cdot}$ radical scavenging assay was performed according to the method described by RE *et al.* (1999) in a 96-well microplate. A stable stock solution of $ABTS^{\cdot}$ radical was obtained by mixing 7.8 mM ABTS and 2.45 mM of potassium persulfate at 37°C for 16 h in the dark. The $ABTS^{\cdot}$ radical stock solution was diluted with 80% ethanol until an absorbance of 0.7±0.02 at 734nm was achieved. 40 µL of varying concentrations of sample extracts (3.12-200 µg/mL) were added to 160 µL of diluted $ABTS^{\cdot}$ stock solution. The mixture was incubated at 25±2°C for 10 min and the absorbance was measured at 734 nm using a Biotek microplate reader. The percentage of free radical scavenging activity was calculated as follows:

$$\% \text{ ABTS Scavenging effect} = \frac{Ac - As}{Ac} x100$$

Where Ac = absorbance of control, As = absorbance of sample.

2.8. Enzyme inhibition assays

2.8.1. α-Amylase inhibition assay

The α-Amylase inhibition assay was performed according to the method described by BERNFELD (1955) with modifications in a 96-well microplate. Briefly, 50 µL of varying concentrations (12.5-200 µg/mL) of ethanolic sample extract was added to 40 µL of cooked starch (1% w/v) and 50 µL of α-amylase enzyme (0.5 mg/mL), 860 µL of 100 mM acetate buffer (pH 6.0) was added to the mixture and incubated at 40°C for 15 min. 0.5 mL of DNS (3,5-dinitrosalicyclic acid) was added after incubation and then placed in a boiling water bath for 5 min. It was then cooled in cold-water bath and the absorbance was recorded at 540 nm. The percentage inhibition of each sample was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{Ac - (As - Ab)}{Ac} x100$$

Where Ac = absorbance of control, Ab = absorbance of blank and As = absorbance of sample.

2.8.2. α-Glucosidase inhibition assay

The α-glucosidase inhibition assay was done according to the method described by MATSUI *et al.* (2001) with modifications, in a 96-well microplate. Reaction mixtures of 20 µL of 4 mM of p-nitrophenyl-α-d-glucopyranoside , 30 µL of 50 mU/mL of α-glucosidase enzyme and 40 µL of different concentrations (2.5-80 µg/mL) of sample extracts (1mg of

each of the freeze-dried sample was reconstituted in 1mL of 50mM sodium acetate buffer)were added together to give a volume of 100 μL in 50 mM sodium acetate buffer (pH 5.8). The mixtures were incubated at 37°C for 30 min after which the reaction was stopped by addition of 50 μL of 0.1M sodium carbonate. Absorbance was measured at 405 nm using a 96-well microplate reader. The inhibition of α-glucosidase in percentage was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{Ac - (As - Ab)}{Ac} x100$$

Where Ac = absorbance of control, Ab = absorbance of blank and As = absorbance of sample.

2.9. Statistical Analysis

All measurements were carried out in triplicate data (n=3). All the results were presented in the form of mean±standard deviation (SD). Data was statistically analyzed by one-way analysis of variance (ANOVA) with IBM SPSS software package (version 21.0). When *F* values were significant, mean differences were compared using Duncan's multiple range test at the 5% level of probability.

3. RESULTS AND DISCUSSION

3.1. Total phenolic and flavonoid contents

The results of the total phenolic and flavonoid contents of the extracts of coconut testa and bean seed coats were compared as shown in Table 1. The TPC ranged from 3.46 to 44.61 GAE (mg) per gram of dry sample with the highest phenolic content being displayed by TCO. All tested bean seed coat extracts were significantly ($p<0.05$) different in their TPC values except BB and RB. In the case of TFC, the values were found to range from 2.30 to 67.60 QE (mg) per gram of dry sample with the highest value (67.60 mg QE/g) being displayed by TCO. Among the bean seed coat extracts, RKB showed the highest TPC and TFC values. A number of previous studies discussed about the phenolic and flavonoid contents of beans. According to WU *et al.* (2004), RKB was ranked the first in the USDA's list of top 20 foods with high antioxidant capacity. However, the differences in phenolic contents of beans might also be due to genetic factors, variation between cultivars, and extraction methods (ZHAO et al., 2014) There was hardly any detailed study to compare the TPC and TFC of the TCO, which was higher than those of other extracts namely, RKB, RB, BEP, and BB. Although RAIHANA *et al*. (2015)previously discussed the food values of several fruit seed wastes, TCO was not mentioned despite it being a by-product of coconut processing. According to MARIKKAR and MADURAPPERUMA (2012) there was hardly any data on TPC of TCO but the fatty acid compositions of various oil samples were available. As SENEVIRATNE *et al*. (2009) compared the distribution of the phenolic contents in coconut oil extracted under two different methods, the phenolic content was increased with the addition of TCO into white coconut kernel. In another study, APPAIAH *et al*. (2014) confirmed that the oil extracted from TCO was composed of phenolics, phytosterols, tocopherols, tocotrienols etc. Generally, the outer layers such as seed coats and testa would have polyphenolic compounds due to their protective role in plants. Moreover, dark colours of seed coats were generally found to correlate with higher phenolic contents (KANATT *et al*., 2011).

Table 1. Total phenolic and flavonoid contents and ferric reducing antioxidant power of coconut testa and different bean seed coats.

Type of extract	Total Phenolic content (mgGAE/g)	Total flavonoid content (mgQE/g)	Reducing Power (FRAP) mmol FeSO4/100g
RKB	21.80±0.51b	24.38±1.22b	204.71±2.87b
RB	5.29±0.47c,d	6.29±1.21c	48.45±7.34d
TCO	44.61±0.56a	67.60±6.99a	546.10±36.90a
BEP	3.46±0.25d	2.30±0.38c	26.70±4.43d
BB	9.19±0.53c	7.35±1.30c	68.68±3.30c

Data represented as mean±sd of triplicate analysis. Mean values within the column with different superscript letters are significantly ($p < 0.05$) different. Abbreviations: RKB, red kidney bean seed coat; RB, red bean seed coat; TCO, testa of coconut; BEP, black-eyed pea seed coat; BB, black bean seed coat.

The HPLC- DAD analysis data shown in Table 2 compared the flavonoid and phenolic acid compositions of TCO and bean seed coat extracts. Phenolic compounds are essential constituents of plants due to their ability to scavenge free radicals; they are known for their alleviation of oxidative stress as well as beneficial effects on human health.

Table 2. Phenolics composition of coconut testa and selected beans seed coats by HPLC-DAD analysis[1].

Phenolic compounds (mg/g)	TCO	RKB	RB	BB	BEP
Gallic acid	0.42±0.03	0.01±0.01	0.16±0.02	0.11±0.02	0.04±0.00
Catechin	-	0.18±0.09	-	-	-
Epigallocatechin gallate	5.747±0.54	-	-	-	-
Chlorogenic acid	0.625±0.24	-	-	-	-
Caffeic acid	1.752±0.08	-	-	-	-
Ferulic acid	-	-	-	-	-
Ellagic acid	-	-	0.91±0.03	4.85±0.02	7.25±0.06
Rutin	-	-			1.210±0.01
Quercetin	-	-	0.17±0.00	0.69±0.27	0.05±0.00
Kaempferol	-	-	-	-	-

[1]Abbreviations: See Table 1.

According to IRONDI *et al*. (2014) the most abundant phenolic acid in plants is caffeic acid followed by chlorogenic acid while quercetin and rutin are two important flavonoids in a number of food items. According to Table 2, the extracts contain varied levels of phenolic compounds which include; gallic acid, ferulic acid, chlorogenic acid, ellagic acid, catechin, epicatechin, caffeic acid, quercetin, rutin and kaempferol. The concentrations of the different phenolic compounds ranged from 0.03-0.25 mg/mL for phenolic acids and 0.05-0.45 mg/mL for flavonoids. Gallic acid was the most common phenolic compound present among the extracts with the highest amount observed in TCO. Previously, SENEVIRATNE *et* al. (2009) noticed that coconut oil extracted by incorporating TCO had compounds such as gallic acid, epigallocatechin, catechin, p-hydroxybenzoic acid, epi-catechin and caffeic acid. The level of gallic acid in RB seed coat and RKB seed coat were quite comparable.

Some phenolic compounds such as ellagic acid, caffeic acid and rutin were noticeably higher in certain extracts while phenolic compounds such as ferulic acid and kaempferol were observed to be absent in all extracts.

3.2. Antioxidant activities

A comparison of the FRAP activities of the extracts of TCO and bean seed coats are presented in Table 1. The FRAP values ranged from 26.70 to 546.10 mmol $FeSO_4$/100g extracts with the highest and lowest values being displayed by TCO and BEP, respectively. According to our literature search, only few studies were available to compare the FRAP value of TCO. Previously, SENEVIRATNE *et al*. (2009) reported the phenolic-dependent antioxidant capacities of coconut oil without giving consideration for FRAP activity. The FRAP values of bean seed coats used in this study were found to range from 26.7 to 204.71 mmol $FeSO_4$/100g. The values displayed significant ($p<0.05$) differences among the extracts of RKB, RB, BB, and BEP. Interestingly, bean seed coat extracts of BEP, RKB and BB had displayed better antioxidant capacities than the seed extracts of the same reported previously by MARATHE *et al.* (2011). BOATENG *et al.* (2008) stated that dark coloured seed coats usually had higher FRAP values than the pale coloured ones, which might be due to higher amount of phenolic compounds in them. According to some other reports, high FRAP values of grains such as red bean were due to the high content of condensed tannins (ZOU and CHANG, 2014). The results of DPPH radical scavenging activities of the extracts of TCO and bean seed coats were compared as shown in Fig. 1.

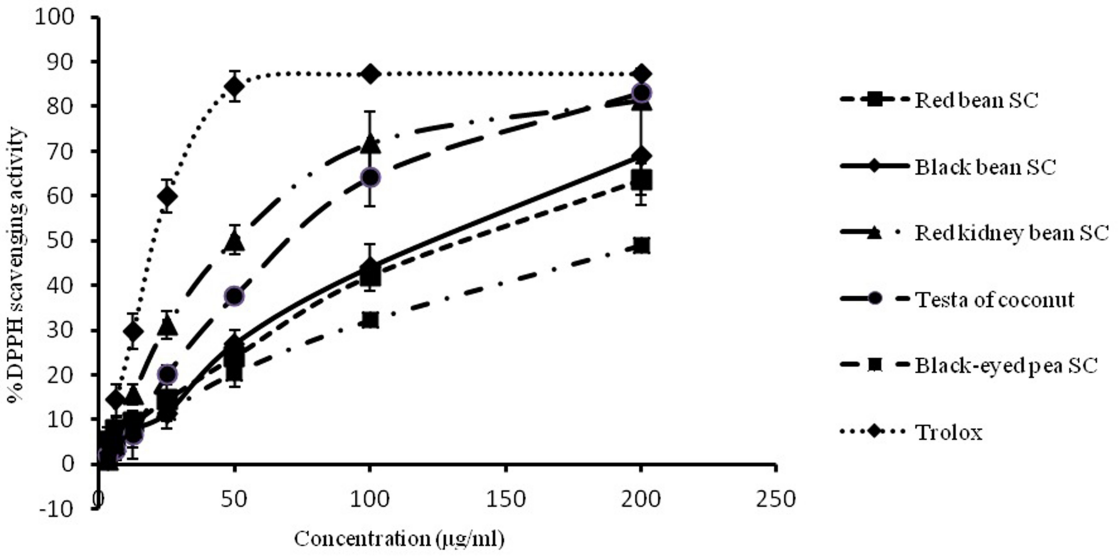

Figure 1. DPPH radical scavenging activities of coconut testa and different bean seed coats. Values represent mean±s.d. of triplicate analysis.

Previously, BOATENG *et al*. (2008) reported the DPPH radical scavenging activities of several common beans, but there was hardly any comparison with TCO. According to Fig. 1, the radical scavenging activities of all extracts were ranged from 48.97 to 83.21% as

observed at 200 μg/mL. The values displayed by TCO and RKB were 83.21% and 81.53%, respectively. The strong radical scavenging potentials of TCO and RKB might be due to their higher phenolic and flavonoid contents as seen before (Table 1).
According to Table 3, there was no significant ($p>0.05$) difference between the EC_{50} values of RB and BB while the EC_{50} of other tested extracts were significantly ($p<0.05$) different with respect to each other.

Table 3. DPPH and ABTS radical scavenging activities of coconut testa and selected bean seed coats.

Type of extract	DPPH EC50 (μg/mL)	ABTS EC50 (μg/mL)
RKB	63.6±3.5c	111.3±0.6c
RB	144.2±6.0b	266.5±36.2b
TCO	47.4±7.0d	125.7±6.7c
BEP	188.2±1.9a	375.7±11.6a
BB	135.4±19.0b	260.5±36.8b
TROLOX	22.4±1.7e	32.0±1.2d

Values represent mean±sd of triplicate analysis. Mean values within the column with different superscript letters are significantly ($p<0.05$) different; Abbreviations: See Table 1.

Previously, GARCIA-LAFUENTE *et al.*(2014) found that the extracts of RB displayed higher radical scavenging activity than those of white ones, which was in agreement with the results obtained in the current study. Separately, HUANG *et al*. (2012) stated that factors such as soil types, genes, temperature, light and agronomic conditions could also affect anthocyanin composition which was an important antioxidant constituent of plants. Seed coats are generally similar to hulls that are rich in polyphenolics and can act as natural antioxidants to counter the formation of free radicals that can lead to the onset of various diseases including diabetes, and cancer (BOATENG *et al*., 2008).
ABTS activities of the extracts of TCO and bean seed coats are shown in Fig. 2.
Both the RKB and TCO displayed maximum scavenging activities of 83.3% and 76.37%, respectively at the concentration of 200 μg/mL. According to Table 1, the phenolic and flavonoid contents of both RKB and TCO extracts were relatively higher than those of any other extract, which might have contributed to their strong antioxidant activity. It has been reported that phenolics with higher molecular weight (e.g. tannins) were greater in ability to scavenge free radicals (SIDDHURAJU and BECKER, 2007) The higher the phenolic content of an extract, the stronger the antioxidant activity that they tend to exhibit (GARCÍA-LAFUENTE *et al.*, 2014). ABTS values of the extracts of the other samples showed significant ($p<0.05$) differences. According to Table 3, there were no ($p>0.05$) significant differences in the EC_{50} values of the RKB and TCO, as well as the EC_{50} values of BB and RB extracts. However, significant ($p<0.05$) differences were observed between the extracts of BEP and other samples. Most of the extracts exhibited strong scavenging ability at all the tested concentrations (3.12 – 200 μg/mL). Extracts of RKB and TCO showed somewhat similar activities.

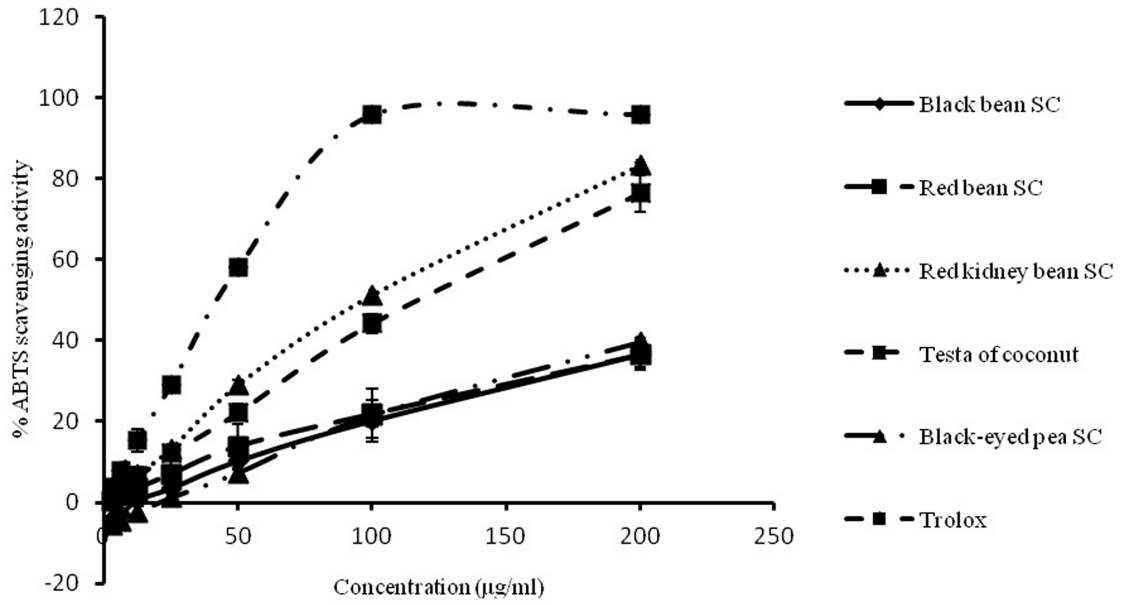

Figure 2. ABTS radical scavenging activities of coconut testa and different bean seed coats. Values represent mean±s.d. of triplicate analysis.

3.3. Enzyme inhibitory activities

3.3.1. α-Amylase inhibitory activity

The α-amylase inhibitory potentials of the extracts of TCO and bean seed coats were compared as shown in Table 4. Inhibition of carbohydrate degrading enzymes is one of the approaches to decrease the rate of glucose released into the bloodstream (ADEMILUYI and OBOH, 2013). MOJICA *et al.*(2015) stated that common beans contain bioactive substances such as phenolics, which help in inhibiting digestive enzymes by binding to these enzymes and modifying their activity. At the highest concentration of 200 μg/mL, the highest activity was displayed by RKB (77.02%) while the least activity was recorded for TCO (24.83%). When the efficacy of various extracts were compared using their IC_{50} values, the values of the test samples were ranged from 120.5 to 612.3 μg/mL while the IC_{50} of acarbose (positive control) was 115.9 µg/mL (Table 4). This indicated that the positive control exhibited stronger inhibition towards α-amylase when compared with the test extracts. Significant ($p<0.05$) differences were noticed among the extracts such as; RB, TCO, BB, and BEP except between RKB and the positive control, which showed no significant difference. In this study, the anti-amylase activity of RKB was significantly higher than that of TCO. Plant-derived phenolic compounds have the potential to inhibit digestive enzymes, thus serving as natural therapeutic agents for the management of diabetes and its associated complications (IRONDI *et al.*, 2014).

3.3.2. α-Glucosidase inhibitory activity

The α-glucosidase inhibitory potentials of extracts of TCO and bean seed coats were compared as shown in Table 4. Reducing the rate at which starch is being broken down and absorbed may be beneficial to manage insulin resistance in diabetic patients (UDDIN *et al.*, 2014). Various extracts showed a dose-dependent increase in their inhibition towards α-glucosidase.

Table 4. α-Amylase and α-Glycosidase inhibitory effects of coconut testa and bean seed coats[1].

Samples	α-Amylase IC50 (μg/mL)	α-Glucosidase IC50 (μg/mL)
RKB	120.5±15.4d	19.9±5. 7c
RB	612.3±113.0a	29.9±1.4b
TCO	532.8±68.0b	4.8±1.4d
BEP	573.6±68.1a,b	32.0±9.5b
BB	319.3±36.0c	14.9±4.2c
Acarbose	115.9±11.0d	43.4±3.7a

[1]Values represent mean±standard deviation of triplicate analysis. Mean values in the same column with different superscript letters are significantly different ($p<0.05$); RKB, red kidney bean seed coat, RB, red bean seed coat, TCO, testa of coconut, BEP, black-eyed pea seed coat, BB, black bean seed coat.

As shown in Table 4, the IC_{50} values of the test extracts in relation α-glucosidase inhibition were ranged from 4.84 to 32.03 μg/mL, with the IC_{50} value of acarbose being significantly ($p<0.05$) higher than those of RB, BB, RKB and BEP. However, IC_{50} values of BB and RKB were not significantly ($p>0.05$) different in the similar way between the IC_{50} values of RB and BEP. Since IC_{50} value of TCO was observed to be significantly lower than those of any other extract, it could be considered as potential alpha-glucosidase inhibitor for the management of diabetes. In fact, its inhibitory potential was even stronger than those of some other legumes reported previously by YAO *et al*. (2013).

4. CONCLUSIONS

In this study, polyphenolic contents, antioxidative properties, and α -amylase and α-glucosidase inhibitory potentials of TCO and selected bean seed coats were compared. Among the different extracts studied, TCO and RKB exhibited better antioxidant properties. This may be partly due to their high phenolic content; thus helping to scavenge free radicals and preventing oxidative damage in biological systems. TCO displayed a mild α-amylase inhibition but a strong α-glucosidase inhibition, which is a key characteristic feature of an effective anti-diabetic agent. Hence, TCO could possibly serve as a base material for preparation of food formulation suitable for diabetic patients.

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