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Comparative analysis of the chemical compositions of indigenous watermelon (*Citrullus lanatus*) seeds from two districts in Limpopo Province, South Africa

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Seeds from different indigenous watermelons were analysed for their chemical composition such as phenolics, proteins, oils, minerals, ash and fibre content as well as antioxidant activity measured in methanol extract using 2.2-diphenyl-1-picrylhydrazyl (DPPH) assay. The morphological variations of watermelons, which revealed diversity in terms of fruit and seed colour were also assessed. Average percentages for lipid content, total proteins, crude fibre, ash and total carbohydrates as 34.4, 16.5, 23.1, 3.99, and 3.16%, respectively, were recorded for Capricorn district and 31.6, 14.9, 22.0, 4.58, and 5.26% were recorded for Sekhukhune landraces. The Capricorn landraces had higher content of flavonoids (0.222 mg/g) than Sekhukhune varieties (0.130 mg/g). But, landraces from Sekhukhune had higher antioxidant activity (46.5%) which corresponded to higher total phenolic content of 0.59 mg/g. Thus, suggest that watermelon seeds may serve as potential source of natural products for food, feed and pharmaceutical applications.

Key words: Amino acids, *Citrullus lanatus*, flavonoids, phenolics, seeds, watermelon.

INTRODUCTION

Citrullus lanatus (watermelon) belongs to the family Cucurbitaceae, also referred to as cucurbits from a large group with approximately 800 species and 130 genera (Najafi et al., 2010). This family includes species like pumpkins, melons, gourds and squashes, that are cultivated worldwide for medicinal and consumption purposes. Plant breeding programmes in watermelons have been essential to develop new varieties of high quality fruit traits, productivity and resistance to biotic and abiotic stress factors. Watermelons have been found to be nutritionally rich due to the ß-carotene and vitamins B, C and E and demonstrated to contain significantly large amounts of lycopene (Choudhury et al., 2015). The phytochemical compounds found in watermelons such as phenolics, tannnins, flavonoids and carotenoids, particularly in seeds have gained interest among

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consumers and scientists (Devasagayam et al., 2004; Addo et al., 2018). Watermelons, including plants such as gooseberries (Chiang et al., 2013), proved to contain large amounts of these phytochemicals and therefore, a good source of antioxidants. Red-flashed genotypes of watermelons were also reported by Choudhury et al. (2015) to contain variably high amounts and different phytochemicals and antioxidants. Among the major sources of phytochemicals, green leafy vegetables and grains are also rich sources of antioxidants apart from being high in energy, protein, essential oils and micronutrients. Furthermore, reports by Oyeleke et al. (2012) and Rahman et al. (2013) indicated that watermelons offer important nutritional and health-promoting compounds, like lycopene. Lycopene, predominantly found in red watermelon and tomato is a carotenoid with strong antioxidant capacity and potential health benefits (Edwards et al., 2003). Watermelon seeds contain high amounts of fatty acids, proteins and vitamins including copper and zinc. Mariod et al. (2009) suggested that watermelons have potential economic benefits especially due to chemicals contained in their seeds. The seeds can be used to prepare a number of consumables including snacks, flour and sauces (Tabiri et al., 2016). However, seeds are often discarded while only the fruit is eaten. The excellent nutritional properties in watermelon seeds have been reported (Badlfu, 1991). In this study, chemical compositions and antioxidant properties in seeds of watermelon fruits collected from two regions (Capricorn and Sekhukhune district) in the Limpopo province, South Africa are reported.

MATERIALS AND METHODS

Plant

C. lanatus landrace fruits used in this study were collected from Sekhukhune (01SDPW, 02SDPW, 03SDPW, and 05SDPW) and Capricorn (06CDGM, 07CDGM, 08CDGM, 09CDGM and 10CDGM) districts during harvesting period in autumn. The districts are situated in the centre of Limpopo province, classified as semi-arid areas, characterised by summer rainfalls and susceptible to frequent droughts. A total of 12 ripe watermelon fruits were randomly collected per landrace, properly washed and sliced to remove the seeds. The seeds were rinsed with dH_2O and air dried by spreading on clean paper towel in an aerated room for 12 h under room temperature. Seeds were randomly separated into 100 seeds samples, weighed and stored under dry conditions until they were analysed.

Seed oil extraction

For seed oil analysis, 10 g of ground seed powder was extracted using n-hexane in a Soxhlet apparatus for 24 h. Crude oil was recovered by evaporating the solvent under a draught of air. Determination of saponification and iodine values was performed according to IAFMM (1981) and George et al. (2013). The values were calculated using the formula: $56.1 (V_2 - V_1) / W$, where V_2 is the volume of HCl used in blank, V_1 is the volume of HCl used in sample and W is the weight of oil.

Estimation of seed protein by Bradford method

Crude proteins and amino acids were analysed as described by the American Association of Chemists (AOAC, 2006). Total proteins were determined from seed powder by colorimetric assay using bovine serum albumin (BSA) as a standard. A solution of 50 µl protein extract was mixed with 5 mL Bradford reagent and absorbance read at 595 nm. Extracts were spotted on $60F_{254}$ TLC plates and developed in 1-butanol-glacial acetic acid-water (4:1:1). TLC plates were sprayed with ninhydrin solution and dried in an oven at 110°C to visualise the spots.

Quantification of phenolics and flavonoids

Watermelon seed powders, 2.0 g, were quantified for total phenolics. Phenols were determined using Folin-Ciocalteu protocol as described by Torres et al. (1987). The analyses were done in triplicates using gallic acid as a standard. The amount of total phenolics was calculated in mg gallic acid equivalents/g dry seed material. For the determination of flavonoids, 500 µl of phenolic extracts was analysed using a protocol by Torres et al. (1987). Total flavonoids were determined as catechin equivalent from the calibration curve of catechin standard solutions and expressed as mg catechin equivalents/g seed material.

Total potential antioxidant activity

The antioxidant activity on watermelon seed extracts was based on their scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals according to a modified assay by Odhav et al. (2007). To determine the time course of antioxidant activity, phenolic extracts were mixed with 200 µM DPPH and allowed to stand for 10 min before absorbance was read at 517 nm. An absorbance versus time graph was plotted to determine the time course of inhibition.

Ash, crude fibre and carbohydrate analysis

Ash content was determined by heating 5.0 g seed powder in porcelain crucibles under a muffle furnace at 500°C for 2 h. The weight of the residual ash content was then calculated as percentage ash content: Ash content (%) = weight of ash / weight of original sample × 100%. Crude fibre was determined by boiling defatted seed powder in 1.3% aqueous sulphuric acid (H_2SO_4) and boiled for 30 min. The filtrate obtained was boiled in 2.5% sodium hydroxide (NaOH) for 30 min. The solution was filtered and residues transferred into a crucible and dried at 105°C to a constant weight. The crude fibre content percentage was calculated as: Crude fibre (%) = weight of dry residue - weight ash / weight of defatted seed material × 100%. The carbohydrate content was determined using the method described by Striegel and Hill (1996) using a portable refractometer REF113 Brix 0-32 ATC for the sugars.

Mineral analysis

The mineral elements, comprising of calcium, magnesium, sulphur, iron, zinc and copper were quantified according to Inuwa et al. (2011). The mineral solution obtained from ashed materials was

Table 1. Fruit and seed physical characteristics of the nine watermelons.

Landrace	Mass per 100 seeds (g)	Colour of fruit	Seed colour
01SDPW	8.5±0.141[g]	Green with cream stripes	Black with brown speckles
02SDPW	8.35±0.778[h]	Green	Black with brown edges
03SDPW	8.55±0.495[f]	Green	Brown with cream stripes
05SDPW	9.25±0.495[d]	Greenish-Grey	Light brown
06CDGM	10.95±0.071[a]	Green	Black with brown edges
07CDGM	8.05±0.212[i]	Green with cream stripes	Black with brown edges
08CDGM	10.55±0.919[b]	Greenish-Grey	Light brown with black speckles
09CDGM	9.35±0.636[c]	Greenish-Grey	Light brown with black tips
10CDGM	8.7±0.141[e]	Green	Black with brown edges

Values are means with standard deviation of three measurements. Values followed by the different letters are significantly different at $p \leq 0.05$ confidence level.

Table 2. Phytochemical characteristics of crude oil of the watermelon seed oils.

Landrace	Oil yield (g)	Saponification value	Iodine value	Oil (%)	Seed oil colour
01SDPW	3.00±0.14[e]	132.40±0.793[e]	136.798±0.449[f]	30.0±0.14[a]	Orange
02SDPW	3.25±0.21[c]	184.57±2.380[c]	134.006±0.269[a]	32.5±0.21[b]	Golden Yellow
03SDPW	3.25±0.07[c]	143.06±1.587[c]	135.085±1.077[e]	32.5±0.07[a]	Orange
05SDPW	3.15±0.35[d]	148.95±0.397[d]	137.687±0.269[d]	31.5±0.35[a]	Orange
06CDGM	3.55±0.07[a]	154.84±0.793[a]	135.149±0.628[c]	35.5±0.07[a]	Golden Yellow
07CDGM	3.00±0.00[e]	135.20±3.967[e]	134.704±0.359[f]	30.0±0.00[b]	Orange
08CDGM	3.35±0.21[b]	161.29±1.190[b]	138.194±0.090[b]	33.5±0.21[a]	Golden Yellow
09CDGM	3.15±0.49[d]	145.30±2.380[d]	138.575±0.269[a]	31.5±0.49[a]	Pale Yellow
10CDGM	4.15±0.35[d]	145.02±3.570[d]	136.862±0.538[d]	41.5±0.35[a]	Orange

Oil yield was expressed as g/10 g sample. Saponification value was measured in mg KOH/g oil, and Iodine value in grams of I_2/100 g oil. Values with same superscript letters are not significantly different within the column and ± values are standard deviations of triplicates.

filtrated and used to determine trace elements (iron, zinc and copper) using Varian Spectra AA atomic absorption spectrometer. Sulphur was determined gravimetrically as barium sulphate ($BaSO_4$) (Mendham et al., 2000). Calcium-magnesium complex was determined by complexiometric titrimetric analysis (Mendham et al., 2000). Crystals of eriochrome black T were used as indicator with the end point being a colour change from wine red to clear blue.

Statistical analysis

Analysis of variance (ANOVA) was done using SPSS version 22 (IBM SPSS Statistics). Results reported in the tables were obtained from three replicates (n=3) and are reported as means ± standard deviation of the mean. Data was used to determine the significance level at p value<0.05.

RESULTS AND DISCUSSION

The results indicate that, Capricorn and Sekhukhune watermelons differed significantly in their morphology and chemical compositions. All fruits were globular with shallow grooves and the skins appeared light to deep green with creamy stripes in colour. The seeds were small, black in colour with crown stripes and brown with creamy white stripes, for the Capricorn and Sekhukhune districts respectively. These characteristics which are typical of the Cucurbitaceae may be due to the genotypes and environmental conditions. The difference in seed mass observed in 06CDGM (10.95 g) was ascribed to bigger seed size and thus demonstrating higher amounts of phytochemicals probably contained within the seeds (Tables 1 and 2). Mariod et al. (2009) reported a positive correlation between seed size and amounts of oils, proteins, carbohydrates and fibre found in the seeds.

Ash, fibre and carbohydrate proximate composition

As shown in Figure 1, the highest estimated percentage compositions for crude fibre, ash and carbohydrate

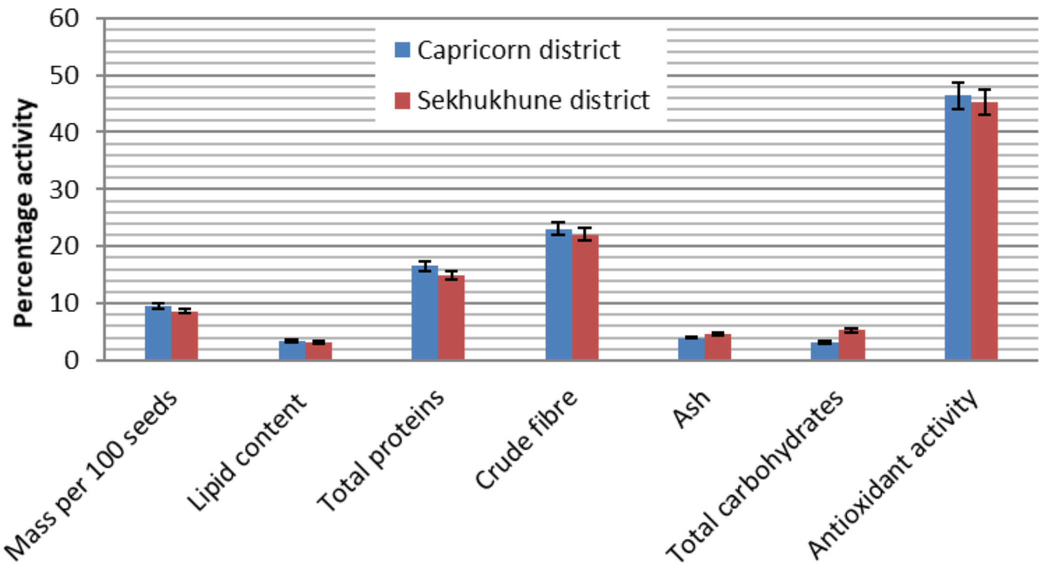

Figure 1. Quantification of the antioxidant activity and nutrient composition of watermelon landraces collected from two districts in Limpopo.

content were 23.1, 4.58 and 5.26%, respectively. Capricorn landraces exhibited high crude fibre (23.1%) than Sekhukhune varieties which had high ash (4.58%) and sugar (5.26%) content. The sugar content obtained in this study was found to be very low as compared to 22.9 and 26.6% reported for other watermelons including *Cucumis melo* (Mehra *et al*. 2015). Ash serves as inorganic content from which mineral elements are obtained. As expected, landraces from Sekhukhune were found to contain higher Fe, Zn, S, Ca and Mg. These findings are favourably comparable with observations made by Tabiri et al. (2016) even though their ash percentages were relatively low (2.0 to 3.0%). Landraces differed in the amount of copper, sulphate and iron. Furthermore, the high ash and fibre indicate the potential to provide higher amounts of minerals. Further research could lead to use of these seeds as good source of essential nutrients used as supplements in manufacturing of foods and feed products.

Quantity and physical characteristics of crude oils

Landraces collected from Capricorn district contained high amounts of oil (34.4%) than Sekhukhune varieties (31.6%) (Table 2). The oil content in the samples significantly differed ($p \leq 0.05$) among all the landraces. Variety 10CDGM was the highest with 4.15 g, followed by 06CDGM with 3.55 g. The lowest yields obtained from 01SDPW and 07CDGM both recorded 3.00 g oil. The colour of oil obtained in 10CDGM was orange (Table 2) showing impurities as reported by Raziq et al. (2012). Light yellow oil is considerable and the physicochemical analysis indicated commensurate saponification and iodine values for other similar oilseed such as pumpkin and *Cucumis* oil (Ibeto et al., 2012).

The lighter the oil, the more valuable and acceptable the oil is as edible vegetable oil. However, the variety with the highest oil mass did not exhibit the highest saponification and iodine values. The saponification value was lower as compared to other oilseeds such as soybean (191 mg KOH/g oil) and sunflower (194 mg KOH/g oil) (Baboli and Kordi, 2010). Generally, watermelons collected from Sekhukhune had higher saponification value but lower iodine value. Such indications imply that the seed oil may be low affecting antioxidants, thus reduced potential use for industrial and medicinal purposes. This variation in the seed oils may have been attributed to environmental factors as indicated by Maranz and Weisman (2003) and Di-Vincenzo et al. (2005).

Protein and amino acid composition

The amount of crude protein content obtained was between 14.1 and 20.4%. Capricorn landraces had 16.5% protein content as compared to samples from Sekhukhune district (14.9%). These amounts were very low when compared with 14.5 to 33.3% reported by Karanja et al. (2013) on other *Cucurbita* species. Furthermore, observations compared poorly with seeds of cashew nuts (22.8%), *Cucumis sativus* (26.7%), and *Cucurbita pepo* (27.5%) reported by Karanja et al. (2013).

The amounts of amino acids varied significantly and leucine was found to be the most abundant amino acid. In comparison, Usman et al. (2010) reported the prevalence of glutamic acid and aspartic acid in watermelon seeds. This implies that variation occurs according to genotype and the geographical and environmental conditions in which watermelons are grown. Threonine was the second most abundant, detected in all landraces except in 01SDPW. The prevalence of methionine was low in landraces from Sekhukhune but was high for Capricorn.

This multi-functional sulfur-containing amino acid plays an important role in the synthesis of essential proteins and stabilisation of specific interactions within proteins and protein complexes. The high abundance of this essential amino acid demonstrates the potential role of watermelon seeds in human biological activities. Therefore, observations indicate that watermelons have attractive amino acid and protein profiles, providing greater prospective for use as food supplements.

Total phenolics, flavonoids and antioxidant activity

Flavonoid content in the watermelon seeds was in the range of 0.015 to 0.347 mg/g of sample. Capricorn landraces had high flavonoid content (0.222 mg/g) than Sekhukhune varieties (0.130 mg/g). Adetutu et al. (2015) reported 0.05 mg/g of flavonoids in watermelon seeds. In contrast, henna, pomegranate and soybean seeds reportedly contain 1.10, 6.79 and 0.580 mg/g, respectively (Cherbi et al., 2016; Elfalleh et al., 2012; Josipovic et al., 2016). However, phenolics quantified were found to be significantly lower than previously reported by other researchers. About 0.57 mg gallic acid equivalents/g was obtained. This is much lower as compared to reports by Etim et al. (2013) and Sharma et al. (2013) with an average of 0.96 mg gallic acid equivalents/g in other *Cucurbita* spp. Xu and Chang (2008) reported fewer flavonoids and phenolics in soybean.

Varghese et al. (2013) reported that the different techniques employed affect the results and also limit the observations, rendering them somehow incomparable. Therefore, differences observed in our study may also be due to the extraction conditions, as well as the genotypes. Antioxidant activity expressed as %DPPH inhibition of the landraces is presented in Figure 1. Capricorn varieties had high scavenging capacity (46.5%) than Sekhukhune (45.3%). Variation in DPPH activity may be due to the high phenolic content found in Capricorn extracts. Rahman et al. (2013) stated that phenols are responsible for high scavenging activity than other phytochemicals. About 56.9% DPPH free radical scavenging activity of watermelons seeds was reported by Oseni and Okoye (2013). Tabiri et al. (2016) reported 94.46, 70.06 and 59.88% scavenging ability of watermelons cultivar Crimson sweet, Black diamond and Charleston gray, correspondingly.

Conclusions

The results suggest that watermelons from Capricorn district have better phytochemicals and antioxidant capacity than those collected from Sekhukhune district. These results further indicate that seed extracts from these watermelon landraces contain relatively high amounts of oil and secondary metabolite contents coupled with a fair antioxidant activity and thus, suitable to enhance the nutritional quality of foods and feeds.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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