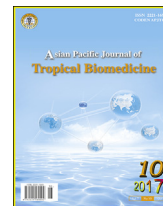


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journal homepage: www.elsevier.com/locate/apjtbOriginal article <http://dx.doi.org/10.1016/j.apjtb.2017.09.008>Phytochemical screening and *in vitro* evaluation of antioxidant and antimicrobial activities of *Kedrostis africana* (L.) Cogn

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

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

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ABSTRACT

Objective: To investigate phytochemical, antioxidant and antimicrobial activities of *Kedrostis africana* (*K. africana*).**Methods:** Dried tubers of *K. africana* were extracted in acetone, water and ethanol. The total phenol, flavonoid, proanthocyanidin and tannin contents were determined spectrometrically. The antioxidant activity was examined using 2,2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt, nitric oxide and hydrogen peroxide assays. The antimicrobial activity was determined by agar dilution method using minimum inhibitory concentration against 3 g positive and three gram negative strains while four fungal strains were also investigated.**Results:** Total phenol, flavonoids, proanthocyanidin and tannin contents ranged from (5.32 ± 0.01) to (10.51 ± 0.01) mg GAE/g; (42.58 ± 0.02) to (529.23 ± 0.01) mg QE/g; (15.05 ± 0.00) to (585.64 ± 0.00) mg CE/g and (0.301 ± 0.010) to (0.937 ± 0.000) mg TAE/g, respectively. The IC₅₀ values of the ethanol extract for 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and hydrogen peroxide were 0.054 and 0.057 mg/mL, respectively, aqueous extract had an IC₅₀ value of 0.135 7 mg/mL for nitric oxide while the acetone extract had an IC₅₀ value of 0.300 mg/mL for 2,2-diphenyl-1-picrylhydrazyl. The ethanol extract demonstrated effective antimicrobial activity against the tested pathogenic species with minimum inhibitory concentrations values ranging from 2.5–5.0 mg/mL for bacteria and (0.312 5–5.000 0) mg/mL for fungi, respectively.**Conclusions:** The tuber of *K. africana* showed potent free radical scavenging property and antimicrobial activity.

1. Introduction

Plants are made up of secondary metabolites which are formed as products of primary metabolism and produced for defense against predators. Examples of such metabolites are tannins, flavonoids and alkaloids; they are known to be the brain behind the healing potentials of plants [1]. Polyphenols are essential in the daily diet of humans because of their ability to scavenge free radicals, which bring about the onset of metabolic disorders, neurodegenerative diseases and

cardiovascular disorders [2]. In addition to their scavenging ability [3], polyphenols also possess antimicrobial activity [4]. In recent times, natural sources of antioxidants are now being focused upon owing to the numerous side effects of synthetic antioxidants [5]. Also, due to the increase in resistance to antibiotics, there is the need to search for new antimicrobial agents [6].

Kedrostis africana (*K. africana*) (L.) Cogn is commonly known as Baboon's cucumber and it belongs to the Cucurbitaceae family. This species is mainly found in Namibia and South Africa (Eastern Cape, Free State, Gauteng, Kwazulu-Natal, Limpopo, Mpumalanga, Northern Cape, North West and Western Cape). This tuberous plant has a water-storage organ, thus making it resistant to drought [7]. *K. africana* tuber is used in Khoi-San and Cape Dutch medicine as an emetic, purgative, diuretic and against dropsy [8]. The crushed fresh bulb is used ethnomedically for the management of obesity in the Eastern Cape of South Africa [9,10].

*Corresponding author: Gloria Aderonke Otunola, Medicinal Plants and Economic Development Research Centre, Department of Botany, University of Fort Hare, Alice 5700, South Africa.

Tel: +27 0 40 602 2320

E-mail: gotunola@ufh.ac.za (G.A. Otunola).

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K. africana has been used traditionally for the management and treatment of a myriad of ailments. However, there is a dearth of information on the evaluation of its polyphenolic contents, antimicrobial and antioxidant properties. Hence this study evaluated these parameters.

2. Materials and methods

2.1. Location and collection of sample

The tubers of *K. africana* used for this study were collected in August 2015 near Baddaford farm in Fort Beaufort which is in the Amathole District Municipality, Eastern Cape, South Africa. This area lies within the latitude 32°43'28.66" and longitude 26°34'5.88". The plant was authenticated by Mr. Tony Dold of 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. Extract preparation

The whole plant was rinsed with deionized water and gently wiped with a paper towel to remove the water and subsequently oven-dried (LABOTEC, South Africa) at 40 °C for 72 h until constant weight was achieved. The dried sample was then ground into powder (Polymix® PX-MFC 90D Switzerland) and stored at 4 °C until needed for the various the various analyses. The ground sample (200 g) was weighed into 3 separate conical flasks containing (2 L) acetone, ethanol, and water respectively, then shaken in an orbital shaker (Orbital Incubator Shaker, Gallenkamp) for 48 h. The crude extracts were filtered under pressure using a Buchner funnel and Whatman No. 1 filter paper. The acetone and ethanol extracts were further concentrated to dryness to remove the solvents under reduced pressure using a rotary evaporator (Strike 202 Steroglass, Italy), while the aqueous filtrate obtained was concentrated using a freeze dryer (Vir Tis benchtop K, Vir Tis Co., Gardiner, NY). The acetone, aqueous and ethanol extracts were stored at 4 °C.

2.3. Reagents and chemicals

Solvents and chemicals used were purchased from Merck and Sigma–Aldrich, Gauteng, South Africa. These included Folin–Ciocalteu reagent, anhydrous sodium carbonate, aluminium trichloride (AlCl₃), sodium nitrite, sodium chloride, 2,2 diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), vanillin, potassium acetate, ferric chloride, butylated hydroxytoluene (BHT), ascorbic acid, rutin, *n*-butanol, diethyl ether, ammonia solution, acetone, ethanol, hydrochloric acid, sodium hydroxide, phosphate buffer, potassium ferricyanide, ammonium molybdate, sodium phosphate, trichloroacetic acid, glacial acetic acid and sodium nitroprusside. All the chemicals used in this study were of analytical grade.

2.4. Phytochemical screening

2.4.1. Determination of polyphenolic content

Total phenol content was determined using the Folin–Ciocalteu's reagent method as described by Fu *et al.* [11] with some modifications. Briefly, 0.5 mL of the various crude extracts (1 mg/mL) and standard gallic acid (20–100 µg/mL) was

dispensed in different test tubes and was mixed thoroughly with 2.5 mL of 10% (v/v) Folin–Ciocalteu's reagent by the aid of a vortex. The reaction mixture was allowed to stand at room temperature for about 5 min, followed by the addition of 2 mL of 7.5% (w/v) anhydrous sodium carbonate. The mixture was vortexed and incubated at 40 °C for 30 min. In the control tube, the extract volume was replaced by methanol. After incubation, the absorbance was measured at 765 nm using a UV-3000 PC spectrophotometer. The experiment was done in triplicate. The phenol content was extrapolated from the gallic acid standard/calibration graph equation; $y = 8.7668x + 0.1977$, $R^2 = 0.9983$, and calculated using the following formula:

$$C = c \times V/m$$

where C = total content of phenolic compounds in mg/g plant extract in gallic acid equivalent (GAE) or mg GAE/g extract, c = the concentration of gallic acid established from the calibration curve in mg/mL, V = the volume of extract in mL and m = the weight of extract used in the assay in g.

2.4.2. Flavonoid determination

The flavonoid content of the crude extracts were determined using the aluminum chloride colorimetric method described by Sen *et al.* [12] with little modification. This method is based on the quantification of the yellow-orange color produced by the interaction of flavonoid with AlCl₃. Briefly, 0.5 mL of extract (diluted in the extraction solvent) was mixed with 2 mL of distilled water and 0.15 mL of 5% sodium nitrite and was allowed to stand for 6 min. Thereafter, 0.15 mL of 10% AlCl₃ and 1 mL of 1 M sodium hydroxide were added to the solution and then made up to 5 mL with distilled water. Absorbance was measured at 420 nm. Flavonoid content was calculated using a quercetin calibration curve equation, $y = 1.1734x + 0.1543$, $R^2 = 0.9698$ and the results were expressed as mg of quercetin equivalent (QE)/g using the formula CV/m in the same manner as described for total phenol.

2.4.3. Proanthocyanidin (condensed tannin)

Proanthocyanidin was determined as described by Kibiti and Afolayan [13]. About 0.5 mL of 1 mg/mL of the extract solution was added to a mixture of 3 mL of 4% w/v vanillin and 1.5 mL of hydrochloric acid, and was then vortexed. The mixture obtained was allowed to stand for 15 min at room temperature and the absorbance was measured at 500 nm using a UV-3000 PC spectrophotometer. The blank had neither the extract nor catechin. The experiment was done in triplicate. Proanthocyanidin content was calculated using the calibration curve equation: $y = 0.9038x + 0.0449$, $R^2 = 0.9951$ and expressed as mg catechin equivalent (CE)/g using the formula, CV/m as earlier described.

2.4.4. Tannin determination

Tannin content was determined as described previously by Noha *et al.* [14]. Plant extract (0.2 g) was dissolved in 20 mL of 50% methanol and placed in a water bath at 80 °C for 1 h. The extract was filtered into 100 mL volumetric flasks. To the filtrate was added 20 mL distilled water, 2.5 mL of Folin Ciocalteu reagent and 10 mL of 17% sodium carbonate. The mixture was thoroughly mixed and made up to 100 mL with distilled water, which was allowed to stand for 20 min and absorbance was read at 760 nm. Tannin content was calculated using tannic acid calibration curve equation: $y = 154.45x$,

$R^2 = 0.9585$ and the results were expressed as mg of tannic acid equivalent (TAE)/g using the formula CV/m in the same manner as described previously.

2.5. Antioxidant assay

The antioxidant capacities of the different extracts were measured using DPPH radical scavenging activity, ABTS radical scavenging activity, hydrogen peroxide and nitric oxide scavenging activities. These measurements compared to two standard antioxidants – BHT and rutin.

2.5.1. ABTS radical scavenging activity

The method described by Hsu *et al.* [15] with slight modification was adopted for the determination of the ABTS activity of the plant. The working solution was prepared by mixing equal volumes of 7 mM ABTS and 2.45 mM potassium persulfate. This was allowed to react for 12 h at room temperature in the dark to release ABTS radicals ($ABTS^+$). The $ABTS^+$ stock solution was further diluted by mixing 1 mL of the $ABTS^+$ solution with about 50 mL of methanol to obtain an absorbance of 0.700 ± 0.006 at 734 nm. Subsequently, 1 mL of the $ABTS^+$ solution was mixed with 1 mL of the plant extract/or standards at different concentrations (0.005–0.080 mg/mL). After 7 min, the reduction in absorbance was measured at 734 nm. The percentage inhibition of $ABTS^+$ by the extract or standard was calculated according to the following equation:

$$\% \text{ inhibition} = [(Abs \text{ control} - Abs \text{ sample}) / (Abs \text{ control})] \times 100$$

2.5.2. DPPH radical scavenging activity assay

The method of Mamta *et al.* [16] with slight modification was used for DPPH radical scavenging activity. Briefly, 1 mL of the different concentrations (0.005–0.080 mg/mL) of the test samples/standards [BHT and vitamin C (Vit C)] were mixed with 1 mL of 0.135 mM DPPH in methanol solution. After shaking vigorously, the reaction mixture was then incubated in the dark at room temperature for 30 min, and the absorbance was measured spectrophotometrically at 517 nm. The scavenging ability of the plant extract was then calculated using the following equation:

$$DPPH \text{ Scavenging activity } (\%) = [(Abs \text{ control} - Abs \text{ sample}) / (Abs \text{ control})] \times 100$$

In this equation, Abs control is the absorbance of DPPH + methanol; Abs sample is the absorbance of DPPH radical + sample/or standard.

2.5.3. Hydrogen peroxide (H_2O_2) scavenging activity

H_2O_2 scavenging activity of the plant extract was determined by the method of Owaisi *et al.* [17] with slight modification. Briefly, plant extracts (1 mL) at different concentrations were mixed with 0.6 mL of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm against blank solution containing phosphate buffer solution without H_2O_2 . The positive controls used were BHT and Vit C. The percentage scavenging of hydrogen peroxide of samples was calculated using the following formula:

$$H_2O_2 \text{ inhibition capacity } (\%) = [1 - (H_2O_2 \text{ abs of sample} / H_2O_2 \text{ abs of blank})] \times 100$$

2.5.4. Nitric oxide scavenging activity

This assay was done according to the procedure of Balakrishnan *et al.* [18]. Briefly, 0.5 mL of extracts/standard drug (BHT and Vit C) at different concentrations (0.025–0.400 mg/mL) was mixed with 2 mL of 10 mM sodium nitroprusside prepared in 0.5 mM phosphate buffer saline (pH 7.4). The mixture was incubated at 25 °C for 2.5 h. After incubation, 0.5 mL of Griess reagent was added and the absorbance was read at 540 nm. A solution containing water instead of the extract/standard was used as a control. The amount of nitric oxide radicals inhibited by the extract was calculated using the following equation:

$$NO \text{ radical scavenging activity } (\%) = [(Abs \text{ control} - Abs \text{ sample}) / (Abs \text{ control})] \times 100$$

Abs control is the absorbance of NO radicals + methanol and Abs sample is the absorbance of NO radical + extract or standard.

2.6. Rationale for micro-organism selection

The bacteria and fungi used for this study were selected based on their roles as opportunistic pathogens of humans and animals and their association with obesity and diabetes described by Vajro *et al.* [19].

2.6.1. Microorganism strains

The selected bacterial strains were three Gram positive strains; *Actinomyces odontolyticus* (ATCC 17929), *Lactobacillus sakei* (ATCC 15521), and *Staphylococcus aureus* (ATCC 18824) and three Gram negative strains; *Enterobacter cloacae* (ATCC 13047), *Pseudomonas aeruginosa* (ATCC 19582) and *Bacteriodes thetaiotomicron* (*B. thetaiotomicron*) (ATCC 29741). The fungal strains used for this investigation were *Candida albicans* (ATCC 10231), *Microsporium gypsum* (ATCC 24102), *Penicillium chrysogenum* (ATCC 10106), and *Trichophyton tonsurans* (*T. tonsurans*) (ATCC 28942).

2.6.2. Bacterial inoculum preparation

The test bacterial strains that were originally maintained on nutrient agar slants were recovered in sterile Mueller Hinton broth and incubated overnight at 37 °C. In order to obtain distinct colonies, the 24 h old cultures were diluted 1:100 v/v in fresh sterile Mueller Hinton broth and cultured on Mueller Hinton agar overnight at 37 °C. The colony suspension method of EUCAST [20] was used for the preparation of the inoculum. Identical colonies from the culture were suspended in 0.85% sterile saline, adjusted with saline and compared with 0.5 McFarland standards to obtain a suspension density equivalent to 10^6 CFU/mL. The suspensions were confirmed by spectrophotometric reading at 600 nm. The cell suspensions were finally diluted 1:100 by transferring 0.1 mL of the bacterial suspension into 9.9 mL of sterile broth to give an approximate inoculum of 10^4 CFU/spot. These suspensions were used for inoculation within 15 min.

For the fungal inoculum preparation, modified method of Therese *et al.* [21] was used for the assay. The fungal strains were freshly sub-cultured on Sabouraud Dextrose Agar and incubated at 25 °C for 72 h. About 1 cm² of three day old spore producing cultures was dropped in sterile distilled water and vortexed for

30 s to release the fungal spores. The spore density of each fungus was adjusted with a spectrophotometer at 580 nm to obtain a final concentration of approximately 10^5 spores/mL. Cell suspensions were finally diluted to 10^4 CFU/spot. For the *Candida* spp., the inocula were prepared by adding 1.0 mL of overnight *Candida* cultures to 9.0 mL of SDB to yield 10^4 CFU/spot of the inoculum as described by Wiegand *et al.* [22].

2.6.3. Agar dilution assay

The methods described by Wiegand *et al.* [22] and the EUCAST [20] which are modifications from the guidelines of the Clinical and Laboratory Standard Institute, were used for this study.

Muller Hinton and Sabouraud Agar were respectively prepared according to the manufacturer's description for antibacterial and antifungal screening. The agar was autoclaved at 121 °C for 15 min and allowed to cool to 50 °C in a water bath. About 1 mL from the 2-fold serial dilutions was added to the molten agar (19 mL) in the water bath, swirled, poured into Petri dishes, and allowed to cool and solidify. About 10 µL each from both the bacterial and fungal inoculum was delivered individually on the solidified agar surfaces to give the desired final inoculum of 1×10^4 CFU/spot. The extracts and standard antibiotic concentrations for the antibacterial and antifungal evaluation ranged from (0.156–5.00) mg/mL. The ciprofloxacin concentration ranged from (2–64) µg/mL, while nystatin (antifungal standard) ranged from (0.5–16.0) µg/mL. Bacteria plates were incubated at 37 °C for 16–24 h while fungi plates were incubated at 30 °C for (48–72) h. The minimum inhibitory concentrations (MICs) were determined as the lowest concentration of extracts inhibiting the visible growth of each organism on the agar plate. The presence of 1 or 2 colonies was disregarded.

2.6.4. Extract preparation

A 100 mg/mL stock solution prepared in a little amount of DMSO and made up with either Muller Hinton or Sabouraud Dextrose Broth for anti-bacterial and fungal respectively was prepared. Two-fold serial dilutions of the extracts (50, 25, 12.5, 6.25, 3.125, 1.562 5 mg/mL) were also prepared. Standard antibiotics (ciprofloxacin and nystatin for antibacterial and fungi respectively) were also prepared by two-fold serial dilutions as described by the Clinical and Laboratory Standard Institute.

2.7. Statistical analysis

All data were expressed as mean \pm standard deviation (SD) of three replications. Statistical analysis was performed by ANOVA. As the data showed significance ($P < 0.05$), a mean separation was done using the Fischer's LSD with the aid of MINITAB 17 statistical package.

3. Results

3.1. Phytochemical content

The total polyphenol, flavonoid, proanthocyanidin and tannin concentrations in tuber extracts of *K. africana* are shown in Table 1.

The flavonoid, proanthocyanidin and total phenolic concentrations were higher in acetone extracts compared to the aqueous and ethanol extracts. The ethanol extract had the highest tannin concentration while aqueous extract had the lowest concentration. The concentrations of total phenol, flavonoid, proanthocyanidin and tannins contents ranged from (5.32 \pm 0.01) to (10.51 \pm 0.01) mg GAE/g; (42.58 \pm 0.02) to (529.23 \pm 0.01) mg QE/g; (15.05 \pm 0.00) to (585.64 \pm 0.00) mg CE/g and (0.301 \pm 0.010) to (0.937 \pm 0.000) mg TAE/g, respectively.

3.2. Extract antioxidant activities

The results of this study showed that the antioxidant activities of the plant extracts compared favorably with those of the standard antioxidants BHT and Vit C employed in the antioxidant assays.

3.2.1. DPPH free radical scavenging assay

The DPPH radical scavenging activities of the extracts and standards are presented in Figure 1. At the maximum concentration tested (0.08 mg/mL), BHT exhibited the highest DPPH scavenging activity followed by Vit C, acetone extract, aqueous extract and ethanol extract in a decreasing order. The concentration of the extracts or standards required to scavenge 50% of the radicals (IC_{50}) were in the following order: BHT > Vit C > acetone extract > ethanol extract > aqueous extract as shown in Table 2.

3.2.2. ABTS radical scavenging assay

The scavenging abilities of the various extracts on ABTS radical are shown in Figure 2. The extracts of *K. africana* showed good ABTS radical scavenging activities (42%–66%) at a concentration of (0.005–0.080) mg/mL. *K. africana* extracts had high scavenging activity, however, the activity was lower than that of BHT and Vit C. The IC_{50} values of the tested extracts/standards were in the following order: BHT > Vit C > ethanol extract > acetone extract > aqueous extract as shown in Table 2.

3.2.3. Nitric oxide scavenging assay

The percentage nitric oxide inhibitory activities of the extracts and standards (Figure 3) were in the order: BHT > aqueous extract > Vit C > ethanol extract > acetone

Table 1

Polyphenolic content of various solvent extracts of *K. africana* (mean \pm SD).

Solvent	Total phenol (mg GAE/g)	Proanthocyanidin (mg CE/g)	Flavonoids (mg QE/g)	Tannins (mg TAE/g)
Aqueous	5.32 \pm 0.00	15.05 \pm 0.00 ^c	42.61 \pm 0.02 ^c	0.301 \pm 0.010 ^c
Acetone	5.98 \pm 0.00 ^b	585.64 \pm 0.00 ^a	529.23 \pm 0.01 ^a	0.496 \pm 0.000 ^b
Ethanol	10.51 \pm 0.01 ^a	222.91 \pm 0.00 ^b	285.78 \pm 0.02 ^b	0.937 \pm 0.020 ^a

mg GAE/g = milligram gallic acid equivalent per gram of extract; mg CE/g = milligram quercetin equivalent per gram of extract; mg TAE/g = milligram tannic acid equivalent per gram of extract; mg QE/g = milligram catechin equivalent per gram of extract. Different Letters (a, b and c) within the same row are significantly different ($P < 0.05$).

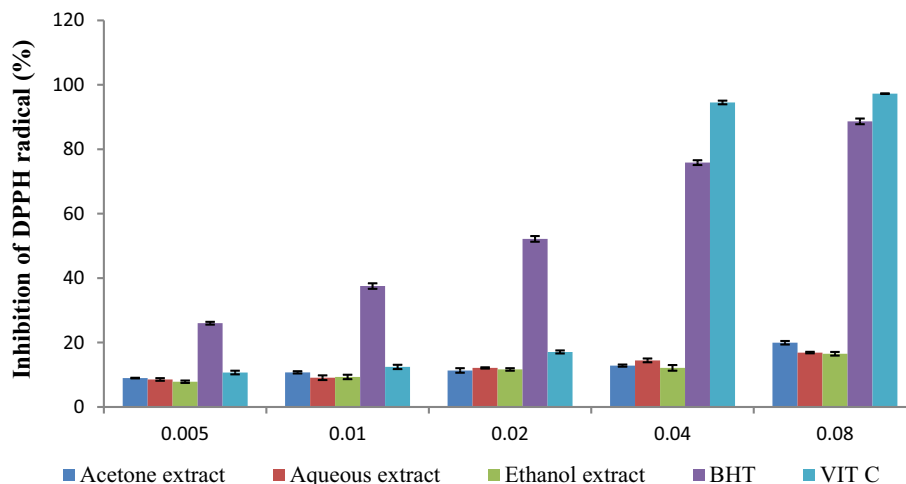


Figure 1. DPPH radical scavenging activity of different extracts of *K. africana*.

Table 2

IC₅₀ values of various extracts of *K. africana* tuber and standard drugs.

Extracts/standard	DPPH		ABTS		Nitric oxide		H ₂ O ₂	
	IC ₅₀	R ²	IC ₅₀	R ²	IC ₅₀	R ²	IC ₅₀	R ²
Aqueous	1.870	0.929 1	0.098	0.977 7	0.136	0.895 6	1.787	0.559 6
Acetone	0.300	0.967 6	0.065	0.984 5	0.620	0.998 9	0.126	0.959 3
Ethanol	0.398	0.937 8	0.054	0.927 4	0.151	0.974 2	0.057	0.936 0
BHT	0.015	0.985 7	0.003	0.941 8	0.029	0.989 7	≪ 0.025	0.724 6
Vit C	0.022	0.795 4	0.032	0.966 7	0.195	0.864 2	≪ 0.025	0.772 9

R² is coefficient of determination; values obtained from the regression lines with 95% confidence level. ≪: Far lesser than the lowest concentration used in the assay.

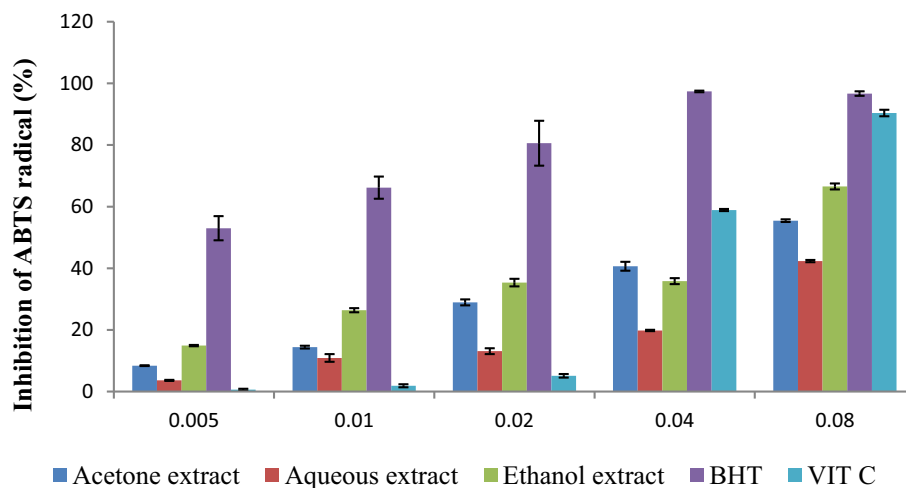


Figure 2. ABTS radical scavenging activity of different extracts of *K. africana*.

extract, at the highest concentration (0.40 mg/mL). The IC₅₀ values of the tested extracts/standards were in the order: BHT > aqueous extract > ethanol extract > Vit C > acetone extract. With aqueous extract possessing the lowest IC₅₀ indicative of its strong nitric oxide scavenging activity as shown in Table 2.

3.2.4. Hydrogen peroxide scavenging assay

The scavenging ability of the plant extracts against hydrogen peroxide is shown in Figure 4. The scavenging activity of the plant in different solvents followed the order: Vit C > aqueous extract > BHT > ethanol extract > acetone extract at the highest

concentration (0.40 mg/mL). The percentage inhibition of hydrogen peroxide in the different solvents was concentration dependent and significantly different ($P < 0.05$). The ethanol extract had highest radical scavenging potential among the extracts used but did not scavenge as much as BHT and Vit C. The IC₅₀ values of the tested extracts/standards were in the order: BHT > Vit C > ethanol extract > acetone extract > aqueous extract (Table 2).

3.3. Bacterial minimum inhibitory concentration (MIC)

The results of the antibacterial MIC using agar dilution are shown in Table 3. The results revealed that both Gram positive

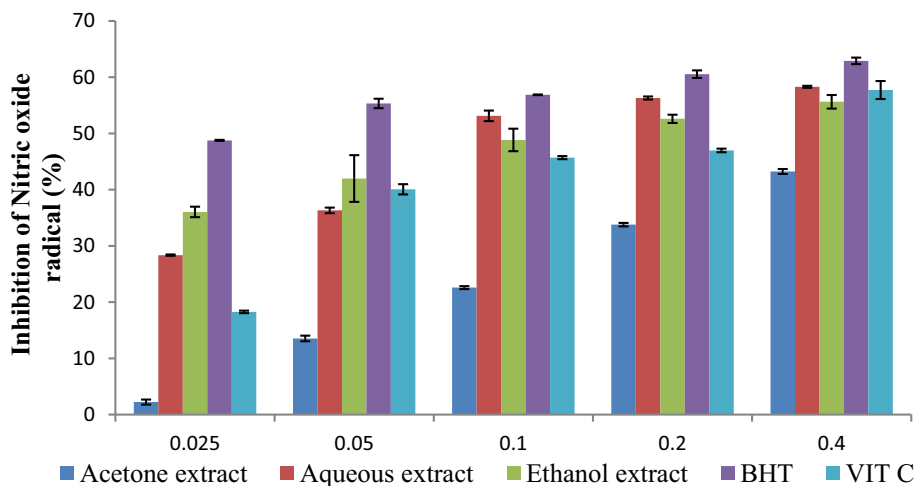


Figure 3. Nitric oxide radical scavenging activity of different extracts of *K. africana*.

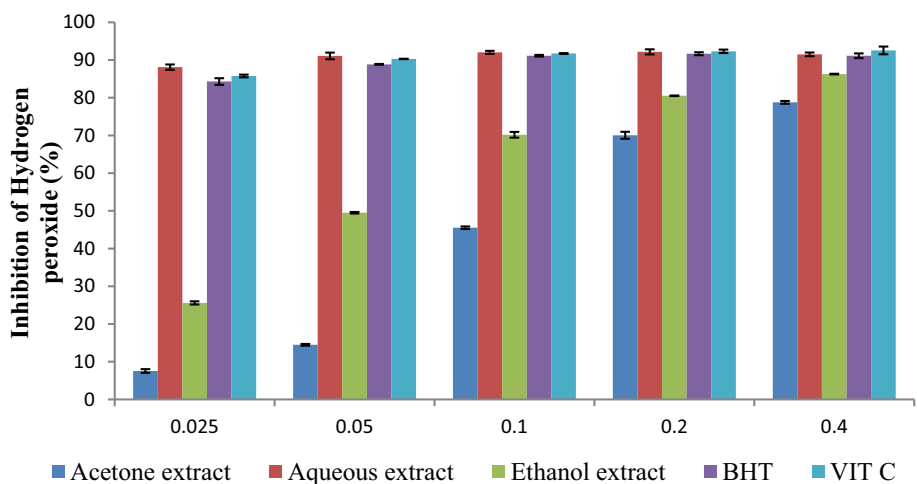


Figure 4. Hydrogen peroxide scavenging activity of different extracts of *K. africana*.

Table 3

MICs of different solvent extracts of *K. africana* on selected Gram negative and Gram positive bacteria.

Bacteria	Gram	Acetone (mg/mL)	Aqueous (mg/mL)	Ethanol (mg/mL)	Ciprofloxacin ($\mu\text{g/mL}$)
<i>B. thetaiotomicron</i>	–	> 5	> 5	> 5	2
<i>Actinomyces odontolyticus</i>	+	> 5	> 5	2.5	2
<i>Lactobacillus sakei</i>	+	> 5	> 5	5	2
<i>Enterobacter cloacae</i>	–	> 5	5	5	2
<i>Pseudomonas aeruginosa</i>	–	> 5	5	2.5	2
<i>Staphylococcus aureus</i>	+	> 5	5	5	32

(+ve) and Gram negative (–ve) bacteria tested were susceptible to the various extracts of *K. africana*. The MIC values ranged from 2.5 mg/mL to > 5 mg/mL for both Gram –ve and Gram +ve bacteria. The acetone extract exhibited the lowest activity on both Gram –ve and Gram +ve bacteria with MIC values greater than 5 mg/mL. The standard drug

(ciprofloxacin) showed higher antibacterial activity with MIC values ranging from 2 $\mu\text{g/mL}$ to 32 $\mu\text{g/mL}$.

The antifungal activities of *K. africana* extracts on some selected fungi associated with obesity are shown in Table 4. The MIC was taken after 3 d incubation. The acetone and ethanol extracts were more active against the four fungi used. Its MIC

Table 4

MIC of different solvent extracts of *K. africana* on selected human pathogenic fungi.

Fungi	Acetone (mg/mL)	Aqueous (mg/mL)	Ethanol (mg/mL)	Nystatin ($\mu\text{g/mL}$)
<i>Microsporium gypsum</i>	5	> 5	5	8
<i>Penicillium chrysogenum</i>	0.312 5	> 5	0.312 5	4
<i>T. tonsurans</i>	> 5	> 5	> 5	4
<i>Candida albicans</i>	0.312 5	> 5	0.312 5	> 16

ranged from (0.312 5–5.000 0) mg/mL except for *T. tonsurans* whose MIC was greater than 5 mg/mL. The aqueous extract showed the least activity among all the extracts used with an MIC value greater than 5 mg/mL for all the fungi used for the study.

4. Discussion

Lately, enhanced diets have been employed to combat certain health conditions in humans. Polyphenolic compounds, inherent in plant extracts elicit a myriad of biological activities among which is their antioxidant ability [23]. At present, several studies have highlighted the potential health benefits of polyphenols and their pharmacological potentials which include antiulcerogenic [24], anti-estrogenic [25], anti-diabetic [26], anticancerogenic [27] and anti-inflammatory effects [28]. Moreover, they rarely produce little or no toxic effect upon their ingestion [29]. Antioxidants play a major role in scavenging oxidative free radicals. Phenols such as flavonoids and terpenoids, chiefly exert their antioxidant potentials by mopping up free radicals and reactive oxygen species [30]. The results of the current study demonstrated that the various extracts of *K. africana* possessed good free radical scavenging activity. The three different extracts used contain an array of polyphenolics with appreciable amounts of proanthocyanidin and flavonoid [30]. In the present study, the ability of the three extracts of *K. africana* to scavenge free radicals. DPPH, ABTS, nitric oxide and hydrogen peroxide were used to determine the antioxidant activity [31]. The IC₅₀ was employed to ascertain antioxidant efficacy of the samples. The lower the IC₅₀ value of the sample, the greater its ability to scavenge the radical. From the results obtained, the synthetic antioxidant BHT and Vit C demonstrated the best antioxidant effectiveness for most of the antioxidant assays. The antioxidant activities of the extracts could be due to high levels of polyphenols present in the various extracts. Compared with the acetone and aqueous extracts, the ethanol extract of *K. africana* tuber elicited superior scavenging ability for ABTS, nitric oxide and hydrogen peroxide assays; the acetone extract scavenged best the DPPH radicals. These results suggest that *K. africana* extracts possess good free radical scavenging potentials and could act as a natural source of antioxidants. In recent times, bacteria have become increasingly resistant to conventional antibiotics [32], at an alarming rate [33]. This has created a desperate need for new antibacterials [33]. We evaluated the antimicrobial activity of *K. africana* plant extracts and determined the MIC. The *K. africana* extracts were found to possess high antimicrobial activity; even crude extracts of this plant demonstrated strong activity against multidrug resistant strains. All the tested bacterial strains showed resistance to the acetone extract. In the aqueous extract, *B. theraiotomicron*, *A. odontolyticus* and *L. sakei* were resistant while *B. theraiotomicron* was resistant to ethanol extract. All the tested fungi strains were resistant to the aqueous extract while the acetone and ethanol extracts were not active against *T. tonsurans*. The resistance exhibited by *T. tonsurans* to the tested crude extracts could be due to the presence of more complex cell wall in fungi. Furthermore, the observed non-activity of some plant extracts used in this study could be attributed to the interference of synergism between active compounds or a very low concentration of the

compounds present in the crude extracts that are active against the various organisms. The choice of different solvents for extraction in this study were based on our previous observations and other reports that water extracts of the plants generally showed little or no antimicrobial activities [34,35]. The weak activity of the aqueous extracts against Gram-positive and Gram-negative bacteria investigated in this study is in agreement with previous reports [36,37]. Generally, Gram-negative bacteria have been reported to be more resistant to plant extracts than the Gram-positive strains [38]. This is also reaffirmed in this study. However, it is worthy of note that the acetone extracts in this study also exhibited weak activity like the aqueous extract against both Gram-positive and Gram-negative bacteria investigated in this study. The results also show that the ethanol extracts have significant antimicrobial activity against both bacteria and fungi tested. According to Farasat *et al.* [39], the antimicrobial effects of medicinal plants could be attributed to the active components in the crude extracts which could act in synergy.

The tuber of *K. africana*, which contains a high amount of flavonoids and phenolic compounds, exhibit high antioxidant and free radical scavenging activities. The *in vitro* assays demonstrate that this plant extract could be a noteworthy resource of an innate antioxidant, which may help in preventing the development of diverse oxidative stress-related diseases. The results of our study revealed superior antioxidant and antimicrobial activities in ethanol extracts of *K. africana*. These results support the notion that a diet rich in herbs and plants can aid in the possible reduction of free radicals and microbial growth and could act as a defense against associated disorders. Although our findings indicate that *K. africana* possess antimicrobial and antioxidant properties, further research on the isolation and formulation of active ingredients from the leaves should be conducted to further its therapeutic applications.

Conflict of interest statement

Authors declare that there is no conflict of interest.

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