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Polyphenolic Content and Radical Scavenging Activities of the Peel, Pulp and Seed of Avocado (*Persea americana* Mill.) Grown in Tanzania

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Abstract

Avocado is a healthy fruit and the consumption is continuously growing worldwide. The fruit contains polyphenolic compounds with antioxidant effects. Globally, research has been devoted to exploring the fruit quality, especially compounds with antioxidant effects, from different avocado-growing sites. However, the fruit quality of the Tanzanian avocado has so far not been investigated. In this study, the contents of polyphenols in peel, pulp and seed of avocados sampled in south-western Tanzania are described. The levels of total polyphenolic and flavonoid contents were measured, and antioxidant activity was evaluated using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The total polyphenolic content was highest in the seed and lowest in the peel (424 and 200 mg GAE/100 g DW, respectively). As for the total flavonoid content, the pulp had the highest value of 36.98 mg RE/100 g DW, while the seed had the lowest value of 32.54 mg RE/100 g DW. The overall average half maximal effective concentration (EC_{50}) values in decreasing order, corresponding to an increasing antiradical activity, were 4.90 (peel), 3.63 (seed) and 3.24 µg/mL (pulp). The seed and peel possessed high levels of total polyphenolic and flavonoid content, thus demonstrating substantial antioxidant capacity. Seed and peel can potentially be processed and included in the diet to provide inexpensive antioxidant ingredients of natural origin. Consumption of the seed will not only improve human health but also reduce the environmental pollution, as many thousand tonnes of avocado seeds are produced in Tanzania per year; a huge amount currently remains as waste.

Keywords: Flavonoid content; Free radicals; EC_{50}; Polyphenolic content.

Introduction

Free radicals such as reactive nitrogen and oxygen species, continuously generated in human bodies by oxidative stress, damage tissues and their increased concentrations are associated with the development of chronic

illnesses such as cancer, cardiovascular, and neurological diseases (Rahman 2007, de Araújo et al. 2016). They are also associated with endothelial and gastrointestinal dysfunction, atherosclerosis and lung disease (Rozner and Garti 2006, de Araújo et al. 2016). Scavenging of the excess free radicals in human bodies is done by the endogenous antioxidant defence system that employs enzymatic and non-enzymatic antioxidants (Sharma et al. 2012). Such system is sometimes overwhelmed and needs to be assisted by the intake of antioxidants that could be synthetic or naturally occurring in foods. Some food crops have been reported to contain high levels of antioxidants that can prevent excess free radical formation, scavenge them and promote their decomposition, thereby preventing the damages associated with them in human bodies. Due to this, there is a growing interest in researching on such foods and also to develop or process them into natural products (Lobo et al. 2010). The use of plant-derived dietary antioxidants to supplement the endogenous antioxidant defence system seems to be the best option as the synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene have been shown to have harmful effects on the liver and to cause carcinogenesis in animal studies (Botterweck et al. 2000, Saad et al. 2007). The long-term intake of synthetic antioxidants has also been linked with skin allergies, gastrointestinal tract problems and increased risks of cancer (Botterweck et al. 2000, Engin et al. 2011).

Avocado, an evergreen tree in the laurel (Lauraceae) family, is widely grown in tropical and subtropical countries due to its edible fruits with outstanding nutritional and health properties (Wang et al. 2010, Alkhalaf et al. 2019). Avocado contains polyphenolic compounds that have antioxidant effects and thus are capable of preventing or delaying the development of degenerative diseases (Alkhalaf et al. 2019). Due to its nutritive values and health benefits, avocado is included in a variety of dishes, from breakfast, through brunch, lunch and dinner, to supper. As a result, the avocado market is rapidly growing globally and was projected to have an annual growth rate of 5.7% between 2020 and 2025, corresponding to a rise from US$ 12.8 billion in 2019 to US$ 17.9 billion by 2025 (Research and Markets 2020).

Due to the health benefits of the avocado fruit, a number of investigations have been carried out to reveal its antioxidant characteristics, in *inter alia*, Colombia (Rosero et al. 2019), Egypt (Alkhalaf et al. 2019), China (Ge et al. 2017) and Japan (Prabath-Pathirana et al. 2013). However, no such study has been carried out on Tanzanian avocados. Thus, this study was undertaken to explore the levels of polyphenol compounds in the peel, pulp and seed of avocado fruits sampled from three major avocado producing regions in south-western Tanzania. The study not only unveils the fruit quality of the Tanzanian avocados with regard to antioxidant characteristics, but also pioneers research on the quality evaluations of avocado in the country.

Materials and Methods

Study area and sampling

The study involved avocado fruits from four districts, namely, Mbozi, Mbeya city, Rungwe and Njombe rural located in the Songwe (for Mbozi), Mbeya (for Mbeya city and Rungwe) and Njombe (for Njombe rural) regions in south-western Tanzania (Figure 1). Sampling of the local avocado fruits was undertaken in August 2018.

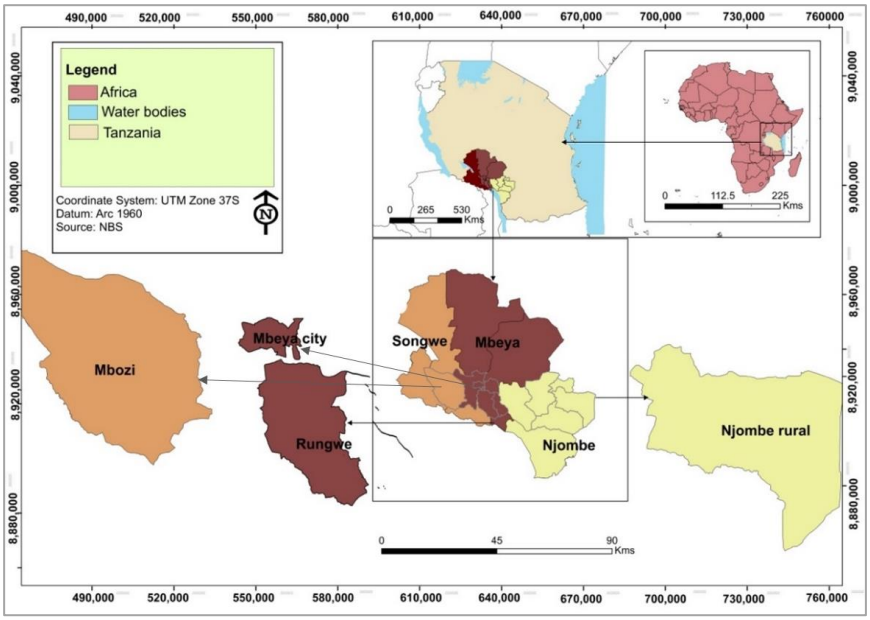

Figure 1: Location map showing study sites (1) Top right: Location of Tanzania in Africa (2) Top middle: Location of the three regions in Tanzania (3) Bottom middle: locations of the four districts where sampling was carried out.

Sampling of the fruits was done from a total of 30 trees in the three regions: Njombe (12 trees), Mbeya (14 trees) and Songwe (4 trees). Three to four healthy mature fruits from different parts of a tree were sampled, labelled and then kept in a black plastic box with lid at room temperature. In the evenings, the fruits were unpacked and then kept on a cold concrete floor to delay ripening. After every 6 days of collection, the obtained fruits were transported in perforated plastic buckets to the Department of Botany, University of Dar es Salaam, Tanzania for laboratory analysis.

Sample preparation

At the Department of Botany, University of Dar es Salaam, the fruits were thoroughly washed with water and then kept at room temperature until ripen. Among the fruits sampled per tree, two fruits that showed optimal ripeness for consumption but no visible damages were selected. Thereafter, the peel, pulp and seed of each of the selected fruits were manually separated, covered with aluminium foil, and stored at −20 °C until extraction.

Extract preparation

The preparation of avocado extracts was done following the method described by Velioglu et al. (1998) with some modifications. About 5 g of each sample (peel, pulp, and seed) was ground using a mortar and pestle, followed by adding 200 ml of 95% ethanol. The ground samples were then agitated in the dark for four hours at 200 rpm, at room temperature, using an orbital shaker (Heidolph Unimax 1010, Shcwabach, Germany). Thereafter, each sample was filtered through a Whatman No. 4 filter paper and then centrifuged at 1500 × g for 15 min. Finally, ethanol was evaporated from the sample by using a rotary evaporator at 40 °C.

Determination of total polyphenolic and flavonoid contents

Determination of the total polyphenolic contents of avocado peel, pulp and seed was done using the Folin-Ciocalteau reagent according to the method described by Kähkönen et al. (1999). Estimation of the total polyphenolic content was performed using the calibration curve for gallic acid, and expressed as mg gallic acid equivalent (GAE)

per 100 g of the dried avocado sample (mg GAE/100 g DW). Assessment of the total flavonoid content was done by a colorimetric assay following the method described by Serra Bonvehí et al. (2001). Rutin, a flavonoid and natural antioxidant, was used as standard and the results were expressed in mg rutin equivalent (RE) per 100 g of the dried avocado sample (mg RE/100 g DW).

DPPH radical scavenging capacity

The free radical-scavenging activities of the avocado peel, pulp and seed extracts were investigated using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical approach, following the method of Masuda et al. (1999) with some modifications as described in Tachibana et al. (2001). Avocado ethanolic extract solutions of the peel, pulp and seed at five different concentrations, i.e., 1, 0.1, 0.001, 0.0001 and 0.00001 mg/mL (extract/ethanol) were prepared. 100 µl of 5 mM DPPH in ethanol was added to 4.9 ml of diluted avocado extract and thoroughly mixed. The avocado extract-DPPH mixtures were incubated in the dark at 37 °C for 30 min and thereafter, the absorbance of each sample was measured spectrophotometrically at 517 nm. The absorbance was measured also for each avocado extract dilution without the addition of DPPH (A_s), and for the DPPH solution without the addition of avocado extract (A_o). The DPPH radical-scavenging activity of each avocado extract was calculated as:

$$DPPH\ radical\ scavenging\ activity\ (\%) = 100[A_0 - (A_1 - A_s)]/A_0$$

where A_0 = absorbance of the control solution containing only DPPH; A_1 = absorbance of the avocado extract solution with added DPPH, and A_s = the absorbance of the avocado extract solution without DPPH.

Data analysis

Total polyphenolic and flavonoid content

The R software version 3.6.3 (R Core Team 2020) was used to reveal differences in total polyphenolic and flavonoid contents between the samples and/or between the regions. Three technical replicates were summarized into a mean value, and a plot of mean values of phenolic content versus mean values of flavonoid content was made to see if they were correlated, and if there were differences between parts of the fruit. To analyse this further, the mean value of the two fruits per tree was calculated and inserted in a model with split-plot design without blocks, and with different number of replicates in the different regions. The main effect was the region, and for each observation in a region we had a factor with three levels as split-plot effect; peel, pulp and seed. Shapiro's test was used to confirm that the assumption of normality in these data was met. Calculations of denominator degrees-of-freedom were done with the Kenward-Roger method, using the package lmerTest (Kuznetsova et al. 2017) and the Tukey's method was used for pairwise comparisons. The level of significance used was 0.05. The results were averaged at the levels of region and sample.

DPPH radical scavenging activity

The DPPH radical scavenging data was used to find EC_{50} based on a logistic function for the observations and compare different parts of the fruit and different regions. The logarithm values of the doses were fitted to the curve, and we used the logarithm with base 10. The EC_{50} on the log-scale was calculated for each single observation using the logistic model. We also calculated EC_{50} on the original scale, the slope of the curve and the standard error of EC_{50} on the log-scale. The calculations were made in the R software using a non-linear regression (nls2) (Grothendieck 2013). The presence of outliers in the estimate of EC_{50} was checked. Analysis of variance (type III analysis of variance with package lmerTest in R) was used to study differences in the mean EC_{50} values of avocado extract from different fruit parts (peel, pulp and seed) and regions. Denominator degrees of freedom were calculated with the Kenward-Roger method, and the Tukey's method was used for pairwise comparisons with 0.05 as the level of significance. The results were averaged at the levels of region and sample. Correlations between total polyphenolic content, total

flavonoid content and antiradical activity defined as the reciprocal of EC_{50} were also computed using the R software.

Results and Discussion

Total polyphenolic and flavonoid contents

Total polyphenolic and flavonoid contents were measured in three technical replicates for each fruit part (peel, pulp and seed). Generally, the mean value of the total polyphenolic content was lowest in the peel (200 mg GAE/100 g DW) and highest in the seed (424 mg GAE/100 g DW; Table 1). The polyphenolic level in the seed was significantly different ($p < 0.05$) from those measured in the pulp and peel. Polyphenolic compounds are acknowledged as ameliorating oxidative damage and the risk of chronic illnesses due to their ability to reduce free radical generation, and also by scavenging them (González et al. 2010).

In the peel, the mean total polyphenolic contents ranged from 184.0 ± 26.2 (Mbeya) to 225.2 ± 28.3 mg GAE/100 g DW (Njombe; Table 1). The total polyphenolic contents in the pulp were lowest (184.8 ± 49.1 mg GAE/100 g DW) and highest (292.1 ± 28.3 mg GAE/100 g DW) in the Songwe and Njombe avocados, respectively. The highest and lowest total polyphenolic contents in the seed were observed in the Songwe (452.0 ± 49.1 mg GAE/100 g DW) and Mbeya avocados (390.1 ± 26.2 mg GAE/100 g DW), respectively. However, all these comparisons between regions were not significantly different ($p > 0.05$). The total polyphenolic contents of avocado peel in our study were within the range of 21.74 to 1,252.31 mg GAE/100 g DW reported by Morais et al. (2015) for the raw freeze-dried and oven-dried peels. In their study, they obtained the lowest value in freeze-dried peel samples suggesting that freeze-drying decreases the total polyphenolic content.

For the seed, the reported values of total polyphenolic contents of other studies ranged from 30.98 (Segovia et al. 2018) to 29,200 mg GAE/100 g DW (Pahua-Ramos et al. 2012). The highest value was obtained for seed flour extracted using methanol/water (75:25 v/v). López-Cobo et al. (2016) evaluated the quantity and types of polyphenolic compounds in avocado peel, pulp and seed, when the fruit was at optimal ripeness for consumption and when it was overripe. They found that the concentrations of the identified polyphenolic compounds were higher in the pulp and seed of overripe fruit than in the optimally ripe fruit, perhaps due to the effect of phenylalanine ammonia-lyase (PAL), which might increase its activity as fruits get ripen. Contrary to that, they found that the levels of polyphenolic compounds were lower in the peel of overripe fruits than in the peel of optimally ripe fruits.

Table 1: Total polyphenolic contents in ethanolic extracts from peel, pulp and seed of avocado. [z] Values are presented as mean ± standard error of the model*, and expressed in mg GAE/100 g DW

Region	Peel (n = 60)	Pulp (n = 60)	Seed (n = 60)
Mbeya	$184.0 \pm 26.2^{A,n}$	$257.9 \pm 26.2^{A,n}$	$390.1 \pm 26.2^{A,m}$
Songwe	$189.3 \pm 49.1^{A,n}$	$184.8 \pm 49.1^{A,n}$	$452.0 \pm 49.1^{A,m}$
Njombe	$225.2 \pm 28.3^{A,n}$	$292.1 \pm 28.3^{A,n}$	$429.0 \pm 28.3^{A,m}$
Grand mean	200^{n}	245^{n}	424^{m}

[z] Numbers sharing the same capital letter in the same column are not significantly different at $p = 0.05$, and in this case there are no significant differences. Numbers sharing the same small letter within a row are not significantly different at $p = 0.05$.
*The model produces the same standard error for some of the mean values.

Rodríguez-Carpena et al. (2011) found lower average polyphenolic levels in the avocado pulp extracts (76 to 100 mg GAE/100 g DW) prepared using ethyl acetate, acetone, and methanol. They found, however, higher average polyphenolic levels in the peel (3293–8797 mg GAE/100 g DW) and seed (1699–6082 mg GAE/100 g DW)

compared to the corresponding values in the present study. The significantly higher polyphenolic contents in the seed compared to the pulp recorded in our study concur with the findings by Ge et al. (2017), Wang et al. (2010) and Kosińska et al. (2012). The total polyphenolic levels observed in the present study are lower than the levels reported by Peschel et al. (2006) for ethanolic extracts of apple, pear and strawberry residues from juice production; i.e., 4156, 1209 and 3874 mg GAE/100 g DW, respectively. Likewise, the total polyphenolic levels recorded in the present study are lower than those reported by Huang et al. (2012) for methanolic extract of blackberry (558 mg GAE/100 g DW) and blueberry (944 mg GAE/100 g DW). However, the total polyphenolic levels recorded in the present study are higher than that reported by Huang et al. (2012) for methanolic extract of strawberry (272 mg GAE/100 g DW).

Flavonoids isolated from avocado extracts display a wide range of antioxidant characteristics. Quercitrin, a flavonoid extracted from avocado leaf and seed, has been shown to inhibit virus cell entry or replication by inhibiting formation of HIV syncytium and viral p24 antigen (Wigg et al. 1996). Afzelin and quercetin 3-*O*-D-arabinopyranoside, flavonoids isolated from avocado leaf, have been shown to inhibit herpes simplex virus type I (HSV-1) (Rodriguez-Saona et al. 1998). In the present work, the total flavonoid contents ranged from 32.54 (in the seed) to 36.98 mg RE/100 g DW (in the pulp; Table 2) although the difference was not statistically significant.

The peel flavonoid levels ranged from 30.21 ± 3.17 (Njombe) to 39.65 ± 3.05 mg RE/100 g DW (Mbeya), although they were not significantly different at $p = 0.05$. The flavonoid levels in the pulp were also highest in Mbeya (44.83 ± 3.05 mg RE/100 g DW) and lowest in Songwe (32.69 ± 5.50 mg RE/100 g DW) and differed significantly between regions. In the seed, the minimum and maximum flavonoid contents were recorded for the Mbeya (25.05 ± 3.05 mg RE/100g DW) and Songwe (43.01 ± 5.50 mg RE/100 g DW) avocados, respectively, and they differed significantly with regions. Available results from other studies on total flavonoids in pulp measured with the rutin standard ranged from 43.85 (Ge et al. 2017) to 913 mg RE/100 g FW (Ikpeme et al. 2014). Contrary to our findings, Ge et al. (2017) reported significantly higher flavonoid contents in the seed (936.60 to 1636.25 mg RE/100 g FW) than in the pulp. The flavonoid compounds isolated from the seed have been found to exhibit strong *in vitro* antioxidant activity and antimicrobial potentials (Rodríguez-Carpena et al. 2011, Kosińska et al. 2012). Such finding suggests that avocado seed could be used as an inexpensive raw material for the production of functional food or as an antioxidant additive (Rodríguez-Carpena et al. 2011, Kosińska et al. 2012).

Table 2: Total flavonoid contents in ethanolic extracts from seed, pulp and peel of avocado. [z] Values are presented as mean ± standard error of the model*, and expressed in mg RE/100 g DW

Region	Peel (n = 60)	Pulp (n = 60)	Seed (n = 60)
Mbeya	$39.65 \pm 3.05^{A,n}$	$44.83 \pm 3.05^{A,n}$	$25.05 \pm 3.05^{B,n}$
Songwe	$38.32 \pm 5.50^{A,n}$	$32.69 \pm 5.50^{AB,n}$	$43.01 \pm 5.50^{A,n}$
Njombe	$30.21 \pm 3.17^{A,n}$	$33.43 \pm 3.17^{B,n}$	$29.58 \pm 3.17^{AB,n}$
Grand mean	36.06^{n}	36.98^{n}	32.54^{n}

[z]Numbers sharing the same capital letter within a column are not significantly different at $p = 0.05$. Numbers sharing the same small letter within a row are not significantly different at $p = 0.05$.

*The model produces the same standard error for some of the mean values.

DPPH radical scavenging activity

Scavenging of the DPPH free radicals is a broadly used approach in evaluation of the antioxidant activities of plant extracts due to its simplicity, quickness, sensitivity and reproducibility (Villaño et al. 2007). A freshly prepared DPPH solution is deep purple with an absorption maximum at 515 nm. When an antioxidant is added, the purple colour fades away (Matthäus et al. 2002). Antioxidant molecules neutralize the DPPH free radicals resulting in a formation of a colourless product 2,2-diphenyl-1-hydrazine or its substitute, and consequently a decrease in absorbance at 515 nm (Matthäus et al. 2002). In this assay, the EC_{50} is widely used for assessment of the antioxidant activity of test samples, and is calculated as the concentration of antioxidants needed to decrease the initial DPPH concentration by 50% (Matthäus et al. 2002). Thus, the lower EC_{50} value the higher antioxidant activity of the test samples.

When analysing the antioxidant characteristics of the avocado samples, our first calculations were made based on one curve per observation, where the EC_{50} was computed on the log10-scale (an example is shown in Figure 2).

Analysis of variance showed that the EC_{50} was generally highest for the peel (4.90 µg/mL) and significantly different from that of the pulp (3.24 µg/mL) ($p < 0.05$, Table 3). In the Mbeya and Njombe samples, the seed displayed the lowest EC_{50} value, i.e., 3.43 and 2.19 µg/mL, respectively, and the pulp recorded the lowest EC_{50} value (2.45 µg/mL) in the Songwe samples.

The EC_{50} values recorded for samples in the present study were lower than the values reported by Rivero-Cruz et al. (2020) for ethanolic extracts of ascorbic acid (43.2 µg/mL), quercetin (9.9 µg/mL) and Trolox (6.3 µg/mL), suggesting a higher antiradical activity of the avocado extracts. Similarly, higher EC_{50} values and hence lower antioxidant activity have been reported for ethanolic extract of blueberry (420 µg/mL), blackberry (440 µg/mL), and strawberry (810 µg/mL) (Huang et al. 2012).

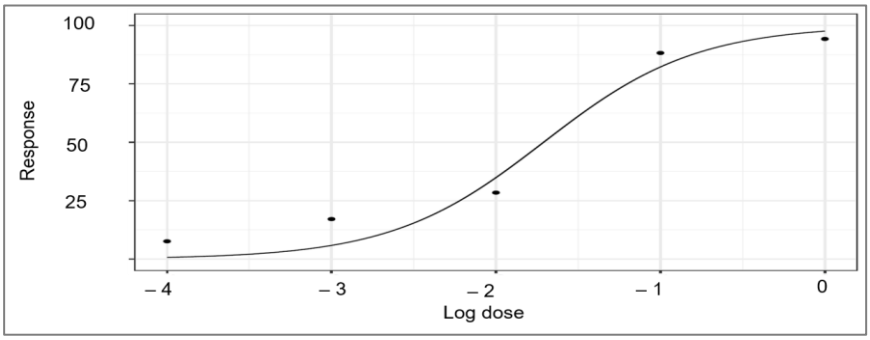

Figure 2: Estimation of the EC_{50} in the peel, pulp and seed of avocado using a logistic model, here illustrated with a logistic curve showing estimation of the EC_{50} of one observation of peel from Songwe.

Table 3: Radical scavenging activity (EC_{50} in µg/mL) of ethanolic extracts from peel, pulp and seed of avocado [z]

Region	Peel	Pulp	Seed
Mbeya	$4.54^{AB,m}$	$5.10^{A,m}$	$3.43^{A,m}$
Songwe	$7.44^{A,m}$	$2.45^{B,n}$	$4.78^{A,mn}$
Njombe	$3.12^{B,m}$	$2.58^{B,m}$	$2.19^{A,m}$
Grand mean	4.90^{m}	3.24^{n}	3.63^{mn}

[z] Numbers sharing the same capital letter within a column are not significantly different at $p = 0.05$. Numbers sharing a small letter within a row are not significantly different at $p = 0.05$.

Analysis of variance showed that there were significant differences between the EC_{50} values for different regions (p = 0.01) and fruit part (p = 0.04) but not with the interaction of region and sample (p = 0.09). Unlike the present study which recorded the highest antiradical activity values in the pulp, Alkhalaf et al. (2019) reported a higher antioxidant capacity in the seed than in the pulp. Similar findings were reported by Gómez et al. (2014), who observed that ethanolic and water extracts of avocado seed had significantly higher antioxidant capacity than that of pulp.

In the present study, there were no significant correlations between total polyphenolic content, total flavonoid content and antioxidant activity (antiradical activity, $1/EC_{50}$). Although a correlation between polyphenolic content and antioxidant capacity was noted in Cai et al. (2004), other studies supported the findings of the present study. For example, Bajpai et al. (2005) did not observe any correlation between total polyphenolic content and antioxidant activity when analysing twenty one species of edible legume/cereals and nineteen species of medicinal plants. Likewise, Sengul et al. (2009) observed no correlation between total polyphenolic content and antioxidant activity when analysing eight species of medicinal plants. Similarly, Juma et al. (2016) did not find any correlation between total polyphenolic content and antioxidant activity of different species of edible mushrooms. The possible reason for the lack of significant correlation between total polyphenolic content, total flavonoid content and antioxidant capacity in the present study could be that there are other non-phenolic/non-flavonoid compounds that contribute to radical scavenging.

Conclusion

This study has demonstrated that avocado seed and peel do possess antioxidant characteristics comparable to what can be found in the pulp. Moreover, the seed has higher levels of total polyphenolics than the pulp. This calls for the transformation of a popular habit of discarding avocado seed and peel and instead trying to find a proper way of processing them and to include them as derived/natural products in human diets.

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Conflicts of Interest

The authors declare no conflict of interest.

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