NAMIBIA JOURNAL FOR RESEARCH SCIENCE & TECHNOLOGY

Namibian Journal for Research, Science and Technology

NCRST
National Commission On Research Science & Technology

Volume 5 Issue 2
DOI: 10.54421/njrst.v5i2

Original Research Article

Nutritional Value and Antimicrobial Activity of Selected Edible Tubers of Namibia

[1*]C. Mukakalisa, [2]R.H. Hans, [2]K.M. Kalili, [1]M.A. Kandawa-Schulz, [3]M. Beukes

*[1]Department of Biochemistry, Microbiology and Biotechnology, Faculty of Agriculture, Engineering and Natural Sciences, University of Namibia, Namibia
[2]Department of Physics, Chemistry and Material Science, Faculty of Agriculture, Engineering and Natural Sciences, University of Namibia, Namibia
[3]Department of Biochemistry, Faculty of Science, Stellenbosch University, South Africa

*Corresponding author: cmukakalisa@unam.na

How to cite this article:
Hans, R., Mukakalisa, C., Kalili, K., Kandawa-Schulz, M., & Beukes, M. (2024). Nutritional Value and Antimicrobial Activity of Selected Edible Tubers of Namibia. *Namibian Journal for Research, Science and Technology*, *5*(2), 37-46. https://doi.org/10.54421/njrst.v5i2.111

ARTICLE INFO

Received: Sept 2022
Accepted: Sept 2023
Published: Nov 2024

Keywords:
edible tubers, proximate content, antimicrobial, antioxidant, antibiofilm, cytotoxicity

ABSTRACT

Indigenous, leafy vegetables have been extensively studied but edible, tuberous vegetables are relatively underexplored in terms of their nutritional and medicinal values. This study aimed to evaluate the macronutrient, total phenolic and flavonoid contents as well as the antioxidant, cytotoxicity, antimicrobial and antibiofilm activities of six edible tubers from Namibia. Proximate analysis revealed that the tubers have a high total carbohydrate and energy content, with the highest values recorded for *Walleria nutans*. The high protein content of 13 g/100 g dry mass recorded for *Coccinea rehmannii*, supports the use of members of this genus as nutritional protein supplements. The best, albeit poor, antimicrobial activity was recorded for the dichloromethane (DCM) extract of *Eulophia hereroensis* with an MIC of 2.5 mg/mL against *Eschericia coli*. Evaluation of the antibiofilm activity against *Staphylococcus aureus* showed that the DCM extract of *Walleria nutans* displayed the best biofilm formation inhibitory activity, whereas *E. hereroensis* exhibited the best biofilm eradication activity. A high total phenolic content and strong antioxidant activity was recorded for the aqueous methanolic extract of *E. hereroensis*, with IC_{50}s of 0.056 ±0.000 and 0.041 ±0.003 mg/mL obtained with the DPPH and H_2O_2 scavenging assays, respectively. All tubers showed little to no cytotoxicity with CC_{50} > 200 µg/mL against the VERO cell line. The macronutrient content, in vitro antioxidant and antimicrobial activities of the selected tuberous vegetables are reported here for the first time. *Eulophia hereroensis* and *W. nutans* showed potential as functional foods and merit an in-depth phytochemical study to isolate and characterize the phenolic compounds and potential antibiofilm agents.

1. Introduction

Positive psychology Of the approximately 23 000 plant species found in the Southern African region, 1740 are reported to be edible (Welcome & Van Wyk, 2019). Indigenous food plants contribute significantly towards food and nutrition security, especially in

developing countries (Shackleton et al., 2009; Bokelmann et al., 2022), but are relatively underutilized and sometimes erroneously referred to as "poor man's vegetables" (Legwaila et al., 2011; Masarirambi et al., 2022). Whatsmore, these vegetables are tolerant to drought, poor soils, local pests and offer further appeal by being easily accessible as well as fast-growing, rendering them available for harvesting before the cultivation of staple crops (Sujatha and Renuga, 2013; Kolberg, 2001). Indigenous, leafy vegetables, for example, *Amaranthus thunbergii*, *Cleome gynandra*, and *Chorcorus olitorius* are rich in minerals, carbohydrates, proteins (Legwaila et al., 2011; Afolayan and Jimoh, 2007) and some reportedly display medicinal properties (Kim et al, 2013). On the other hand, indigenous root and tuber vegetables, of which the most commonly consumed are yam, cassava, sweet potatoes and potatoes, are also nutritionally diverse (Lyimo et al., 2007; Maliro,2001) but unlike their leafy counterparts have not been extensively studied.

The ability to produce heat-resistant toxins, to be heat-resistant (Bintsis, 2017) and the development of biofilms, that is, to offer protection against the human hosts' immune system (Gajewska and Chajęcka-Wierzchowska, 2020), are among the survival strategies developed by some foodborne bacteria. Of concern is the development of antimicrobial resistance, which serves as the key motivator for the continued search for novel plant-based drugs which exert their activity through new mechanisms of action. ESKAPE microorganisms such as *Staphylococcus aureus* and *Klebsiella pneumoniae* are characterized by their ability to form biofilms which, in addition to contributing towards their virulence (Gajewska and Chajęcka-Wierzchowska, 2020), serve as a barrier against most antibiotics and sterilizing agents (Bi et al, 2021). The multifunctional properties of indigenous vegetables, high in natural antioxidants such as phenolics and flavonoids, is further evidence by reports that their consumption has been associated with reduced incidences of diseases like cancer, cardiovascular as well as cerebrovascular diseases (Das et al., 2012; Campos et al, 2006).

The tubers selected for this study (Fig. 1) included: *Brachystelma gymnopodum* and *Fockea angustifolia* (Apocynaceae)*; Coccinea rehmannii* and *Trochomeria macrocarpa* (Cucurbitaceae); *Eulophia hereroensis* (Orchidaceae) and *Walleria nutans* (Tecophilaeaceae). These edible tuberous vegetables are frequently consumed by the Jul'hoansi ethnic group of the Khoisan who resides in the Nyae Nyae Conservancy in the Otjozondjupa region of Namibia (Leffers, 2003), and their nutritional, medicinal and phytochemical properties are unexplored. This study was therefore aimed at evaluating the proximate composition, antimicrobial activity as well as the antibiofilm activity of tuber extracts against *S. aureus,* a foodborne pathogen and prolific biofilm producer. It was further aimed at evaluating the antioxidant and cytotoxic activities, as well as to quantify the phenolic and flavonoid contents of the indigenous tubers.

Figure 1. Indigenous tuberous vegetables selected for the study

2. Materials and Methods

2.1 Reagents and Solvents

This All the reagents and solvents used in the study were of analytical grade. Folin-Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, quercetin, ascorbic acid (vitamin C), aluminum chloride, sodium carbonate, sodium nitrite, sodium hydroxide, hydrogen peroxide (30% H_2O_2), iron (III) chloride, dimethyl sulfoxide, were purchased from Sigma-Aldrich (Germany). Cultures of *Candida albicans* ATCC13933, *Escherichia coli* ATCC700928, *Staphylococcus aureus* ATC12600, and *Klebsiella pneumoniae* ATCC10556 as well as the cell lines used for cytotoxicity analysis, Vero (ATCC® CCL-81), and HT-29 (ATCC® HTB-38), were obtained from the American Type Culture Collection (ATCC).

2.2 Collection and Preparation of Plant Material

Fresh plant samples were collected in Tsumkwe (19°35′12.2″ S, 20°30′39.9″E) and surrounding villages, in the Otjozondjupa region of Namibia (research/collection permit number: 1941/2014) during January 2016. For each tuber, a sample of the whole plant was collected, pressed and submitted for taxonomic identification at the National Botanical Research Institute (NBRI) of Namibia (National Herbarium of Namibia, WIND ID report number: 2015/372). The tubers were cut into thin discs and placed in a -80°C freezer overnight. The frozen tubers were freeze-dried using a Biobase freeze-dryer, ground to a powder and stored in dark airtight bottles at -4°C in the fridge until needed for analysis.

2.3 Proximate Analysis

Proximate content of the tubers was determined using

standard methods of the Association of Official Analytical Chemists (AOAC, 1999).

2.4 In vitro Antimicrobial Activity

2.4.1 Preparation of tuber extracts for antimicrobial activity testing

Powdered plant material was successively extracted with organic solvents in the order of increasing polarity using a method described by Jeyasselan and his colleagues (2012) with few modifications. Briefly, 100 g of the powdered tuber was soaked in 300 mL hexane and place on an orbital shaker at room temperature for 48 hours. Filtration of the extract with a double layered muslin cloth was followed by filtration using Whatman No.1 filter paper. The resulting residue was air dried and used for further extraction with dichloromethane (DCM) followed by ethyl acetate, acetone, ethanol and double distilled water using the same procedure. The solvents were evaporated under reduced pressure using a rotary evaporator and the percentage yield of the extracts calculated.

2.4.2 Test Microorganisms and Growth Conditions

The microorganisms used for this study included *Candida albicans* ATCC13933, *Escherichia coli* ATCC700928, *Staphylococcus aureus* ATC12600, and *Klebsiella pneumoniaea* ATCC10556. They were rehydrated in nutrient broth for 48 hours. Stock cultures were prepared and kept at -70ºC until needed. The strains were grown in nutrient broth at 37ºC. To standardize the inoculum, the culture was grown overnight on an agar plate. One or two colonies was transferred into a test tube containing 6 mL broth and incubated at 37ºC.

2.4.3 Agar Disc Diffusion Method

The disc diffusion method used was reported by Pochapshi and colleagues (2011), with few modifications. The extracts were diluted to different concentrations (1 mg/mL to 10 mg/mL), and 10 µL of each concentration was spotted on a disc, allowed to dry at room temperature then placed on a previously inoculated nutrient agar plate. Plates were incubated at 37°C and inhibition zones (IZ) recorded.

2.4.4 Minimum Inhibitory Concentration (MIC)

Agar disc diffusion method was used with different concentrations. The lowest concentration to give a clear zone was determined as the MIC. The test was carried out in triplicate.

2.5 Antibiofilm Assay

2.5.1 Biofilm Inhibition of *S. aureus* by the DCM extracts of *E. hereroensis, W. nutans* and *T. macrocarpa*

The DCM extracts of *Eulophia hereroensis, Walleria nutans* and *Trochomeria macrocarpa* were tested for biofilm inhibitory activity using a method described by Mutalib and colleagues (2015) with minor modifications. To a sterile 96-well plate, 150 µL of broth and 50 µL were added to each well. An aliquot of 50 µL of the fresh bacterial suspension, adjusted with 0.5 McFarland, was added to each well. Positive control (bacterial suspension in broth) and negative control (extract in broth) were included. Following incubation at 37°C for 24 h, the content of each well was gently removed by tapping the plates. The wells were washed with 200 µL of sterile distilled water to remove free-floating bacteria. Biofilms formed by adherent cells in plate were stained with 0.1% crystal violet (Sigma-Aldrich) and incubated at room temperature for 30 minutes. Excess stain was rinsed off by thorough washing with distilled water, taking care not to remove the biofilm, and plates were fixed with 200 µL of 70% ethanol. Optical densities (OD_{630}) of stained adherent bacteria were measured. Percentage inhibition was then calculated using:

$$\%\ inhibition = \frac{(C - T)}{C} \times 100$$

Where C is the OD of wells with strain and T the OD of wells with strain and test sample. The experiment was performed triplicates. $MBIC_{50}$ were calculated using the Graphpad prism software.

2.5.2 Biofilm Reduction/Eradication of *S. Aureus* by the DCM extracts of *E. hereroensis, W. nutans* and *T. macrocarpa*

The reduction or removal of biofilm by the DCM extracts of *E. hereroensis, W. nutans* and *T. macrocarpa* was analyzed using a method described by Abidi, Sherwani, Bibi and Kazmi (2014) with few modifications. To a sterile 96-well microplate, 200 µL of strain was added and incubated overnight (200 µL of media was also incubated to be used as blank). The plate was washed with distilled water to remove planktonic cells. An aliquot of 200 µL of extract was added to the wells (except for control and blank) and incubated for 15 minutes at room temperature. The plate was again washed to remove planktonic cells. Next, 125 µL of 0.1% crystal violet was added to stain the biofilm and incubated at room temperature for 10 minutes. The excess stain was removed and the stain was stabilized with 200 µL of 95% ethanol and incubated at room temperature for 15 minutes. The solution was transferred to a new 96-well plate and OD was measured at 630 nm. The percentage reduction/removal was calculated from the blank, control and test ODs, using the equation below:

$$\%\ reduction = \frac{(C - B) - (T - B)}{(C - B)} \times 100$$

Where C is the OD of the strain, B the OD of the broth and T the OD of strain + extract. The experiment was performed in triplicate. $MBEC_{50}$ were calculated using the Graphpad prism software.

2.6 In vitro Antioxidant activity

2.6.1 Extract preparation

Powdered plant material was extracted with 80% aqueous methanol according to the method described by Josipović and coworkers (2016) with few modifications. Briefly, 100 g of each powder was soaked in 300 ml 80% methanol and place in the shaker at room temperature for 48 hours. They were first filtered with double layered muslin cloth and then through a Whatman No1 filter paper. The solvent was evaporated under reduced

pressure using a rotary evaporator. The percentage yield was calculated for all extracts.

2.6.2 Reducing power assay

The reducing power of tuber extracts was determined according to the procedure described by Saima and coworkers (2013) with few modifications. Various concentrations of 1 mL extracts in deionized water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1% w/v); the mixture was incubated at 50°C for 20 min. Then 2.5 mL of 10% (w/v) trichloroacetic acid was added and the mixture was centrifuged at 3000 g for 10 min at 5°C. The upper layer of the solution (2.5 mL) was mixed with 2.5 mL of distilled water and ferric chloride solution (0.5 mL, 0.1% w/v), and absorbance was measured at 700 nm.

2.6.3 Free radical scavenging assay

The effect of extracts on the DPPH free radical was determined using the method adopted by Anwar and colleagues (2010) with minor modification. The assay was done in 96-well microplate, where 100 µL of DPPH prepared in methanol (0.135 mM) was mixed with 100 µL of different concentrations of extracts solutions. The mixture was left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. The scavenging ability of the plant extract on DPPH was calculated using the equation:

$$DPPH\ scavenging\ activity\ (\%) = \frac{Acontrol - Atest}{Acontrol} \times 100$$

Where $A_{control}$ is the absorbance of DPPH + methanol; A_{sample} is the absorbance of DPPH radical + sample. Data was reported as IC_{50}.

2.6.4 Hydrogen peroxide (H_2O_2) scavenging assay

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method described by Keser et al. (2012) with few modifications. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer saline (pH 7.4). Different concentrations of extracts and ascorbic acid in distilled water (1 mL) were added to a hydrogen peroxide solution (2 mL). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both extracts and standard compound were calculated:

$$\%\ Scavenged = \frac{Acontrol - Atest}{Acontrol} \times 100$$

Where $A_{control}$ is the absorbance of the control and A_{test} is the absorbance of extracts or standards. Data was reported as IC_{50}

2.7 Total phenolic content

Total phenolic content of 80% aqueous methanol extracts was determined using the Folin-Ciocalteu method described by Saeed and coworkers (2012). A 1 mL of sample (1 mg/mL) was mixed with 1 mL of Folin-Ciocalteu's phenol reagent and left to stand at room temperature for 5 minutes. Then 10 mL of a 7% Na_2CO_3 solution was added to the mixture followed by the addition of 13 mL of deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at room temperature; the absorbance was measured at 750 nm. Gallic acid was used as standard. TPC was determined from the extrapolation of the calibration curve, and was expressed as milligrams of Gallic acid equivalents (GAE) per g of dried sample.

2.8 Total flavonoid content

Total flavonoid content was determined using the aluminium chloride colorimetric method described by Josipović and colleagues (2016). A solution of 1 mg/mL of extract was prepared for the test. To 1 mL of extract, 4 mL of distilled water and 0.3 mL of 5 % sodium nitrite solution was added. After 5 minutes, 0.3 mL of 10% aluminum chloride was added. After 6 minutes, 2 mL of 1 M sodium hydroxide was added. Finally, volume was adjusted to 10 mL with distilled water, mixed well and the absorbance was measured at 510 nm. Quercetin was used as standard. TFC was determined from the extrapolation of the calibration curve, and was expressed as milligrams of quercetin equivalents (QE) per g of dried sample

Total phenolic (TPC) and flavonoid (TFC) contents were determined using gallic acid and quercetin standard curves, respectively, and a formula described by Kalita et al (2013).

2.9 Cytotoxicity

2.9.1 Cell culture

Vero (ATCC® CCL-81), and HT-29 (ATCC® HTB-38) cells were obtained from the American Type Culture Collection (ATCC). They were delivered on dry ice then placed in -80°C freezer prior to assay. The cells were maintained in Minimum Essential Medium, with Earle's salts (MEM) with 10% FBS, and antibiotic mixture under defined conditions of temperature at 37°C, 95% humidity and 5% CO_2.

2.9.2 Cell viability assay

Confluent cells were treated with trypsin and collected, counted and diluted to $1x10^4$ cells/mL for analysis. The MTT assay protocol used was reported by Kapewangolo (2015).

To a 96-well plate, 100 µL of 10% media was added. Exactly 100 µL of the test samples were added to row B and a serial dilution was done to the rest of the rows. The first row (A) was left for the blank (containing media only) and for cells without treatment. To the wells, 100 µL of cells was added, and then plate incubated at 37°C, 5% CO_2 for 72 hours. After incubation, 20 µL of MTT was added (in the dark) and plate was incubated at room temperature for 2 hours. To the plate, 50 µL of MTT solubilizing reagent was added to dissolve the formazan, left to stand for 15 min and absorption read at 550 nm. The percentage cell viability was calculated using following formula

$$\%\textbf{Cell viability} = \frac{\textbf{Optical density of compound}}{\textbf{optical density of control}} \times 100$$

Cells were treated at 1×10^4 cells/mL with compound concentrations ranging from 3.125 to 200 µg/mL for the test sample and 0.3125 to 20 µg/mL for the positive control, auranofin.

2.10 Statistical Analysis

All data were collected in triplicate and reported as means with ± standard deviation (SD). $MBIC_{50}$ and $MBEC_{50}$ were calculated for the antibiofilm activity of the extracts. IC_{50} and CC_{50} were determined and reported for antioxidant and cytotoxic activities, respectively.

3. Results and Discussion

3.1 Proximate Analysis

Table 2: Proximate content of the indigenous tubers under investigation

Moisture (% FW)	9.3±1.24	80±1.9	92±0.24	97±1.80	85±1.45	81±0.92
Dry matter (% FW)	7±1.02	14±0.7	8±0.31	3±0.91	17±1.12	19±2.1
Ash (g/100 g DW)	5.7±1.0	8.1±0.09	4±0.32	12±0.09	13±1.25	2.5±0.04
Crude protein (g/100g DW)	5.2±0.42	13±1.03	7.1±1.05	6.4±0.19	9.6±1.53	3.9±0.08
Crude fat (g/100 g DW)	0.8±0.001	0.4±0.003	0.5±0.003	3.2±0.04	0.8±0.001	1.0±0.005
Total carbohydrate (g/100 g DW)	88±1.35	78±2.32	88±0.85	78±0.33	77±1.23	93±1.42
Energy (kcal/100 g DW)	380.0	367.6	384.9	366.4	353.6	396.6

Mean values (n = 3) with ± standard deviation. FW = fresh weight. DW = dry weight. *B. gymnopodum* (BG), *F. angustifolia* (FA), *C. rehmannii* (CR), *T. macrocarpa* (TM), *E. hereroensis* (EH) and *W. nutans* (WN)

Table 1, outlines the proximate content and energy value of the tubers under study. Root and tuber plants are known to have a low protein content, ranging from 1 to 2% of dry mass (Chandrasekara and Kumar, 2016). This study recorded a high protein content for *C. rehmannii* (13±1.03%), *T. macrocarpa* (9.6±1.53%) and *E. hereroensis* (7.1±1.05%), which evidenced the potential of these tubers to serve as protein supplements for the management of kwashiorkor, a disease caused by severe protein malnutrition (Schönfeldt and Hall, 2012). Also noted, is the high total carbohydrate content recorded for the tubers, with values ranging from 77 to 93 g/100 g dry weight, and for which *W. nutans* deserve special mention.

3.3 Antimicrobial Activity

As shown in table 2, all the solvent extracts of *C. rehmannii* and *F. angustifolia* were devoid of antimicrobial activity. The DCM extracts of the remaining tubers, with the exception of *B. gymnopodum*, showed poor to moderate activity against the four strains, with *E. hereroensis* exhibiting the best activity with an MIC of 2.5 mg/mL against *E. coli* and 5 mg/mL against *K. pneumoniae*. These results are supported by previous reports on the activity displayed by the hydroethanolic root extract of *E. hereroensis* against *K. pneumoniae* (Chinsamy, 2012). The results obtained further indicated, that all water extracts were devoid of activity against the different strains, which is in support of previous reports on the lack of antimicrobial activity of water extracts, albeit it the solvent of choice for the preparation of herbal formulations (Igbinosa et al, 2009; Javale and Sabnis, 2009).

Based on the antimicrobial activity profile of the DCM extracts (Table 2), the minimum biofilm inhibitory concentration ($MBIC_{50}$) and minimum biofilm eradication concentration ($MBEC_{50}$) of the DCM extracts of *W. nutans, E. hereroensis, T. macrocarpa* (Table 3), was recorded. The biofilm inhibition/eradication activity of the extracts showed a dosage dependent action. *Eulophia hereroensis* displayed the best eradication activity against the preformed biofilm, while *W. nutans* exhibited the best biofilm inhibitory activity and could serve as a potential source of antibiofilm agents. The biofilm formation ability of microorganisms has been implicated in the development of antibiotic resistance and can prolong or complicate the treatment of life-threatening bacterial infections (Marić and Vraneš, 2007). Of note is the renewed interest directed towards plant extracts as a source of potential antibiofilm drugs (Nogueira et al, 2017)

Table 1 Antimicrobial activity of the solvent extracts for the different tubers

Sample	Solvent	Inhibition zone (mm)				Minimum inhibition concentration (mg/mL)			
		E. coli	*C. albicans*	*K. pneumoniae*	*S. aureus*	*E. coli*	*C. albicans*	*K. pneumoniae*	*S. aureus*
B. gymnopodum	Hexane	-	-	-	-	-	-	-	-
	DCM	-	-	-	-	-	-	-	-
	Ethyl acetate	5.5 (±0.7)	5.5 (±0.7)	-	-	10	10	-	-
	Acetone	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-
	Water	-	-	-	-	-	-	-	-
E. hereroensis	Hexane	-	-	-	-	-	-	-	-
	DCM	10.5 (±0.7)	9.0 (±0.7)	10.0 (±0.0)	12.5 (±1.4)	2.5	10	5	10
	Ethyl acetate	8.5 (±2.1)	6.5 (±0.7)	7.0 (±0.0)	10.5 (±1.4)	10	10	10	10
	Acetone	7.5 (±0.7)	5.5 (±0.7)	5.5 (±0.7)	7.0 (±0.7)	10	10	10	10
	Ethanol	-	-	-	-	-	-	-	-
	Water	-	-	-	-	-	-	-	-
T. macrocarpa	Hexane	-	-	-	-	-	-	-	-
	DCM	7.5 (±0.7)	8.5 (±2.1)	6.5 (±0.7)	11.5 (±1.4)	5	10	10	10
	Ethyl acetate	-	-	-	-	-	-	-	-
	Acetone	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-
	Water	-	-	-	-	-	-	-	-
W. nutans	Hexane	-	-	-	-	-	-	-	-
	DCM	7.0 (±0.00)	5.5 (±0.7)	5.5 (±0.7)	11.5 (±1.4)	10	10	10	10
	Ethyl acetate	5.5 (±0.7)	-	-	-	10	-	-	-
	Acetone	5.5 (±0.7)	5.5 (±0.7)	5.5 (±0.00)	-	10	10	10	-
	Ethanol	-	-	-	-	-	-	-	-
	Water	-	-	-	-	-	-	-	-

Table 3: Biofilm activity of three tuber extracts against S. aureus

	$MBIC_{50}$ and $MBEC_{50}$(mg/mL)	
Sample	**Biofilm inhibition**	**Biofilm reduction**
EH	1.960±0.040	0.785±0.023
TM	>5	8.347±0.786
WN	1.348±0.224	2.463±0.170

3.4 Antioxidant activity

The antioxidant activity of the 80% aqueous methanol extract of the tubers is reported here for the first time, and was evaluated using the DPPH free radical scavenging, hydrogen peroxide scavenging, and reducing power assays. As shown in figure 2, the reducing power of the extracts is dose-dependent with the best activity recorded for the extract of *T. macrocarpa*. The extracts showed potential as antioxidants, with *C. rehmannii, E. hereroensis* and *W. nutans* displaying almost equipotent activity at the different concentrations tested.

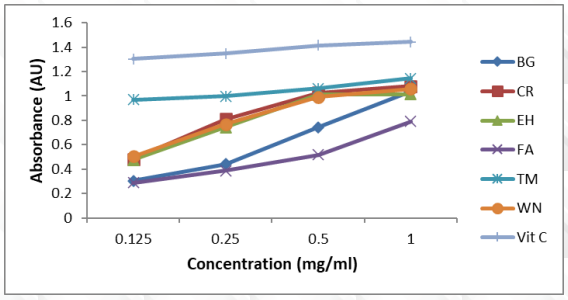

Figure 2 The reducing power of the 80% aqueous methanol extracts of the indigenous tubers under study

For both the hydrogen peroxide and DPPH scavenging assays, the activity of the tuber extracts demonstrated a concentration-dependent response (Table 4). The IC_{50} value obtained with the DPPH assay ranged from 0.033±0.001 mg/mL to 0.232 ±0.001 mg/mL, indicating strong antioxidant activity. The best activity was observed for *B. gymnopodum* with an IC_{50} of 0.033 mg/mL followed by *C. rehmannii* and *E. hereroensis*, which displayed almost equipotent activity, with IC_{50}s of 0.054 ±0.001 and 0.056 ±0.0003, respectively.

Table 4 IC_{50} values obtained with the DPPH and H_2O_2 scavenging assays as well as the total phenolic and flavonoid contents for the six tubers

Sample	IC_{50} (mg/mL)		TPC	TFC
	DPPH scavenging assay	H_2O_2 scavenging assay	µg GAE/g dry sample	µg QE/g dry sample
B. gymnopodum	0.033 ±0.001	0.219 ±0.011	1626±12.47	431±6.90
C. rehmannii	0.054 ±0.001	0.122 ±0.002	520±1.35	174±1.35
E. hereroensis	0.056 ±0.0003	0.041 ±0.002	3822±7.05	527±29.87
F. angustifolia	0.066 ±0.001	0.086 ±0.016	3334±1.26	457±7.05
T. macrocarpa	0.073 ±0.001	0.080 ±0.0002	761±6.90	321±1.26
W. nutans	0.232 ±0.001	0.092 ±0.003	570±29.87	460±12.47

Although hydrogen peroxide is considered a weak oxidizing agent, it has the ability to deactivate enzymes through oxidation of their thiol groups (Keser et al, 2012). *Eulophia hereroensis* showed the best scavenging activity with an IC_{50} of 0.041±0.002 mg/mL followed by *T. macrocarpa* with an IC_{50} of 0.080±0.0002 mg/mL. Most of these different tubers showed promising antioxidant activity with IC_{50} values less than the reference value of 100 µg/mL according to Jadid and colleagues (Jahid et al, 2017).

3.5 Phenolic Content

Phenolic compounds represent a universal class of phytochemicals, and their dietary intake offer a range of health benefits, including the prevention of chronic diseases (Das et al., 2012; Campos et al, 2006). *Eulophia hereroensis* showed the highest TPC and TFC with values of 3822 µg GAE/g dry sample and 527 µg QE/g dry sample, respectively (Table 4).

3.6 Cytotoxicity Testing

The toxicity effect of the crude 80% methanol extracts was evaluated using the MTT assay with auranofin as positive control. Cell viability of the extracts were reported. Figures 3 show the effect of the extracts on two cell lines, Vero African green monkey kidney cell line (ATCC®CCL-81™) and a human colorectal adenocarcinoma cell line, HT-29 (ATCC®HTB-38™).

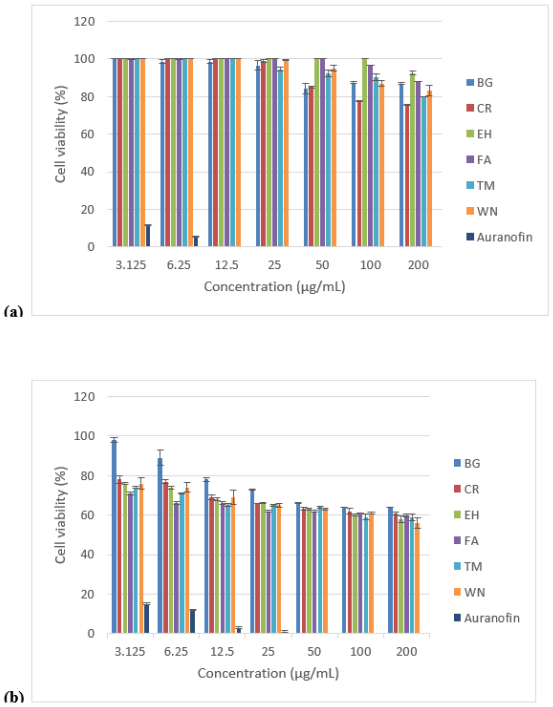

Figure 3 Cytotoxic activity of tuber extracts against (a) Vero and HT-29 cell lines.

The extracts demonstrated a dose-dependent response with a 50% cytotoxic concentration (CC50) value > 200 µg/mL. Auranofin was used as a standard control and exhibited a CC50 value of < 3.125 µg/mL.

According to the American National Cancer Institute (NCI), a CC50 < 30 µg/mL for crude extracts can be considered cytotoxic (Talib and Mahasneh, 2010), therefore, as shown in figure 3(a), the hydromethanolic extracts of all the tubers are non-toxic. Figure 3(b) showed, that extracts of E. hereroensis and W. nutans gave a high cell decline with viabilities of 58±1.77% and 56±2.83%, respectively, which revealed their potential to serve as cancer-protective agents, as reported for most plant-based foods with good antioxidant activities (Raffoul et al, 2012).

4. Conclusions

The findings of this study revealed that the tubers of *E. hereroensis* and *W. nutans* are sources of macronutrients and potential antimicrobials, demonstrating their dual purpose as food and medicine. This study singled out three tubers, namely *B. gymnopodum*, *E. hereroensis* and *W. nutans* for further analysis to characterize the dietary phenolic compounds t and antibiofilm agents, as well as to expand screening in other cancer models, for example, cervical and breast cancers.

5. Acknowledgements

The authors are grateful to the Southern African Biochemistry and Informatics for Natural Products (SABINA) for funding the project. The financial contribution of the National Commission on Research

and Science and Technology (NCRST) and National Research Foundation (NRF) Collaborative Research Programme towards the plant collections trips is also acknowledged.

References

AOAC. (1999). Official methods of analysis, 16th edn. Association of Official Analytical Chemists, Washington.

Abidi, S. H., Ahmed, K., Sherwani, S. K., & Kazmi, S. U. (2014). Reduction and removal of *Pseudomonas aeruginosa* biofilm by natural agents. *Int J Chem Pharm Sci*, *5*, 28-34.

Afolayan, A. J., & Jimoh, F. O. (2009). Nutritional quality of some wild leafy vegetables in South Africa. *International journal of food sciences and nutrition*, *60*(5), 424-431 https://doi.org/10.1080/09637480701777928.

Anwar, F., Qayyum, H. M. A., Hussain, A. I., & Iqbal, S. (2010). Antioxidant activity of 100% and 80% methanol extracts from barley seeds (*Hordeum vulgare* L.): stabilization of sunflower oil. *Grasas y aceites*, *61*(3), 237-243.

Bi, Y., Xia, G., Shi, C., Wan, J., Liu, L., Chen, Y., ... & Liu, R. (2021). Therapeutic strategies against bacterial biofilms. *Fundamental Research*, *1*(2), 193-212.

Bintsis, T. (2017). Foodborne pathogens. *AIMS microbiology*, *3*(3), 529.

Bokelmann, W., Huyskens-Keil, S., Ferenczi, Z., & Stöber, S. (2022). The Role of Indigenous Vegetables to Improve Food and Nutrition Security: Experiences From the Project HORTINLEA in Kenya (2014–2018), doi: 10.3389/fsufs.2022.806420.

Campos, D., Noratto, G., Chirinos, R., Arbizu, C., Roca, W., & Cisneros-Zevallos, L. (2006). Antioxidant capacity and secondary metabolites in four species of Andean tuber crops: native potato (Solanum sp.), mashua (*Tropaeolum tuberosum* Ruiz & Pavón), Oca (*Oxalis tuberosa* Molina) and ulluco (*Ullucus tuberosus* Caldas). *Journal of the Science of Food and Agriculture*, *86*(10), 1481-1488, https://doi.org/10.1002/jsfa.2529

Chandrasekara, A., & Josheph Kumar, T. (2016). Roots and tuber crops as functional foods: a review on phytochemical constituents and their potential health benefits. *International journal of food science*, 2016, doi: 10.1155/2016/3631647.

Chinsamy. M. (2012). South African medicinal orchids: A pharmacological and phytochemical evaluation. University of KwaZulu-Natal.South Africa

Das, A., Chaudhuri, D., Mandal, N., & Chatterjee, A. (2012). Study of antioxidant and reactive oxygen species scavenging activity of the edible tuber of "greater yam" (*Dioscorea alata* L.) from north-east India. *flora*, *11*, 12.

Dincer, S., Uslu, F.M. & Delikl, A. (2020) Antibiotic Resistance in Biofilm. in *Bacterial Biofilms*, IntechOpen S. Dincer, M. S. Özdenefe, and A. Arkut, Eds. p. 13.

Gajewska, J., & Chajęcka-Wierzchowska, W. (2020). Biofilm formation ability and presence of adhesion genes among coagulase-negative and coagulase-positive staphylococci isolates from raw cow's milk. *Pathogens*, *9*(8), 654, doi: 10.3390/pathogens9080654.

Igbinosa, O. O., Igbinosa, E. O., & Aiyegoro, O. A. (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *African journal of pharmacy and pharmacology*, *3*(2), 058-062.

Jadid, N., Hidayati, D., Hartanti, S. R., Arraniry, B. A., Rachman, R. Y., & Wikanta, W. (2017, June). Antioxidant activities of different solvent extracts of *Piper retrofractum* Vahl. using DPPH assay. In *AIP conference proceedings* (Vol. 1854, No. 1, p. 020019). AIP Publishing LLC, doi: 10.1063/1.4985410.

Javale, P., & Sabnis, S. (2010). Antimicrobial properties and phytochemical analysis of *Emblica officinalis*. *Asian J Exp Biol Sci*, *91*, 111.

Jeyaseelan, E. C., Jenothiny, S., Pathmanathan, M. K., & Jeyadevan, J. P. (2012). Antibacterial activity of sequentially extracted organic solvent extracts of fruits, flowers and leaves of *Lawsonia inermis* L. from Jaffna. *Asian Pacific journal of tropical biomedicine*, *2*(10), 798-802.

Josipović, A., Sudar, R., Sudarić, A., Jurković, V., Matoša Kočar, M., & Markulj Kulundžić, A. (2016). Total phenolic and total flavonoid content variability of soybean genotypes in eastern Croatia. *Croatian journal of food science and technology*, *8*(2), 60-65.

Kalita, P., Tapan, B. K., Pal, T. K., & Kalita, R. (2013). Estimation of total flavonoids content (TFC) and antioxidant activities of methanolic whole plant extract of *Biophytum sensitivum* Linn. *Journal of Drug delivery and Therapeutics*, *3*(4), 33-37.

Kapewangolo, P., Omolo, J. J., Bruwer, R., Fonteh, P., & Meyer, D. (2015). Antioxidant and anti-inflammatory activity of *Ocimum labiatum* extract and isolated labdane diterpenoid. *Journal of Inflammation*, *12*(1), 1-13.

Keser, S., Celik, S., Turkoglu, S., Yilmaz, O., & Turkoglu, I. (2012). Hydrogen peroxide radical scavenging and total antioxidant activity of hawthorn. *Chem J*, *2*(1), 9-12.

Kim, S. J., Cho, A. R., & Han, J. (2013). Antioxidant and antimicrobial activities of leafy green vegetable extracts and their applications to meat product preservation. *Food control*, *29*(1), 112-120, doi: 10.1016/j.foodcont.2012.05.060.

Kolberg, H. (2001). Indigenous Namibian leafy vegetables: A literature survey and project proposal. *Agricola*, *12*, 54-60. [Online]. Available: https://www.nbri.org.na/sites/default/files/Agricola2001_No12_12_Kolberg.PDF.

Leffers, A. (2003). *Gemsbok bean & Kalahari truffle: Traditional plant use by Jul'hoansi in north-eastern Namibia*. Gamsberg Macmillan.

Legwaila, G. M., Mojeremane, W., Madisa, M. E., Mmolotsi, R. M., & Rampart, M. (2011). Potential of traditional food plants in rural household food security in Botswana.

Lyimo, M. E., Gimbi, D. M., & Shayo, N. B. (2007, May). Nutritional evaluation of composite flour based on root and tuber crops and sensory acceptability of the developed products. In *Proceedings of the 13th ISTRC symposium* (pp. 514-520)..

Maric, S. (2007). Characteristics and significance of microbial biofilm formation. *Period. Biol.*, *109*, 1-7, doi: 10.1016/j.fmre.2021.02.003.

Maliro, M. F. (2001). The role and potential of traditional tuber crops in Malawi. In *Combating Desertification with Plants* (pp. 55-64). Springer, Boston, MA, doi: 10.1007/978-1-4615-1327-8_5.

Masarirambi, M. T., Nxumalo, K. A., Kunene, E. N., Dlamini, D. V., Mpofu, M., Manwa, L., ... & Bwembya, G. C. (2020). Traditional/indigenous vegetables of the kingdom of eswatini: Biodiversity and their importance: A review. *J. Exp. Agric. Int*, *42*, 204-215 doi: 10.9734/jeai/2020/v42i930602.

Mutalib, L. Y., Nuraddin, S. M., & Aka, S. T. H. (2015). Phytochemical screening, antibacterial and antibiofilm evaluation of *Lagenaria siceraria* fruit growing in Kurdistan Region\Iraq. *J. Pharmacogn. Phytochem*, *4*(1), 45-49.

Nogueira, J. W. A., Costa, R. A., da Cunha, M. T., & Cavalcante, T. T. A. (2017). Antibiofilm activity of natural substances derived from plants. *African Journal of Microbiology Research*, *11*(26), 1051-1060.

Pochapski, M. T., Fosquiera, E. C., Esmerino, L. A., Dos Santos, E. B., Farago, P. V., Santos, F. A., & Groppo, F. C. (2011). Phytochemical screening, antioxidant, and antimicrobial activities of the crude leaves' extract from *Ipomoea batatas* (L.) Lam. *Pharmacognosy magazine*, *7*(26), 165.

Raffoul, J. J., Kucuk, O., Sarkar, F. H., & Hillman, G. G. (2012). Dietary agents in cancer chemoprevention and treatment. *Journal of Oncology*, *2012,*

doi: 10.1155/2012/749310.

Saeed, N., Khan, M. R., & Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC complementary and alternative medicine*, *12*(1), 1-12.

Saima, N., Bushra, S., & Muhammad, S. (2013). Alteration in antioxidant and antimicrobial attributes of leaves of Zizyphus species in response to maturation. *Journal of Medicinal Plants Research*, *7*(2), 61-70.

Schönfeldt, H. C., & Hall, N. G. (2012). Dietary protein quality and malnutrition in Africa. *British Journal of Nutrition*, *108*(S2), S69-S76, doi: 10.1017/S0007114512002553

Shackleton, C. M., Pasquini, M. W., & Drescher, A. W. (Eds.). (2009). *African indigenous vegetables in urban agriculture*. Routledge.

Sujatha, S., & Renuga, F. B. (2013). Medicinal and edible tubers from forty two settlements of Tribals from Pechiparai Social forest in Kanyakumari District, India. *Scholars Academic Journal of Biosciences*, *1*(5), 213-216.

Talib, W. H., & Mahasneh, A. M. (2010). Antiproliferative activity of plant extracts used against cancer in traditional medicine. *Scientia pharmaceutica*, *78*(1), 33-46, doi: 10.3797/scipharm.0912-11.

Welcome, A. K., & Van Wyk, B. E. (2019). An inventory and analysis of the food plants of southern Africa. *South African Journal of Botany*, *122*, 136-179, doi: 10.1016/j.sajb.2018.11.003.

Abbreviations

BG	*Brachystelma gymnopodum*
CC_{50}	50% Cytotoxic Concentration
CR	*Coccinea rehmannii*
DCM	Dichloromethane
DPPH	1,1-diphenyl-2-picrylhydrazyl
EH	*Eulophia hereroensis*
ESKAPE	*Enterococcus faecium; Staphylococcus aureus; Klebsiella pneumoniae*; *Acinetobacter baumannii; Pseudomonas aeruginosa* and *Enterobacter* spp
FA	*Fockea angustifolia*
GAE	gallic acid equivalents
H_2O_2	hydrogen peroxide
IZ	inhibition zones
IC_{50}	50% inhibitory concentration
$MBEC_{50}$	Minimum concentration that eradicates 50% of biofilm
$MBIC_{50}$	Minimum concentration that inhibits biofilm formation by 50%
MIC	Minimum inhibitory concentration
MTT	3-(4, 5-dimethyl thiazolyl-2,5-diphenyl-tetrazolium bromide
NCI	National Cancer Institute
NCRST	National Commission on Research and Science and Technology
NRF	National Research Foundation
QE	quercetin equivalents
SABINA	Southern African Biochemistry and Informatics for Natural Products
TFC	Total flavonoid content
TM	*Trochomeria macrocarpa*
TPC	Total phenolic content
WN	*Walleria nutans*