Heliyon

In vitro α-amylase, α-glucosidase, lipase inhibitory and cytotoxic activities of tuber extracts of *Kedrostis africana* (L.) Cogn

Jeremiah Oshiomame Unuofin[*]**, Gloria Aderonke Otunola, Anthony Jide Afolayan**

Medicinal Plants and Economic Development (MPED) Research Centre, Department of Botany, University of Fort Hare, Alice 5700, South Africa

[*] Corresponding author.

E-mail address: unuofinjeremiah@gmail.com (J.O. Unuofin).

Abstract

Kedrostis africana, is a tuberous plant commonly used by traditional healers in the Eastern Cape Province of South Africa for the management of obesity. The aim of this study was to investigate the antiobesity and cytotoxic effects of *Kedrostis africana* extracts in vitro The α-amylase, α-glucosidase and lipase inhibitory activities of aqueous and ethanol extracts of *Kedrostis africana* tuber were investigated while the cytotoxic effects of these extracts were analyzed using Hoechst 33342 and propidium iodide (PI) dual staining in combination with Molecular Devices ImageXpress Micro XLS Widefield microscope for high content analysis on human cervical (HeLa) cell line. The ethanol extract exhibited the strongest inhibitory effect on pancreatic lipase (IC$_{50}$ = 381.86 μg/ml) and on α-glucosidase (IC$_{50}$ = 157.99 μg/mL) while the aqueous extract has strongest α-amylase (IC$_{50}$ = 439.45 μg/ml). Both tuber extracts were found nontoxic at tested concentrations on HeLa cell lines as confirmed by the Hoechst 33342 and propidium iodide dual staining respectively. This study revealed that both the aqueous and ethanol tuber extract of *K. africana* exerts a certain degree

of inhibitory effect on α-amylase, α-glucosidase and lipase and were also nontoxic to HeLa cell line at tested concentrations.

1. Introduction

Obesity is a metabolic disorder which results from excessive accumulation of body fat, and it is associated with a myriad of comorbidities, such as the onset of cardiovascular diseases (especially heart diseases and stroke), that caused most death in 2012; diverse types of cancer (colon, kidney, liver, breast, pancreatic, ovarian, prostate, and endometrial cancers); osteoarthritis, hypertension and type 2 diabetes mellitus are responsible for most deaths (Wanderley and Ferreira, 2010; Kitahara et al., 2014; Gallagher and LeRoith, 2015; WHO, 2015).

Within the space of 1980 and 2014, the world's obesity prevalence has increased yearly. According to data from the World Health Organization report of 2014, more than 1.9 billion adults were overweight and, among them, more than 600 million were obese (WHO, 2015). The solution to obesity epidemic requires a multifaceted approach. Some obese people have difficulty losing weight through diet and exercise alone. Therefore the use of medications is employed, they control body weight by restricting energy absorption and cause weight loss (Boniglia et al., 2008). However, effect is noticed after a long while (Kaukua et al., 2003) and their weight are regained when treatment is discontinued. Also, some of these anti-obesity drugs approved and marketed have now been withdrawn due to adverse effects such as abdominal pain, bloating, flatulence, oily stools, diarrhea, and decreasing in fat soluble vitamins absorption (Kang and Park, 2012). It is, therefore, necessary to develop an alternative source of medicine from natural products or plant based sources as this would help in the discovery and development of new drugs with no side effects. A number of natural products possess the ability to induce body weight reduction and prevent diet induced obesity (Birari and Bhutani, 2007; Mohamed et al., 2014; Yun, 2010). These natural products possess inhibitors of digestive enzymes that interfere with the hydrolysis and absorption of dietary carbohydrates and lipids (Wang et al., 2010; Garza et al., 2011; Gu et al., 2011). The main enzymes involved in the hydrolysis of carbohydrates and fat are α-amylase, α-glucosidase and pancreatic lipase (Gu et al., 2011; Nair et al., 2013). There is a possibility that plants with strong α-amylase, α-glucosidase and lipase inhibitory activities may have potential toxic and carcinogenic effects, thus rendering them unsuitable for therapeutic applications (Fennell et al., 2004). Hence, it is imperative to examine the potential cytotoxic activity in order ascertain the safety limits of medicinal plant extracts. According to Ahmad et al. (2006), some plants' extracts possess bioactivity, but these activities are neutralized by their

cytotoxicity activity; therefore is necessary to critically evaluate plant extracts so as to determine their overall efficacy.

Kedrostis africana (L.) Cogn (Cucurbitaceae) commonly called baboon's cucumber is indigenous to Nambia and South Africa (Eastern Cape, Free State, Gauteng, Kwazulu-Natal, Limpopo, Mpumalanga, Northern Cape, North West and Western Cape) (Eggli, 2002). Locally, it is called "uthuvana/uthuvishe" by the Xhosa-speaking South Africans (Dold and Cocks, 1999). The plant is traditionally used by the Khoi-San and Cape Dutch medicine as an emetic, purgative, diuretic and against dropsy (van Wyk, 2008). In the Eastern Cape of South Africa, the crushed fresh bulb is used for the management of obesity in folklore (Afolayan and Mbaebie, 2010; George and Nimmi, 2011).

According to Unuofin et al. (2017a), this plant serves as a rich source of minerals and proximate contents. Also, certain biological activities such as antioxidant, antibacterial, fungicidal and larvicidal activities have been ascribed to different solvent extracts of *K. africana* (Unuofin et al., 2017b,c).

Kedrostis africana has been used in folk medicine for the management of obesity, but scientific evaluation is still lacking in this regard. Therefore, in this study, α-amylase, α-glucosidase, pancreatic lipase inhibitory activities were screened in vitro, and cytotoxicity was evaluated against HeLa cell line of aqueous and ethanol extracts of *K. africana*.

2. Materials and methods

2.1. Location and collection of sample

K. africana tuber used for this study was collected in August 2015 in Fort Beaufort in the Amathole District Municipality, Eastern Cape, South Africa. It was authenticated by Mr Tony Dold, Selmar Schonland Herbarium, Rhodes University, South Africa, and a voucher specimen (Unuofin Med, 2015/2) was prepared and deposited in the Giffen Herbarium, University of Fort Hare.

2.2. Preparation of extracts

The oven-dried powder of the tuber were extracted using ethanol and water and shaken in an orbital shaker (Orbital Incubator Shaker, Gallenkamp) for 48 hours. The extracts were filtered using a Whatman No. 1 filter paper. The ethanol extract was concentrated under vacuum to dryness while the aqueous extract was concentrated using a freeze dryer (Vir Tis benchtop K, Vir Tis Co., Gardiner, NY).

2.3. Chemicals

Melphalan, RPMI1640 medium, 10% fetal bovine serum, dimethyl sulfoxide (DMSO), Hoechst 33342, Propidium iodide, Phospahte Buffer Saline (PBS),

para-Nitrophenol palmitate (pNPP), gum arabic, sodium deoxycholate, Triton X-100, Tris-HCl buffer (pH 8.0), monosodium and disodium phosphate, p-nitro-phenyl-α-D-glucopyranoside (PNP-GLUC), sodium carbonate, epigallocatechin gallate (EGCG), α-glucosidase, α-amylase, isopropanol, Triton X-100, Tris-HCl buffer, porcine pancreatic lipase, orlistat.

2.4. *In vitro* cytotoxic activity of plant extracts (Hoechst/PI staining)

The plant extracts were solubilised in 1% DMSO to a final concentration of 50 000 μg/mL. Samples were then stored at 4 °C until required. HeLa (cervical cancer) were used for the screening process. Cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum. Cells were seeded in 200 μL aliquots at a cell density of 3×10^4 cells/mL (i.e. 6 000 cells per well) in 96 well plates and left overnight to attach. For treatment of the cell line, the medium was replaced with fresh medium containing three concentrations (50, 100 and 200 μg/mL) of extract. Treated cells were incubated at 37 °C in a humidified 5% CO_2 incubator for 48 hours. Treatment medium was gently removed prior to the addition of the Hoechst/PI staining and replaced with 100 μl PBS containing bisBenzamide H 33342 trihydrochloride (Hoechst 33342) at a concentration of 5 μg/mL. Cells were stained for 20 minutes at 37°C. Propidium Iodide was added at a concentration of 10 μg/mL using 10 μL per well of a 110 μg/mL stock directly before imaging. Image acquisition was performed using an ImageXpress Micro XLS Widefield High-Content Analysis System (Molecular Devices®). This microscope is Africa's first high content analysis system and thus the accuracy and sensitivity were compared to the crystal violet assay, a more traditional method of quantifying cell density. The images were analyzed using the MetaXpress® High-Content Image Acquisition & Analysis Software and Multi-wavelength Cell Scoring analysis module.

The average cell number was calculated using MetaXpress® software.

% cell density = A570 nm of treated cells/A570 nm of untreated cells X 100.

2.5. Alpha-amylase inhibitory activity of plant extracts

The α-amylase inhibitory activity of the extract was carried out according to the standard method with minor modification (Ademiluyi and Oboh, 2013). Ten microliter of porcine pancreatic amylase solution (0.1 mg/mL) were mixed with 30 μL of plant extracts or phosphate buffer (the control), or positive control (acarbose, 64 μg/mL) and pre-incubated at 37 °C for 10 minutes. Then, 40 μL starch solution was added to initiate reaction and incubation was done at 37 °C for 30 minutes, then 20 μL of 1 M

HCl and 75 μL iodine reagent were added to the 96-well plate. The absorbance of the mixture was measured at 580 nm.

α-amylase inhibitory activity was measured using the formula:

% α-amylase inhibition = [1- (A/B)] X 100

Where A = absorbance of test well (plant extract or acarbose)B = absorbance of enzyme control

Two controls (without enzyme and substrate) were used to ascertain if any reaction took place in their absent. This was done to exclude false positive results, as some plants extracts have been reported to contain traces of α-amylase or starch.

2.6. Alpha-glucosidase inhibitory activity of plant extracts

The α-glucosidase inhibitory activity was determined by a colorimetric assay utilizing a well established protocol (You et al., 2011) with some modifications. Forty microliter of α-glucosidase solution (50 μg/mL) were mixed with 10 μL of plant extract or Epigallocatechin gallate (EGCG) as positive control and pre-incubated at 37 °C for 5 minutes. Then, 10 μL of PNP-GLUC was then added to initiate reaction and incubation was done at 37 °C for 30 min, after which 50 μL of sodium carbonate solution were added to the 96-well plate to terminate the reaction. The absorbance of the mixture was measured at 405 nm.

α-glucosidase inhibitory activity was measured using the formula:

% α-glucosidase inhibition = [1- (A/B)] X 100.

Where A = absorbance of test well (plant extract or positive control)B = absorbance of enzyme control

2.7. Pancreatic lipase inhibitory activity of plant extracts

The inhibitory activity against pancreatic lipase was measured using p-nitrophenyl butyrate (p-NPB) as a substrate with a modified method from Zhang et al. (2008). 10 μL of extracts (prepared at concentrations of 50, 100 and 200 μg/mL), positive control (Orlistat, 100 μM) and DMSO (negative control vehicle used to dissolve the extracts) were pipetted into respective wells of a 96-well plate. Freshly prepared porcine pancreatic lipase was added at four times the volume of the test samples, positive and negative controls (40 μL). The plates were initially incubated at 37 °C for 15 minutes. Thereafter, 170 μL of the substrate solution was added to the wells. The plate was then incubated at 37 °C for 25 minutes and the absorbance was read at 405 nm.

Percentage lipase inhibition was calculated as:

% Lipase inhibition = [1- (A/B)] X 100.

Where A = absorbance of test well (plant extract or orlistat)B = absorbance of enzyme control

2.8. Data analysis

The statistical analysis was done on MINITAB version 17 for Windows. Data were expressed as mean $\pm$ SD of five replicates and were subjected to one-way analysis of variance (ANOVA) followed by Fischer's Least Significant Difference (LSD) to determine significant differences in all the parameters. Values were considered statistically significant at $p < 0.05$.

3. Result

3.1. Cytotoxicity activity of extracts on HeLa cell lines

Cytotoxicity screening was performed on HeLa cell line. Cell numbers were determined using Hoechst 33342/PI staining. Fig. 1 shows the fluorescent micrographs that were captured during this experiment. The ethanol extract had more cell death due to the cytotoxic nature, this was depicted with the cell numbers greatly reducing (blue colour) and the number of dead cells stained positive (red colour) for PI is increased when compared with the aqueous extract and the untreated sample.

Fig. 2 displays the cytotoxicity activity of *K. africana* extracts on HeLa cell line as confirmed by Hoechst 33342/PI staining. As indicated in Fig. 2, the aqueous extract of *K. africana* did not cause any significant decrease in cell number across all tested concentrations (50$-$200 μg/mL). However, the ethanol extract displayed a noticeable increase in cell death at all the test concentration with a more significant ($p < 0.005$) cell death at the lowest concentration (50 μg/mL). The IC_{50} values for both the aqueous and ethanol extracts are greater than 200 μg/mL which is the highest concentration tested (Table 1).

3.2. α-amylase inhibitory activity

Both the aqueous and ethanol extracts of *K. africana* exhibited weak α-amylase inhibitory activity at the tested concentrations (50, 100, or 200 μg/mL) with inhibition in a concentration-dependent manner (Fig. 3). Although, the percentage inhibition of the positive control (acarbose) was $92.98 \pm 1.37\%$ at 64 μg/mL, it ranged from $19.85 \pm 0.37\%$ to $31.64 \pm 1.11\%$ and $13.91 \pm 1.55\%$ to $20.14 \pm 0.63\%$ for the aqueous and ethanol extracts, respectively. However, both extracts (aqueous and ethanol) had lower activities than acarbose. The IC_{50} values are depicted in Table 2.

3.3. α-Glucosidase inhibitory activity

The alpha-glucosidase inhibitory activity of the aqueous and ethanol extracts of *K. africana* was observed to be in a concentration-dependent manner. A stronger
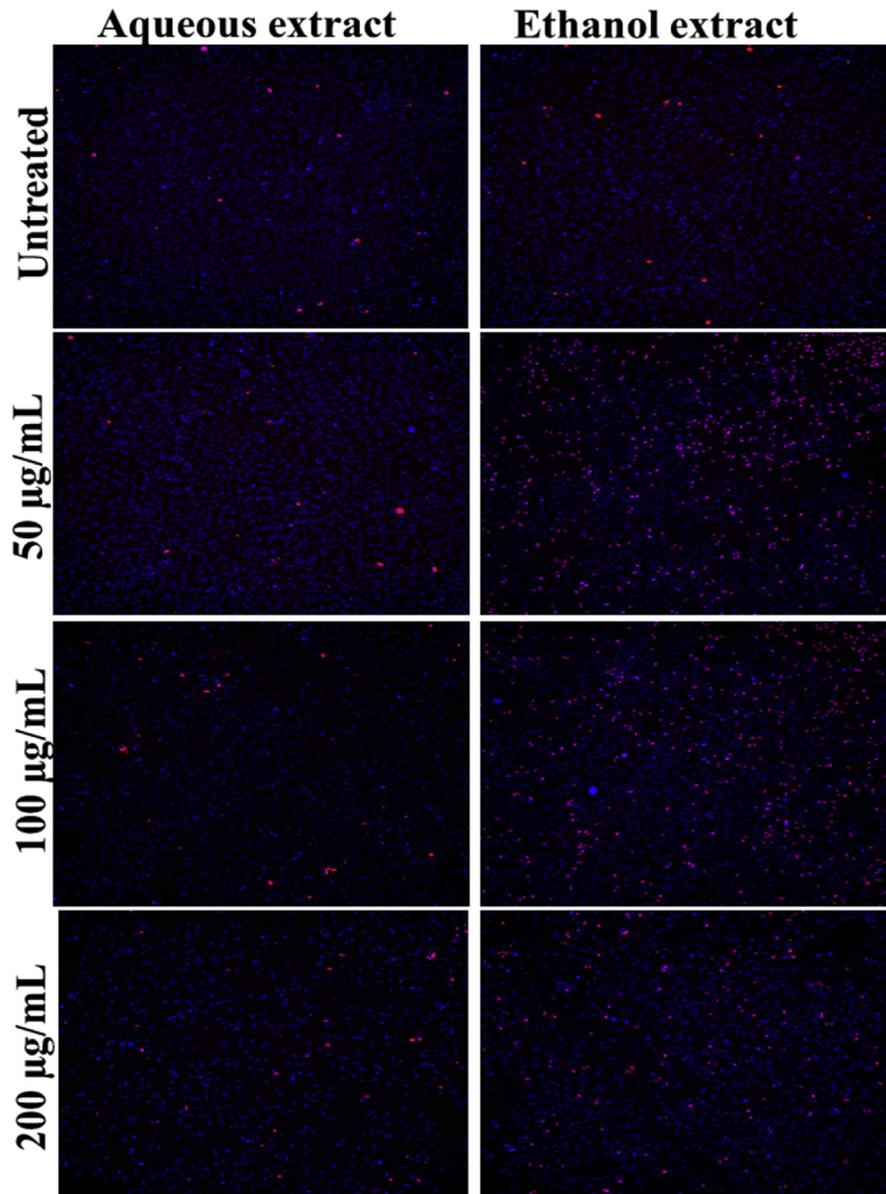
Fig. 1. Fluorescent micrographs captured during the cytotoxicity screening assay comparing the untreated sample with the aqueous and ethanol extract of *K. africana* for HeLa cells using Hoechst 33342 (blue) and propidium iodide (red).

inhibition was observed when compared with α-amylase inhibition (Fig. 4). The percentage α-glucosidase inhibition ranged from $24.57 \pm 6.22\%$ to $33.25 \pm 1.91\%$ in the aqueous extract and from $30.07 \pm 8.84\%$ to $54.30 \pm 0.79\%$ in the ethanol extract. The ethanol extract had a better α-glucosidase inhibitory activity with IC_{50} values of 157.99 ± 5.41 µg/mL while the aqueous extract IC_{50} value was 486 ± 4.64 µg/mL (Table 2).

A)

B)
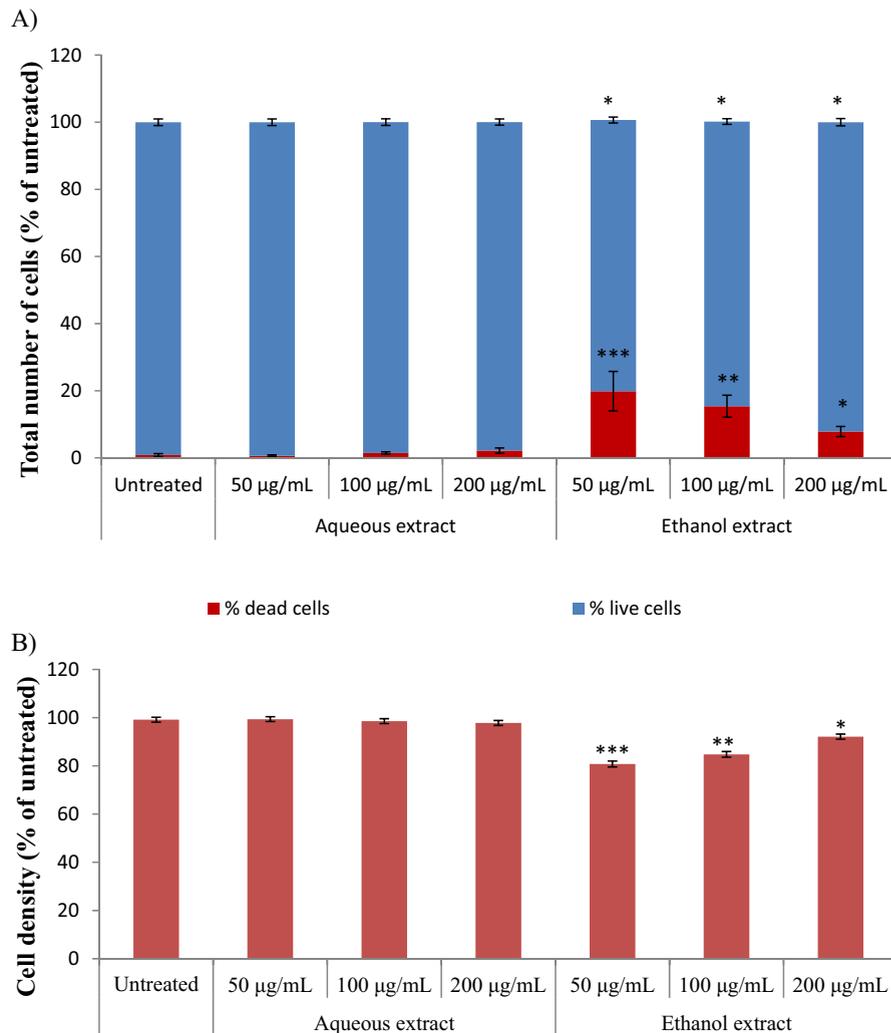
Fig. 2. Cytotoxicity screening of *Kedrostis africana* (A) cell number with (B) cell density for HeLa cells; live- and dead cell numbers were obtained from Hoechst 33342 and PI staining, respectively and cell density from crystal violet staining. Results are reported as means $\pm$ SD where each experiment was performed three times, each in triplicate (*$p < 0.05$; **$p < 0.01$; ***$p < 0.005$ compared to untreated sample).

Table 1. IC_{50} values (µg/ml) for cytotoxic activity of *Kedrostis africana* extracts on HeLa cells after 48 hours exposure.

Name of extract	Cytotoxicity IC_{50} (µg/mL)
Aqueous	>200
Ethanol	>200

Data are presented as mean $\pm$ SD values of triplicate determinations.
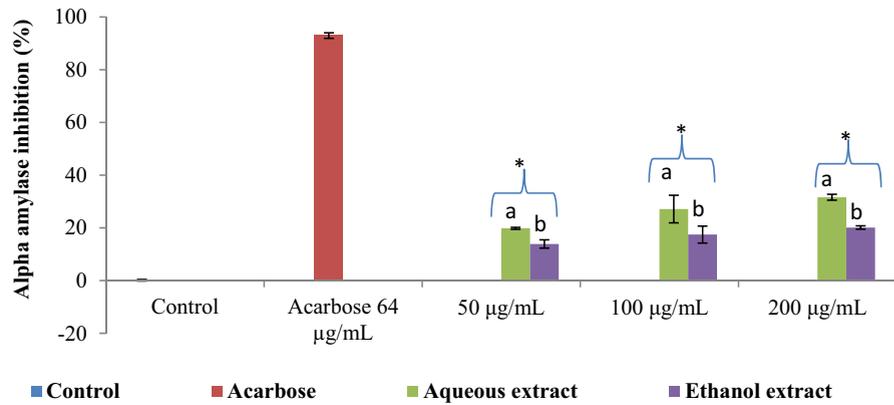
Fig. 3. α-amylase inhibitory activity of the aqueous and ethanol extracts of *Kedrostis africana*. Values are mean $\pm$ SD (n = 3). Mean separation by LSD ($p < 0.05$). Set of bars (the same concentration) with different alphabets are different. *Lower than acarbose (positive control). SD: Standard deviation; LSD: Least Significant Difference.

Table 2. IC$_{50}$ values for α-amylase, α-glucosidase and lipase inhibition activity of *Kedrostis africana* extracts.

Name of extract	IC$_{50}$ values (μg/mL)		
	α-amylase	α-glucosidase	Lipase
Aqueous	439.45 $\pm$ 1.95	486.92 $\pm$ 4.64	842.48 $\pm$ 2.24
Ethanol	949.75 $\pm$ 3.68	157.99 $\pm$ 5.41	381.86 $\pm$ 3.48

Data are presented as mean $\pm$ SD values of triplicate determinations.
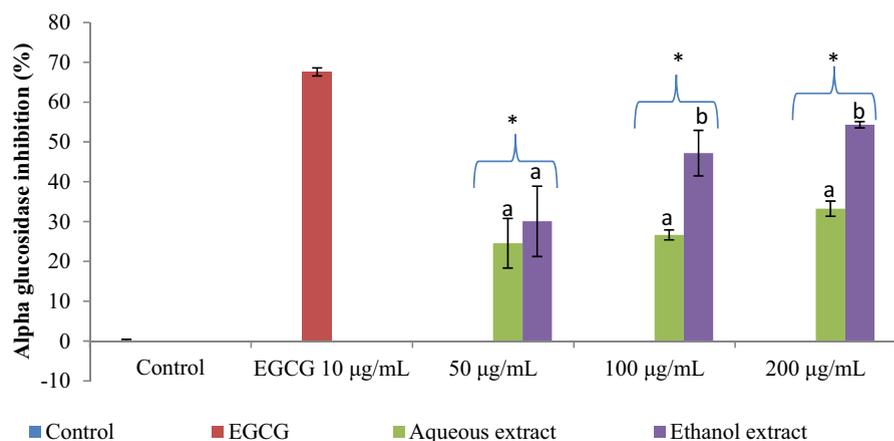
Fig. 4. α-Glucosidase inhibitory activity of the aqueous and ethanol extracts of *Kedrostis africana*. Values are mean $\pm$ SD (n = 3). Mean separation by LSD ($p < 0.05$). Set of bars (the same concentration) with different alphabets are different. *Lower than the positive control EGCG: Epigallocatechin gallate; SD: Standard deviation; LSD: Least Significant Difference.
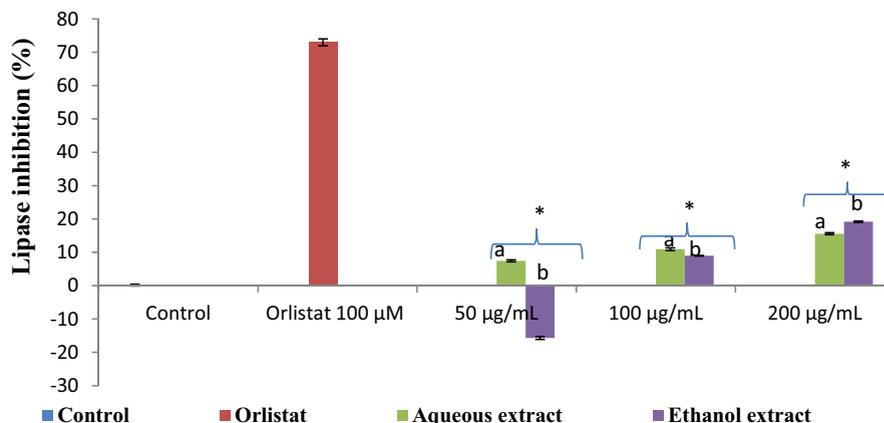
Fig. 5. Lipase inhibitory activity of the aqueous and ethanol extracts of *Kedrostis africana*. Values are mean ± SD (n = 3). Mean separation by LSD (p < 0.05). Set of bars (the same concentration) with different alphabets are different. *Lower than the positive control (Orlistat). SD: Standard deviation; LSD: Least Significant Difference.

3.4. Porcine pancreatic lipase (PPL) inhibitory activity

Lipase inhibitory activity of the aqueous and ethanol extracts of *K. africana* exhibited inhibitory activity at the tested concentrations (50, 100, or 200 μg/mL) in a concentration-dependent manner (Fig. 5). Although, the percentage inhibition of the positive control (acarbose) was 72.98 ± 1.37% at 64 μg/mL, it ranged from 7.43 ± 0.32% to 15.58 ± 0.31% and -15.69 ± 0.43% to 19.17 ± 0.21% for the aqueous and ethanol extracts, respectively. However, both extracts (aqueous and ethanol) had lower activities than orlistat. The ethanol extract had a better lipase inhibitory activity with IC_{50} values of 381.86 ± 3.48 μg/mL while the aqueous extract IC_{50} value was 842.48 ± 2.24 μg/mL (Table 2).

4. Discussion

Over the years, there has been an increasing search for natural products having potent bioactive compounds with low toxicity and possess ability to oxidize fats, control appetite, regulate levels of hormones related to obesity and inhibit digestive enzymes involved in the absorption of carbohydrates and lipids (Cho et al., 2010; Rains et al., 2011) when compared with synthetic compounds.

In this study, we evaluated the *in vitro* cytotoxicity and inhibitory activities against α-amylase, α-glucosidase and lipase of aqueous and ethanol extracts of *K. africana*. Although a number of studies have reported that some medicinal plants possess α-amylase, α-glucosidase and lipase inhibitory activities (Shirwaikar et al., 2005; Ortiz-Andrade et al., 2007) an attribute similar to that of *K. africana*, a wild plant used in folklorically for the management of obesity in the Eastern Cape, South Africa. However, we observed that there is still lacking of information on *K. africana*

cytotoxicity and its abilities to inhibit α-amylase, α-glucosidase and lipase activities *in vitro*.

Different scientific reports have shown that there is a positive correlation between the effects of flavonoids and polyphenols content on inhibitory potentials of alpha-amylase and alpha-glucosidase (Pereira et al., 2011; Adisakwattana et al., 2012; Kam et al., 2013). Flavonoids and their derivatives such as luteolin and kaempferol possess the ability of blocking glucose absorption, inhibiting sodium dependent glucose transporter-1 which improves glucose tolerance (Thouri et al. 2017). Also, high levels of polyphenolic compounds have been shown to reduce the potency of alpha-amylase and alpha-glucosidase by either interacting or inhibiting specific position of the enzyme (Rohn et al., 2002). According to Unuofin et al. (2017b), the tuber extracts of *K. africana* have strong free radical scavenging potential and high polyphenolic contents which have been implicated in enzyme inhibitory capacity. The aqueous extract showed higher inhibition against α-amylase and weaker inhibition against glucosidase while the ethanol extract had a low inhibitory effect against α-amylase and stronger inhibitory activity against α-glucosidase, thence the consumption of both extracts could serve as an antidiabetic agent for the treatment of postprandial hyperglycemia with little or no side effects. The lack of correlation in the result with the above statement could be attributed to the differences in the mechanisms involved and also the inference of other phytochemicals. Our findings are in line with previous reports which stated that mild α-amylase inhibition is permitted in order to prevent the abnormal bacterial fermentation which results from the presence of undigested carbohydrates in the colon and thus promote flatulence and diarrhoea (Etxeberria et al., 2012; Lordana et al., 2013; Nagmoti and Juvekar, 2013). According to Chen et al. (2008), the inhibition of α-glucosidase aids gastric emptying, which brings about satiety and weight loss, which are useful in the treatment of obesity. Thus, the strong alpha-glucosidase activity of the extracts could aid in bringing about satiety and weight loss in obese subjects. Hence, the ability of natural products/medicinal plants in inhibiting α-amylase and α-glucosidase could serve as an alternative therapy for the treatment of obesity in place of synthetic drugs (Mc Dougall et al., 2005).

Pancreatic lipase is the most important enzyme responsible for digestion of dietary fat, slowing down the deposition of fat into adipose tissue and suppression of weight gain which is of beneficial effects to overweight and obesity (Podsędek et al., 2014; Dechakhamphu and Wongchum, 2015). In this study, both crude extracts (aqueous and ethanol) exhibited moderate inhibitory activity against pancreatic lipase. It is noteworthy that at 50 μg/mL of the ethanol extract, their was a stimulatory effect against lipase. Orlistat was more potent than the crude extracts may due to the presence of both active and non active components. Reports have shown that plants rich in phytochemicals such saponins and polyphenol can inhibit pancreatic lipase and

lighten weight gain in high-fat diets induced murine models (Han et al., 2002, 2005; Li et al., 2007).

Natural products/medicinal plants are usually thought to be safer than pharmaceuticals. According to the United States America National Cancer Institute of Plant Screening Program, any plant extract with an IC_{50} value equal to or lesser than 20 μg/mL after an incubation period 48 hrs is generally considered to have an active cytotoxic effect (Lee and Houghton, 2005; Malek et al., 2009). From the above statement, it can be deduced that both extracts screened using HeLa cells are not cytotoxic because their IC_{50} values are far greater than 20 μg/mL. These relatively low toxicity result of all the extracts from this study suggests that these extracts may safely use for anti-obesity treatment.

5. Conclusion

Data obtained from this study suggest that both the aqueous and ethanol tuber extract of *K. africana* exerts mild inhibitory effect on α-amylase, α-glucosidase and lipase with nontoxic effect to HeLa cell line. Further, the results revealed that both extracts could be a good candidate for anti-obesity evaluations in experimental animals. In addition, these results support the traditional use of *K. africana* in the management of obesity.

Declarations

Author contribution statement

Jeremiah Oshiomame Unuofin: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Gloria Aderonke Otunola: Conceived and designed the experiments; Wrote the paper.

Anthony Jide Afolayan: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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