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Phytochemical screening and *in vitro* evaluation of antioxidant and antimicrobial activities of *Kedrostis africana* (L.) Cogn

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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 IC_{50} 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 IC_{50} value of 0.135 7 mg/mL for nitric oxide while the acetone extract had an IC_{50} 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 additional 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 Nambia 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].

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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 ($AlCl_3$), 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 $AlCl_3$. 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% $AlCl_3$ 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: $y = 154.45x$,

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