ORIGINAL ARTICLE

Evaluation of the *in vitro* Antibacterial and Antioxidant Activities, Cytotoxic Effects, and Phytochemical Screening of Root Extracts of *Kedrostis capensis* A. Meeuse and *Trachyandra asperata* Kunth. from Lesotho

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Abstract

Background: The high mortality rate due to infectious diseases has recently been associated with the development of resistance to antibiotics by several strains of microorganisms worldwide. One of the pathophysiological conditions of mammalian tissues in response to infectious organisms is oxidative stress. Natural extracts or their chemical constituents as antioxidants are known to be very effective at preventing the destructive processes caused by oxidative stress. The main aim of the current study was to evaluate *in vitro* antibacterial, antioxidant, and cytotoxic effects of ethanolic root extracts of *Kedrostis capensis* A. Meeuse (Cucurbitaceae) and *Trachyandra asperata* Kunth. (Xanthorrhoeaceae). **Materials and Methods:** The assessments of antibacterial and antioxidant effects were performed using well diffusion method and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity assay, respectively, while cytotoxic potentials were evaluated by employing the brine shrimp toxicity model. **Results:** *K. capensis* extract possessed bactericidal activity against *Escherichia coli* and *Staphylococcus aureus* and bacteriostatic activity against *Pseudomonas aeruginosa* with minimum inhibition concentration (MIC) values ranging from 62.5 to 500 µm/mL and minimum bactericidal concentration (MBC) values varying from 125 to 1000 µm/mL, while *T. asperata* extract was only bacteriostatic against *S. aureus* with MIC value of 500 µm/mL and MBC value greater than 1000 µm/mL. Both *K. capensis* and *T. asperata* extracts scavenged DPPH free radicals in a concentration-dependent manner with the half maximal inhibitory concentration (IC_{50}) values of 113.68 ± 0.11 µg/mL and 134.63 ± 0.27 µg/mL, respectively. *K. capensis* extract exhibited a relatively higher toxicity potential with 50% lethality concentration (LC_{50}) value of 102.37 ± 0.19 µg/mL, while *T. asperata* was non-toxic with lower LC_{50} value of 522.75 ± 0.35 µg/mL. Obtained results suggest that *K. capensis* and *T. asperata* ethanolic root extracts possess bioactive secondary metabolites with antibacterial, antioxidant, and cytotoxicity properties with potential pharmaceutical applications.

Key words: Antibacterial, Antioxidant, Cytotoxicity, *Kedrostis capensis*, *Trachyandra asperata*

INTRODUCTION

Plants are often described as the massive storage of pharmacologically active chemical compounds.[1] The flora of Lesotho, which is known to encompass about 200 plant families, provides up to 303 species that are used in folk remedies.[2] Even though Basotho relied on traditional medicinal herbs for the treatment of

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Received: 07-10-2021
Revised: 24-11-2021
Accepted: 30-11-2021

various ailments since time immemorial,[3] the majority of those herbs are especially underexplored for the production of novel secondary metabolites with potential pharmaceutical applications. Studies have revealed that frequently encountered medicines in clinical settings such as cardiac glycoside digoxin as well as opium alkaloid morphine were discovered through systematic examination of inexpensive traditional herbs and associated traditional knowledge.[2,4] In fact, more than a third of medicines approved by the Food and Drug Administration are of natural origin.[5]

The high mortality rate due to infectious disease has recently been associated with the development of resistance to antibiotics by several strains of microorganisms worldwide, thus calling for an immediate discovery of new antibiotics against resistant bacteria from local herbs.[6] Natural extracts or their chemical constituents as antioxidants are known to be very effective at preventing the destructive processes caused by oxidative stress.[7,8] A plant-based antioxidant polyphenol, quercetin, enhanced the cytotoxic effects and therapeutic response of various chemotherapeutics in the *in vitro* and *in vivo* models against resistant cancer types.[9] Therefore, the identification of antibiotics and antioxidants from plant sources is imperative as they are potential novel secondary metabolites important for pharmaceutical applications.

Kedrostis capensis A. Meeuse belongs to the family Cucurbitaceae.[10] It is native to Lesotho, South Africa, and Namibia.[11] The plant species is vernacularly known as *sesepa-sa-linoha* in Sesotho.[3,12] It is a perennial, prostrate herb with stems annually produced from a tuberous subglobose up to 10 cm thick rootstock, when young suberect and short, but later prostrate and attain a length of up to 40–50 cm.[10] The leaves are usually second, almost invariably firm, grayish-green, ovate to oblong in outline.[10] The tubular roots of *K. capensis* have been reported, in Lesotho folk remedies, to be effective against urinary tract infections,[12] colic,[13] coughs and colds, asthma, chest pain, diarrhea, smallpox, skin diseases, and snakebite.[3] *Trachyandra asperata* Kunth. belongs to the Xanthorrhoeaceae family.[14] It is native to South Africa and Lesotho.[15] The plant species is vernacularly known as *tsilatsila* in Sesotho.[3] It is a grass-like perennial plant with a short basal rootstock. The leaves form a basal rosette and the inflorescence that may be branched and can reach a height of 20 cm.[16] It is found in sandy habitats with water and lots of sun, and is hardly noticeable when interplanted with grasses.[16] The root powder of *T. asperata* has been used in Lesotho traditional medicine to alleviate shoulder pain and for internal and external wounds.[3]

In our previous study,[1] we reported the establishment of a prototype library of crude extracts derived from samples of medicinal plants of Lesotho used for the treatment of infectious diseases as our effort to advance search for novel secondary metabolites with potential pharmaceutical applications. In spite of their wide usage in folk remedies, *K. capensis* and *T. asperata* are still underexplored. Therefore, this study was aimed to evaluate the *in vitro* antibacterial, antioxidant, and cytotoxic effects of ethanolic root extracts of *K. capensis* and *T. asperata*. The assessments of antibacterial and antioxidant effects were performed using well diffusion method and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity assay, respectively, while cytotoxic potentials were evaluated by employing the brine shrimp toxicity model and results were reported in this paper. To the best of our knowledge, this is the first report of the potential antibacterial, antioxidant, and cytotoxic effects of *K. capensis* and *T. asperata* root extracts.

MATERIALS AND METHODS

Plant materials

The study received ethical approval from the National University of Lesotho (NUL), Research and Ethics Committee (ID134-2021). The samples of *K. capensis* and *T. asperata* roots were harvested from their natural populations in Lesotho from Maseru and Mokhotlong districts, respectively, in January 2021. The samples were authenticated by the expert Botanist from NUL Department of Biology and the voucher specimen was deposited in NUL Pharmacy department. The samples were cleaned, dried in a dark room, reduced to powder of 1.0 mm maximum particle size, and packaged in plastic bottles before extraction.[3]

Chemicals and reagents

All the chemicals and reagents used were at least of analytical grade of purity and used directly. The organic solvent used for extraction and experimental procedures (ethanol, methanol, dimethyl sulfoxide [DMSO], and ethyl acetate) as well as sodium chloride (NaCl), sodium sulfate (Na_2SO_4), and Whatman No.1 filter paper were purchased from Chembi Pty Ltd. (Maseru, Lesotho). DPPH, *L*-ascorbic acid, Mueller-Hinton agar, Mueller-Hinton broth, ciprofloxacin 5 µg discs, agar plates, scintillation vials, gelatine, ferric chloride ($FeCl_3$), hydrochloric acid (HCl), potassium iodide, copper acetate, acetic anhydride, and concentrated sulfuric acid (H_2SO_4) were obtained from Prestige Laboratory Supplies (Pty) Ltd. (South Africa). Brine shrimp (*Artemia franciscana*) eggs were purchased from Ocean Nutrition™ (South Africa) and potassium dichromate (>99% purity) was sourced from Sigma-Aldrich (South Africa).

Extraction

The extracts were prepared using Soxhlet extractor followed by liquid-liquid fractionation according to the procedure reported by us.[1] Each of the 44.29 g of *K. capensis* and 50.22 g of *T. asperata* samples was refluxed at 78°C using 500 mL of solvent systems; ethanol/water (9:1 v/v), in Soxhlet extraction apparatus Ace Glass Incorporated, Vineland, NJ.

After 24 h, the ethanol was allowed to evaporate and leave the aqueous layer residual. The aqueous layer residue was extracted by vigorous agitation 3 times with ethyl acetate 50 mL using a separatory funnel. Combined organic layer was washed first with deionized water 10 mL and then with saturated NaCl solution 10 mL, dried over Na_2SO_4, filtered through a Whatman No. 1 filter paper, and concentrated under reduced pressure at 45°C using 0.25–2.l rotary evaporator ROVA-100 from MRC Laboratory Equipment manufacturer Beijing, China. The residual organic solvent was further removed *in vacuo* to produce crude extracts 2.25 g (5.08% yield) *K. capensis* and 2.98 g (5.96% yield) *T. asperata*. The extracts were stored in labeled air-tight 20 mL scintillation vials and refrigerated at below 4°C before analysis.

Test organisms

The following bacterial strains were used in this study: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC23922, and *Pseudomonas aeruginosa* ATCC27853. These organisms were obtained from the National Reference Laboratory, Maseru, Lesotho. All the bacterial strains were maintained at 4°C on Mueller-Hinton agar and subcultured every 15 days.

Well diffusion assay

The antibacterial activities of the crude extracts were determined using the well diffusion method according to the reported procedure.[17] All bacteria were grown on a Mueller-Hinton agar slant at 37°C for 18–24 h, and the bacterial suspension was prepared using a sterile normal saline solution (0.85% NaCl) equivalent to a 0.5 McFarland turbidity standard (corresponding to 1.5×10^8 CFU/mL). The bacterial suspension (100 µL) was spread onto Mueller-Hinton agar plates. A stock solution of each extract was prepared at a concentration of 13.1 mg/mL by dissolving each 131 mg of the extract into 10 mL of DMSO. The hole of 6 mm diameter was punched aseptically with a sterile cork borer and the volume of 20 µL of each extract solution was introduced into the wells. The plates were incubated at 37°C for 18–24 h. Antibacterial activity was measured based on the diameter expressed in mm of the clear zone on the well. A well containing DMSO without an extract was used as the negative control. For a standard antibiotic, ciprofloxacin (5 µg/disc) was used as positive control. Each experiment was carried out in triplicates and mean values were reported.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC values of the extracts were determined using the broth macrodilution method.[17] A bacterial inoculum was standardized at 0.5 McFarland and diluted 1:100 in normal saline to obtain a final concentration 1×10^6 CFU/mL. The extracts were dissolved in DMSO to make a concentration of 1000 µg/mL and were then diluted in 2-fold dilutions to obtain different concentrations ranging from 1000 to 1.95 µg/mL. To each 6 mL tube, 1 mL of Mueller-Hinton broth, 1 mL of diluted extract, and 20 µL of bacterial suspension were added. The negative control contained 1 mL of Mueller-Hinton broth, 1 mL DMSO without the extract, and 20 µL of bacterial suspension. The inoculated tubes were vertex and incubated at 37°C for 24 h. The MIC was the lowest concentration of an extract that completely inhibited the bacterial growth in the tube as detected by the unaided eye. Determination of MBC was carried out by streaking the mixture from each MIC assay negative well on a Mueller-Hinton agar plate and incubating 37°C for 24 h. The lowest concentration that resulted in no visible cell growth was defined as the MBC value. All experiments were performed in triplicate.

DPPH radical scavenging activity

DPPH radical scavenging activity was performed according to the method described in literature.[18] Briefly, a 0.1 mM solution of DPPH in methanol was prepared by dissolving 3.94 mg of DPPH in 100 mL of methanol and stored in a dark cupboard to minimize chemical degradation. A stock solution of the extract was prepared by dissolving 3.75 mg of the extract in 15 mL of DMSO to produce a concentration of 250 µg/mL. Serial dilutions with DMSO were prepared to produce lower concentrations ranging from 250, 125, 62.5, and 31.25 µg/mL. The 0 µg/mL solution (solution without extract) served as negative control. The 1.0 mL of DPPH solution was added to 3.0 mL of extract solution at different concentrations and vortexed. Similarly, a stock solution of *L*-ascorbic acid, which served as positive control, was also prepared by dissolution of 0.15 mg of *L*-ascorbic acid in 15 mL of DMSO. Serial dilutions with DMSO were prepared accordingly to produce lower concentrations of 10.0, 5.0, 2.5, and 1.25 µg/mL. The solution without the ascorbic acid (0 µg/mL) also served as the negative control. The 1.0 mL of DPPH solution was added to 3.0 mL of ascorbic acid solutions and vortexed. The solutions were incubated at 37°C for 30 min and the absorbance was measured at 517 nm UV–Visible Spectrophotometer (Apex Scientific UV–Visible Spectroscopy, South Africa) against the corresponding blank solution. The ability of the extracts and/or ascorbate standard to scavenge DPPH radical was calculated using the given equation below:

$$\text{DPPH radical scavenging activity (\%)} = ((A_{cont} - A_{test})/A_{cont}) \times 100$$

A_{cont} = Absorbance of the negative control
A_{test} = Absorbance in the presence of the extract or positive control

The IC_{50} values were generated using Microsoft Excel by plotting the exact concentration versus %inhibition of DPPH free radical.

Hatching shrimp

The artificial sea water was made by dissolving 32 g of Tropic Marine® Sea Salt in 1 L of deionized water. This was added into an inverted plastic bottle after which the brine shrimp eggs were added for hatching. Regular airflow was supplied into the water to continually disperse the eggs. Moreover, a concentrated light was supplied from a lamp (220–240 V) source as the eggs got incubated for 24 h at room temperature.[19]

Brine shrimp lethality assay

The potential for cytotoxicity of *K. capensis* and *T. asperata* extracts was assessed by the brine shrimp lethality test. Since plant extracts showing LC_{50} values above 500 µg/mL are considered practically non-toxic,[20] the extract was initially tested at 1000 µg/mL and subsequently assessed for artemicidal LC_{50} value at concentrations from 1000 to 15.625 µg/mL at 2-fold dilutions in DMSO. In 48-well plates, 50 µL of the extract was incubated with 30–50 nauplii in 450 µL of the sea salt water. The wells were observed under the stereo microscope (Olympus) for any dead nauplii at the beginning of experiment and the extract-induced mortality was recorded in 24 h. The morphological changes were assessed using the stereo microscope (Olympus) mounted with a Dino-Eye camera. Negative control consisted of nauplii in just sea salt water while potassium dichromate was used as a positive control. The experiment was done in triplicate. The LC_{50} value was calculated with SPSS Statistics v27 package using the probit analysis method.[19]

Qualitative phytochemical screening

Qualitative phytochemical screening was conducted to determine the presence of various active phytochemicals including polyphenols, tannins, phlobatannins, alkaloids, terpenoids, sterols, flavonoids, glycosides, saponins, and quinones, using the method described by Mugomeri *et al*.[21] Gelatin test: Precipitation of gelatin on addition of 1% gelatin solution into the extract in NaCl confirmed the presence of tannins. $FeCl_3$ test: The appearance of the red coloration when four drops of dilute $FeCl_3$ solution were added into the extract indicated the presence of phenols. Wagner's test: Formation of reddish-brown precipitate confirmed presence of alkaloids after the extract's filtrate from dilute HCl was treated with Wagner's reagent, iodine in potassium iodide. Froth test: Frothing which persisted for 60–120 s was formed after 1 mL extract was diluted with 10 mL of water and agitated for 2 min. This indicated the presence of saponins. Test for diterpenes: The green coloration appeared after the extracts were dissolved in water and four drops of copper acetate solution. This was indicative of the presence of diterpenes. Test for phlobatannins: The appearance of red precipitate confirmed the presence of phlobatannins after 2 mL aqueous extract solution was added into dilute HCl. Liebermann–Burchard test: The appearance of red, then blue and finally green coloration indicated the presence of sterols after 2 mL ethanolic extract was mixed with chloroform followed by the addition of 2 mL acetic anhydride and two drops of concentrated H_2SO_4. Test for quinones: Appearance of red coloration after 1 mL of concentrated H_2SO_4 was added to 1 mL of extract confirmed the presence of quinones. Test for flavonoids: The extract solution (1 mL) was taken in a test tube and added few drop of dilute NaOH solution. The appearance of an intense yellow color that becomes colorless on addition of a few drop of dilute acid indicated the presence of flavonoids. 3,5-Dinitrobenzoic acid test: To the alcoholic solution of the extract, few drops of NaOH followed by 2% solution of 3,5-dinitro benzoic acid are added. Formation of pink color indicates presence of cardiac glycosides.

Statistical analysis

All assays were done in triplicates and results were expressed as the mean±SD. The probit regression (SPSS Statistics v27) was used to determine mean lethality concentrations. The GraphPad Prism 5 and Microsoft Excel were used to compute IC_{50} values for the antioxidant activities. Statistical comparisons were performed with one-way analysis of variance, and $P < 0.05$ was regarded statistically significant.

RESULTS

Antibacterial activity of extracts

The antimicrobial activity of *K. capensis* and *T. asperata* extracts was evaluated against three pathogenic bacterial strains using the well diffusion method.[17] The ethanolic root extract of *K. capensis* exhibited antibacterial activity against all test organisms, one Gram positive and two Gram negative, at concentration of 13.1 mg/mL in comparison to ciprofloxacin (5 µg/disc). On the other hand, *T. asperata* extract exhibited antibacterial activity to only Gram-positive bacteria and showed no activity against Gram-negative bacterial strains. The results of the inhibition zone of the extracts against the three pathogens are shown in Table 1 and Figure 1a-d. *K. capensis* exhibited strong antibacterial activity against *E. coli* (25.92 ± 0.15 mm), followed by *S. aureus* (22.38 ± 0.22 mm) and *P. aeruginosa* (19.44 ± 0.56 mm). *T. asperata* exhibited moderately weak antibacterial activity against *S. aureus* (17.33 ± 0.42 mm) and no inhibitory activity was observed against *E. coli* and *P. aeruginosa*.

The antibacterial activities of *K. capensis* and *T. asperata* extracts were further quantitatively evaluated using the broth

macrodilution method.[17] The MIC and MBC values of the extracts are shown in Table 1. Based on the results in Table 1, *K. capensis* extract was effective against all tested bacterial species with MIC values ranging from 62.5 to 500 µm/mL and MBC values varying from 125 to 1000 µm/mL. The MIC values of *K. capensis* extract against *E. coli*, *S. aureus,* and *P. aeruginosa* were 62.5 µm/mL, 125 µm/mL, and 500 µm/mL, respectively. Again, *K. capensis* extract exhibited the bactericidal activity, with MBC values of 125 µm/mL for *E. coli*, 250 µm/mL for *S. aureus,* and 1000 µm/mL for *P. aeruginosa*. Finally, *T. asperata* extract was effective against only one bacterial species, *S. aureus* with MIC value of 500 µm/mL and MBC value >1000 µm/mL.

DPPH radical scavenging activity assay

K. capensis and *T. asperata* extracts scavenged DPPH free radicals in a concentration-dependent manner. The percentages of scavenging activity at varying concentrations of *K. capensis* extract were in the range 15.38–98.69% for concentrations of 31.25–250 µg/mL, results are presented in Figure 2a. The IC_{50} value in the DPPH assay of *K. capensis* extract was 113.68 ± 0.11 µg/mL with the 95% confidence interval between 40.12 and 163.01 µm/mL, as shown in Table 2. The percentages of scavenging activity at varying concentrations of *T. asperata* extract were in the range 18.18–81.16% for concentrations of 31.25–250 µg/mL, results shown in Figure 2b. The IC_{50} value in the DPPH assay of *T. asperata* extract was 134.63 ± 0.27 µg/mL with the 95% confidence interval between 51.42 and 169.40 µm/mL, as shown in Table 2. The standard antioxidant was *L*-ascorbic acid which has the IC_{50} value of 2.33 ± 0.09 µg/mL with the 95% confidence interval between 0.26 and 45.84 µm/mL, as shown in Table 2. This lower IC_{50} value indicates a high potency for antioxidant activity.

Brine shrimp lethality assay

The Figures 3a and 3b displayed the brine shrimp % mortality rates for the ethanolic root crude extracts of *K. capensis* and *T. asperata* at concentrations 1000, 500, 250, 125, 62.5, and 31.25 µg/mL. The LC_{50} concentration values for both *K. capensis* and *T. asperata* extract and that of potassium dichromate standard are summarized in Table 2. While *K. capensis* exhibited a relatively higher toxicity potential with the LC_{50} value of 102.37 ± 0.19 µg/mL, *T. asperata* extract showed low toxicity potential with LC_{50} value of 522.75 ± 0.35 µg/mL. Unlike a positive control, potassium dichromate, that showed toxic effects mostly on the *antennae*, *K. capensis* extract affected most morphological structures of *A. franciscana* and induced more morphological alterations than *T. asperata,* as shown in Figure 4a-d. The cytotoxic effects could be observable on the antennae used for locomotory and food uptake purposes, labrum on the ventral sides used also for food uptake, and the digestive tract useful in food digestion and absorption of nutrients.

Qualitative phytochemical screening

The qualitative phytochemical screening of *K. capensis* and *T. asperata* extracts indicated the presence of various active phytochemicals ranging alkaloids, polyphenols, tannins, phlobatannins, flavonoids, glycosides, terpenoids,

Table 1: Antibacterial activity of *K. capensis* and *T. asperata* root extracts based on well diffusion, MIC, and MBC assays

Bacteria	*K. capensis* extract			*T. asperata* extract			CPFX		
	DIZ (mm)	MIC (µg/mL)	MBC (µg/mL)	DIZ (mm)	MIC (µg/mL)	MBC (µg/mL)	DIZ (mm)	MIC (µg/mL)	MBC (µg/mL)
S. aureus	22.38±0.22	125	250	17.33±0.42	500	>1000	35.89±0.11	NT	NT
E. coli	25.92±0.15	62.5	125	0.00	NT	NT	34.31±0.37	NT	NT
P. aeruginosa	19.44±0.56	500	1000	0.00	NT	NT	33.15±0.39	NT	NT

Values are mean±SD of three replications. DIZ: Diameter of inhibition zone, MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, NT: Not tested; CPFX: Ciprofloxacin. *K. capensis*: *Kedrostis capensis*, *T. asperata*: *Trachyandra asperata*, *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*

Table 2: DPPH radical scavenging activity and brine shrimp lethality assays of *K. capensis* and *T. asperata* root extracts

Extract/control	DPPH radical scavenging activity		Brine shrimp lethality	
	IC_{50}±SD (µg/mL)	95% confidence interval	LC_{50}±SD (µg/mL)	95% confidence interval
K. capensis extract	113.68±0.11	40.12–163.01	102.37±0.19	76.45–139.14
T. asperata extract	134.63±0.27	51.42–169.40	522.75±0.35	458.62–606.47
Positive control*	2.33±0.09	0.26–45.84	1.62±0.04	0.04–65.74

*DPPH radical scavenging -*L*-ascorbic acid standard. *Brine shrimp lethality – potassium dichromate standard. *K. capensis*: *Kedrostis capensis*, *T. asperata*: *Trachyandra asperata*, DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate

sterols, saponins, and quinones, as shown in Table 3. Based on Table 3, *K. capensis* demonstrated the presence of alkaloids, polyphenols, phlobatannins, flavonoids, glycosides, terpenoids, sterols, and quinones. The *T. asperata* demonstrated the presence of alkaloids, polyphenols, tannins, phlobatannins, terpenoids, sterols, saponins and quinones.

DISCUSSION

Medicinal plants have been dispensed by traditional healers and herbalists in Lesotho to treat various ailments and can provide novel secondary metabolites with potential pharmaceutical applications. The studies to evaluate their efficacies began in late 1990s whereby Shale *et al*. examined antibacterial activities of 16 indigenous plants. It was revealed that six species had high antibacterial activities against both Gram-positive and Gram-negative bacteria, with a wider spectrum against Gram-positive bacteria.[22] In our previous study that established a prototype library of crude extracts, the preliminary antimicrobial assay of five medicinal plants against *S. aureus* using the well diffusion method showed high antibacterial activities, confirming previously known data.[1] In the current study, the biological properties of ethanolic root extracts of *K. capensis* and *T. asperata* were evaluated for the 1st time.

The results from the well diffusion assay of *K. capensis* extract revealed a broad-spectrum inhibitory activity (based on the inhibition diameter) against Gram-positive and Gram-negative bacteria such as *E. coli* (25.92 mm) followed by *S. aureus* (22.38 mm) and *P. aeruginosa* (19.44 mm). In the MIC and MBC assays, *K. capensis* extract produced the lowest MIC value of 62.5 µm/mL against *E. coli*, followed by *S. aureus* (125 µm/mL), while *P. aeruginosa* had the highest MIC of 500 µm/mL. The lowest MBC was 125 µm/mL for *E. coli* and *S. aureus* (250 µm/mL). On the other hand, *T. asperata* extract was only effective against Gram-positive *S. aureus* (17.33 mm). These results indicated that *K. capensis* extract possessed bactericidal activity against *E. coli* and *S. aureus* and bacteriostatic activity against *P. aeruginosa* while *T. asperata* extract was only bacteriostatic against *S. aureus*.

Although there are no reports of antibacterial activities of *K. capensis* and *T. asperata* in literature, other studies have

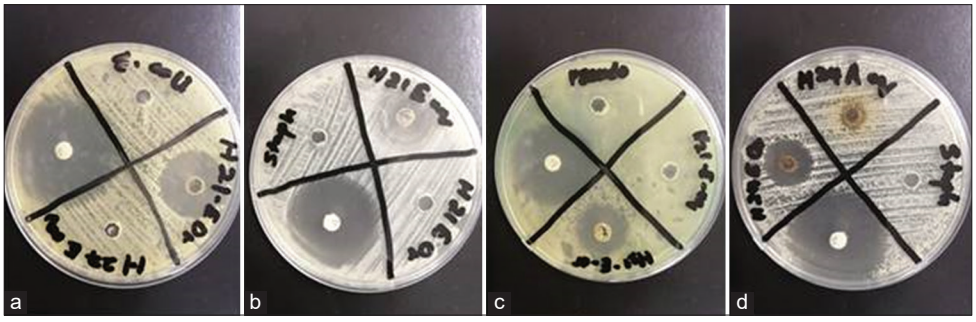

Figure 1: Antibacterial activity based on well diffusion. (a) Inhibition zone of *Kedrostis capensis* extract and ciprofloxacin 5 µg/disc against *Escherichia coli*, (b) inhibition zone of *K. capensis* extract and ciprofloxacin 5 µg/disc against *Staphylococcus aureus*, (c) inhibition zone of *K. capensis* extract and ciprofloxacin 5 µg/disc against *Pseudomonas aeruginosa*, (d) inhibition zone of *Trachyandra asperata* extract and ciprofloxacin 5 µg/disc against *S. aureus*

Table 3: Qualitative phytochemical screening of *K. capensis* and *T. asperata* root extracts

Test No	Chemical test	Phytochemical class	Extract	
			K. capensis	*T. asperata*
1	Wagner's test	Alkaloids	+	+
2	Test for flavonoids	Flavonoids	+	-
3	3,5-Dinitrobenzoic acid test	Glycosides	+	–
4	Test for phlobatannins	Phlobatannins	+	+
5	$FeCl_3$ test	Polyphenols	+	+
6	Test for quinones	Quinones	+	+
7	Froth test	Saponins	–	+
8	Liebermann–Burchard test	Sterols	+	+
9	Gelatin test	Tannins	–	+
10	Test for diterpenes	Terpenoids	+	+

(+) – Present; (-) – absent. *K. capensis*: *Kedrostis capensis*, *T. asperata*: *Trachyandra asperata*. $FeCl_3$: Ferric chloride

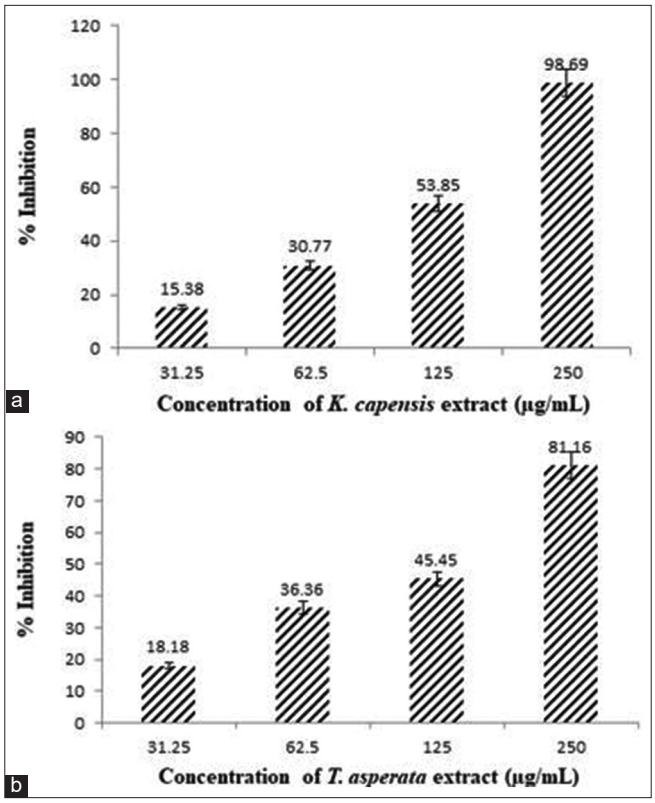

Figure 2: Antioxidant activity. (a) 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity of *Kedrostis capensis* root extract, (b) DPPH radical scavenging activity of *Trachyandra asperata* root extract; error bars indicate±SD

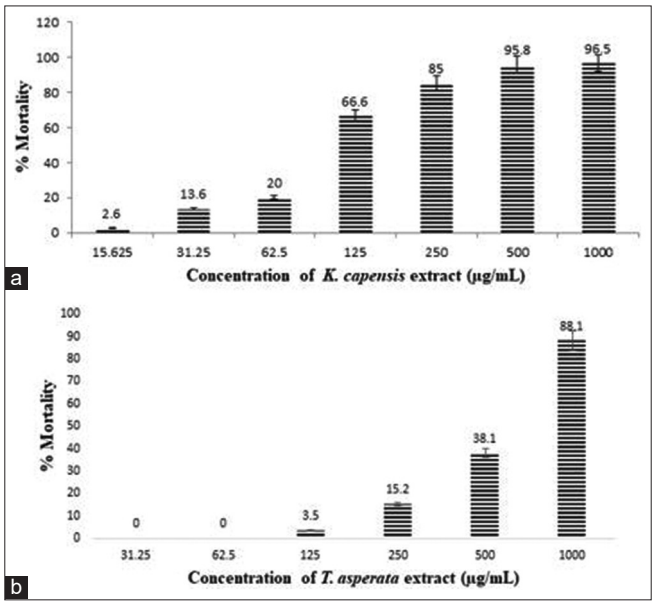

Figure 3: Brine shrimp lethality assay. (a) Brine shrimp concentration-dependent mortality caused by *Kedrostis capensis* root extract, (b) brine shrimp concentration-dependent mortality caused by *Trachyandra asperata* root extract; error bars indicate±SD

reported the antibacterial activities of their close relatives such as *Kedrostis foetidissima* (Jacq.) Cogn. and *Trachyandra tortilis* (Baker) Oberm., respectively. One study revealed that *K. foetidissima* stem extracts 50 µg/disc exhibited high zones of inhibitions of 12.6 mm for *P. aeruginosa* followed by *S. aureus* (8.2 mm) and *E. coli* (6.4 mm) when using agar diffusion technique.[23] In other studies, extracts of *K. foetidissima* produced higher zones of inhibition against *P. aeruginosa*, *S. aureus,* and *E. coli*.[24,25] While on the other hand, *T. tortilis* showed slight antibacterial activity in a study by Wheat (2013).[26]

Antioxidants fight against free radicals and protect humans from various diseases.[7,8] The antioxidant potential of *K. capensis* and *T. asperata* root extracts in the current study was determined using DPPH radical scavenging activity. Interestingly, *K. capensis* and *T. asperata* extract exhibited antioxidant activities in a dose-dependent manner with relatively lower IC_{50} values of 113.68 ± 0.11 and 134.63 ± 0.27 µg/mL, respectively. These results showed that *K. capensis* and *T. asperata* extracts had potential *in vitro* antioxidant activity which could be linked to their flavonoids and phenolic contents. There are no reports of antioxidant activities of *K. capensis* and *T. asperata* from literature, however, studies on their close relatives, *K. foetidissima* and *T. tortilis,* respectively, revealed potent to slight antioxidant activities.[26,27] In another study, *Trachyandra ciliata* (L.f.) Kunth., a species closely related to *T. asperata*, has shown potent antioxidant activity.[28]

According to Moshi *et al*.,[20] plant extracts showing the LC_{50} values >500 µg/mL on brine shrimp are considered non-toxic and Kabubii *et al*., 2015, categorized it as low toxicity potential.[29] In the present study, *K. capensis* showed relatively higher cytotoxic effects with LC_{50} value of 102.37 ± 0.19 µg/mL while *T. asperata* extract showed lower toxicity potential with the LC_{50} value of 522.75 ± 0.35 µg/mL. In addition, *K. capensis* extract affected most morphological structures of *A. franciscana* and induced more morphological alterations than *T. asperata*. The cytotoxic effects could be observable on the antennae used for locomotory and food uptake purposes, labrum on the ventral sides also used for food uptake, and the digestive tract useful in food digestion and absorption of nutrients. These results suggest that *K. capensis* extract was cytotoxic while *T. asperata* extract was non-toxic. A species closely related to *K. capensis* known as *Kedrostis africana* (L.) Cogn. has been previously reported to be moderately toxic on brine shrimp assay with LC_{50} values of 298 and 489 µg/mL for the aqueous and ethanolic extracts, respectively.[30]

The results of qualitative phytochemical analysis suggest that *K. capensis* and *T. asperata* extracts contain bioactive phytochemicals such as polyphenols, tannins, phlobatannins, alkaloids, terpenoids, flavonoids, glycosides, sterols,

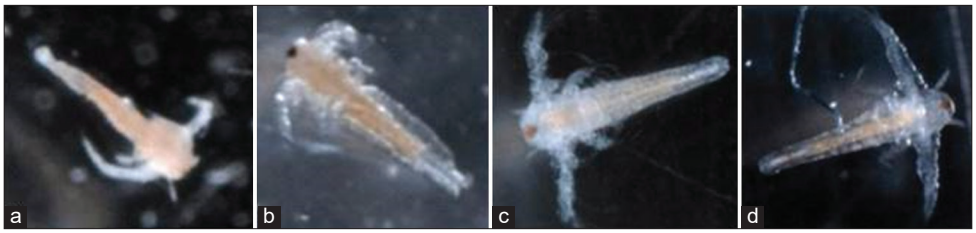

Figure 4: *Artemia* morphological change. (a) Caused by *Kedrostis capensis* extract, (b) caused by *Trachyandra asperata* extract, (c) caused by potassium dichromate standard (positive control), (d) negative control

saponins, and quinones which could explain the high biological activities. There are no previous studies carried out for preliminary phytochemical screening of *K. capensis* and *T. asperata,* however, the phytochemical characterization of their relatively well-studied close species *K. foetidissima* and *T. ciliate* revealed the presence of flavonoids, tannins, triterpenoids, phenols, steroids, glycosides, cardiac glycosides, as well as saponins.[24,28] To the best of our knowledge, this is the first report on the *in vitro* antibacterial, antioxidant, and cytotoxic effects of *K. capensis* and *T. asperata*, thus, we suggest that these plant species may be useful as potential sources of novel secondary metabolites with potential pharmaceutical applications.

CONCLUSION

The ethanolic root extracts from *K. capensis* and *T. asperata* showed antibacterial activity against tested bacterial pathogens. *K. capensis* extract possessed bactericidal activity against *E. coli* and *S. aureus* and bacteriostatic activity against *P. aeruginosa* while *T. asperata* extract was only bacteriostatic against *S. aureus*. The extracts also exhibited potential *in vitro* antioxidant activity which could be linked to their flavonoids and phenolic contents. In addition, *K. capensis* extract exhibited higher cytotoxic effects while *T. asperata* extract was non-toxic. *K. capensis* extract affected most morphological structures of *A. franciscana* and induced more morphological alterations observable on the antennae used for locomotory and food uptake purposes among others. The qualitative phytochemical analysis of *K. capensis* and *T. asperata* extracts revealed the presence of bioactive phytochemicals such as polyphenols, tannins, phlobatannins, alkaloids, terpenoids, flavonoids, glycosides, sterols, saponins, and quinones. Therefore, our results suggest that *K. capensis* and *T. asperata* ethanolic root extracts possess bioactive secondary metabolites with antibacterial, antioxidant, and cytotoxicity properties with potential pharmaceutical applications. The antibacterial, antioxidant, and cytotoxic effects of medicinal plants are possibly due to synergistic actions of active phytochemicals present. Our efforts to determine the actual active principles are still continuing and the results will be communicated in the due course.

ACKNOWLEDGMENTS

Thanks given to Mr. Moretloa Polaki from the Department of Biology at the National University of Lesotho for authentication and much useful advice regarding locating plant species used in the study.

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Source of Support: Nil. **Conflicts of Interest:** None declared.