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ORIGINAL ARTICLE

Phytochemical and Antimicrobial Evaluation of *Lauridia tetragona* (L.F) R.H. Archer: A Medicinal Plant Used for the Management of Dysentery in the Eastern Cape Province of South Africa

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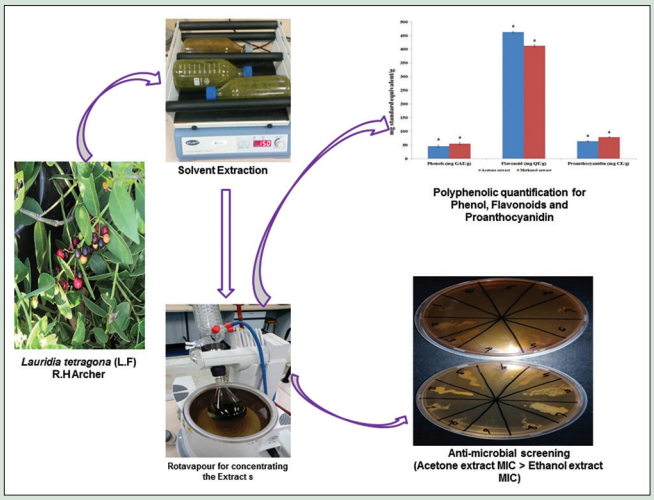
ABSTRACT

Objective: The present investigation evaluated the *in vitro* phytochemical and antimicrobial potential of the methanol and acetone leaf extracts of *Lauridia tetragona* (L.F), a plant consumed as a herb in South Africa. **Materials and Methods:** Spectrophotometry assays using Folin–Ciocalteu, aluminum chloride, and vanillin–hydrochloric acid were used for the determination of phenols, flavonoids, and proanthocyanidin contents of the extracts, respectively. The agar dilution technique was used for the antimicrobial evaluation. The inhibitory activity of the extracts was tested on five Gram-negative and five Gram-positive bacteria and four fungi. **Results:** The methanol extract had a higher phenol (54.87 ± 4.01 mg gallic acid equivalent [GAE]/g) and proanthocyanidin (78.55 ± 0.83 mg catechin equivalent [CE]/g) content than the acetone extracts with 45.27 ± 3.93 mg GAE/g and 63.54 ± 1.67 mg CE/g, respectively. The acetone extract, however, had higher flavonoid content (462.45 ± 1.93 mg quercetin equivalent [QE]/g) than the methanol extract (412.20 ± 3.85 mg QE/g). The minimum inhibitory concentration (MIC) of the antibacterial assay ranged from 2.5 to 5 mg/mL. All the bacteria except *Staphylococcus aureus* were susceptible to the acetone extract. Five bacteria, three Gram positive and two Gram negative, were resistant while the remaining five were susceptible to the methanol extract. Conversely, all the fungi tested were susceptible to both extracts with a MIC which ranged from 0.63 to 10 mg/mL. **Conclusion:** The results obtained revealed that Gram-positive and Gram-negative bacteria and fungi showed some degree of susceptibility to the plant extracts. This gives an indication of broad-spectrum activity exhibited by the crude extracts of *L. tetragona* and supports its ethnomedicinal usage.

Key words: Antimicrobial, *Celastraceae*, dysentery, *Lauridia tetragona*, phytochemical, spectrophotometry

SUMMARY

- This study evaluated the quantitative polyphenolic content and antimicrobial potential of *Lauridia tetragona*
- The study revealed a high polyphenolic content with the methanol extract having the highest phenol and proanthocyanidin content than the acetone extract
- The acetone extract had a better antimicrobial activity than the methanol extract
- The study validated the ethnomedicinal application of *L. tetragona* for the management of gastrointestinal tract infections.



Abbreviations Used: GAE: Gallic acid equivalent; QE: Quercetin equivalent; CE: Catechin equivalent; DMSO: Dimethyl sulfoxide; MIC: Minimum inhibitory concentration.

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INTRODUCTION

Dysentery is diarrhea that contains blood; it is a chronic disease which causes intestinal inflammation because of severe diarrhea with mucus or blood in the feces. The severity of diarrhea ranges from asymptomatic to severe dehydration, resulting in death. Diarrhea is a leading cause of malnutrition and the second leading cause of death in children under 5 years. It is both preventable and treatable.[1] This ailment may have a negative impact on both physical fitness and mental development.

The most common cause of diarrhea is by infection of the intestines due to viruses, bacteria, and, less often, parasites. Noninfectious causes include adverse effects from medications, acute abdominal processes, gastroenterological, and endocrine diseases.[2] Lack of proper sanitary facilities causing wastes been washed into both surface and underground aquifers, unhygienic habits of not washing hands after toileting, and

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preparing food with unwashed hands are also contributing factors to the scourge.[2-4]

According to Chola *et al*.,[5] diarrhea is one of the leading causes of morbidity and mortality in children under 5 years in South Africa. Although the burden status in South Africa is not explicitly known, data from the 2012 official report from the Statistics South Africa (SSA) placed the death rate of under 5 years at around 20%.[6] Liu *et al.*[7] and Dorrington *et al.*[8] placed the death rate at 8% and 13%, respectively. The General Household Survey conducted by the SSA[6] as cited by Chola *et al*.[5] reported over 60,000 cases of childhood diarrhea per month and an approximate 9000 child death that the same year.

In the recent past, overutilization, reliance, and abuse of antibiotics have resulted in the emergence of resistant strains of several groups of micro-organisms. Due to the increase in multidrug-resistant (MDR) organisms, researchers are now looking for new lead molecules as an alternative against MDR organisms.[9] Plants have been a major source in the search for alternative antibiotic agents because they are a major source of active chemical constituents against diseases. Most of the world's population still relies on alternative medicines for the treatment of many serious diseases.[10] Plant extracts are known to have antispasmodic effects, delay gastrointestinal transit, suppress gut motility, stimulate water adsorption, or reduce electrolyte secretion.[11] All these activities may explain the benefits of using certain plants in the treatment of diarrhea.[12]

Most medicinal properties of plants are attributed to the presence of phenolic compounds on them.[13] Phenolic compounds comprise large classes of plant secondary metabolites with a diversity of structural compositions: phenols, flavonoids, proanthocyanidins, and coumarins.[14,15] They are synthesized in plants in response to ecological variations. They are present in plants consumed as human diet and herbal medicine, giving peculiar colors to fruits, juices, and wine, and act as substrates for enzymatic browning and flavoring.[14] Their roles in human health and diseases are based on the findings from research.[16,17] There is a growing interest by natural product scientists in the utilization of phytochemicals in therapeutics and are intensifying efforts toward the evaluation of these valuable medicinal plants.

Lauridia tetragona (L.F) R.H. Archer also known as climbing saffron and Bob-cherry is a shrub, which belongs to the *Celastraceae* family. It is found in the Eastern Cape, KwaZulu-Natal, Limpopo, Mpumalanga, and Western Cape Provinces of South Africa.[18,19] Although much is unknown about this plant, it is consumed as a herb in South Africa. An ethnobotanical survey of plants used in the treatment of dysentery carried out in Amathole District Municipality of South Africa revealed the use of *L. tetragona* as a herb for the treatment of dysentery, diarrhea, and diabetes.[20] Despite the folkloric uses of this plant, no scientific investigation has been reported to validate these claims. This study, therefore, was aimed at evaluating the phytochemical and antimicrobial activities of *L. tetragona* leaves extracted with acetone and methanol.

MATERIALS AND METHODS

Plant material

Matured leaves of *L. tetragona* were collected from Woody Cape, Backpackers, Port Alfred Area of the Eastern Cape Province. The plant was identified and authenticated by a plant taxonomist at the University of Fort Hare, Alice, South Africa, and a voucher specimen (Win 2016/01) was prepared and deposited at the Giffen's herbarium.

Extraction methods

The leaves of the sample were rinsed gently in running tap water to remove dirt and dust, oven-dried to constant weight at 40°C, and pulverized. The pulverized sample was extracted separately in acetone and methanol and shaken in an orbital shaker (Orbital Incubator Shaker, Gallenkamp) for 48 h. The extracts were further concentrated to dryness to remove the solvents under reduced pressure using a rotary evaporator. The resulting extracts were reconstituted in methanol to give the desired concentrations used in the phytochemical study.

In vitro quantitative phytochemical evaluation

The phenol, flavonoid, and proanthocyanidin contents were determined as described by Ohikhena *et al*.[13]

Phenolic acid determination

Phenol determination was estimated spectrophotometrically using the Folin–Ciocalteu method. In brief, 0.5 mL of the extracts (1 mg/mL), standard gallic acid (0.02 mg/mL to 0.1 mg/mL), and methanol (control) were pipetted in different test tubes. 2½ mL of 10% Folin–Ciocalteu reagent was added, and the mixture was vortexed. Thereafter, 2 mL of 7.5% anhydrous sodium carbonate was added to the solution after allowing it to stand for about 5 min, vortexed, and incubated at 40°C for 30 min. After incubation, the absorbance was measured at 765 nm. The phenol content was extrapolated from the gallic acid standard curve equation: y = 9.8809x, R^2 =0.9977 and was expressed as mg gallic acid equivalent (GAE)/g from the equation CV/m; where "C" is the concentration as derived from the calibration curve equation in mg/mL, "V" is the volume of the extract used in the assay in mL, and "m" is the mass of the extract used in the assay in "g."

Flavonoid determination

The flavonoid content of the extracts was determined by the aluminum chloride colorimetric assay. In brief, 0.5 mL (1 mg/mL) aliquots of the extracts, different concentrations (0.2–1 mg/mL) of standard quercetin, and methanol (control) were placed in different test tubes. 2 mL of distilled water and 0.15 mL of 5% sodium nitrite were added. The mixture was allowed to stand for 6 min after which 0.15 mL of 10% $AlCl_3$ was added and allowed to stand for another 5 min, followed by the addition of 1 mL of 1 M sodium hydroxide. The solution was made up to 5 mL with distilled water and measured using a spectrophotometer at 420 nm. The flavonoid content was calculated using the quercetin standard calibration curve equation: y = 1.3068x, R^2 = 0.9927 and was expressed as mg of quercetin equivalent (QE)/g using the formula CV/m as described above in phenol.

Proanthocyanidin (condensed tannin)

To 0.5 mL of 1 mg/mL of the extracts, different concentrations (0.02–1 mg/mL) of the standard catechin and methanol (control) were added 3 mL of 4% vanillin–methanol and 1.5 mL of hydrochloric acid and vortexed. The mixture was allowed to stand for 15 min at room temperature. The absorbance was measured at 500 nm using a spectrophotometer. Proanthocyanidin content was evaluated using the catechin standard calibration curve equation: y = 1.1993x + 0.0158, R^2 = 0.9957 and was expressed as mg catechin equivalent (CE)/g using the formula, CV/m as earlier described.

Antimicrobial assay

Microbial strains

All the organisms used in this study were obtained from the Medicinal Plants and Economic Development Research Centre, University of Fort Hare, South Africa. Five Gram-positive strains such as *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (OK), *Bacillus subtilis* KZN, *Bacillus cereus,* and *Streptococcus pyogenes* and five Gram-negative strains such as *Vibrio cholera, Klebsiella pneumonia* (ATCC 4352), *Pseudomonas aeruginosa* (ATCC 19582), *Salmonella typhi* (OK), and *Escherichia coli* (ATCC 8739) were used for the antibacterial activity.

The fungi isolates used are *Trichophyton mucoides* ATCC 201382, *Trichophyton tonsurans* ATCC 28942, *Candida albicans* (ATCC 10231), and *Aspergillus niger* ATCC 16888.

Preparation of inoculum

The bacteria inoculums, extracts/standard drugs preparations, and agar dilution assays were prepared as described by Wiegand *et al.*[21] and the European Committee on Antimicrobial Susceptibility Testing,[22] which are modifications from the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Direct colony suspension method was used in preparing the inoculum. Bacteria and fungi inoculum suspensions of 1×10^6 CFU/mL and 5×10^5 CFU/mL, respectively, were prepared.

Preparation of extract

Five milliliters stock solution of 500 mg/mL of the extract was prepared by dissolving it with 1 mL of DMSO and made up with either Mueller–Hinton or Sabouraud Dextrose Broth for antibacterial and fungal assays, respectively. Two-fold serial dilutions of the stock (250, 125, 62.5, 31.23, 15.625, and 7.8125 mg/mL) were also prepared with the appropriate broth. Standard drugs (ciprofloxacin and nystatin for antibacterial and fungi, respectively) were prepared with the appropriate broth in 2-fold serial dilutions according to the guidelines of the CLSI.

Agar dilution assay

Mueller–Hinton and Sabouraud Dextrose Agar were prepared according to the manufacturer's description for antibacterial and fungi screening. The agar was autoclaved at 121°C for 15 min and allowed to cool to 50°C in a water bath. In brief, 0.5 mL from the stock and 2-fold serial dilutions were added to the molten agar (24.5 mL), swirled and poured into petri dishes, and allowed to cool and solidify to give the required concentrations. Ten microliters each from both the prepared bacteria and fungi inoculums was delivered on the solidified agar surface to give the desired final inoculums of 1×10^4 CFU/spot and 1×10^3 CFU/spot, respectively. The extracts' final concentrations for the antibacterial assay ranged from 5 mg/mL to 0.1563 mg/mL and from 10 mg/mL to 0.3125 mg/mL for the antifungal assay. The concentration of ciprofloxacin (antibacterial standard) ranged from 64 µg/mL to 2 µg/mL while nystatin (antifungal standard) ranged from 16 µg/mL to 0.5 µg/mL. Bacteria plates were incubated at 37°C, and the readings were taken between 16 and 20 h of incubation. Fungi plates were incubated at 30°C, and the readings were taken after 2–3 days.

RESULTS

Polyphenolic screening

The results of the phytochemical evaluation are shown in Figure 1. Phenolic content was quantified as the amount of the standard gallic acid in milligram equivalent present in a Gram of the extract (mg GAE/g). Although there was no significant difference in the phenolic contents of both extracts, the methanol extract had more phenolic content (54.89 ± 4.01 mg GAE/g) than the acetone extract which had a content of 45.27 ± 3.93 mg GAE/g.

The flavonoid content of the different extract fractions was quantified in mg QE/g. The acetone extract had a nonsignificant ($P > 0.05$) higher composition (462.45 ± 1.93 mg QE/g) than the methanol extract which had a composition of 412.20 ± 3.85 mg CE/g) [Figure 1]. The proanthocyanidin also shown in Figure 1 as mg CE/g) revealed the methanol extract to have a higher composition of 78.55 ± 0.83 mg CE/g while the acetone extract had a composition of 63.57 ± 1.67 mg CE/g.

Antimicrobial assay

The results of the antibacterial minimum inhibitory concentration (MIC) are shown in Table 1. All the bacteria except *S. aureus* were susceptible to the acetone extract with a MIC which ranged from 0.625 to 5 mg/mL. *S. aureus*, *B. subtilis, S. pyogenes, K. pneumonia,* and *P. aeruginosa* were resistant to the methanol extract at the concentrations tested. *E. coli* was more susceptible to the acetone extract with a MIC of 0.625 mg/mL and 2.5 mg/mL in the methanol extract. *E. faecalis, B. cereus, P. aeruginosa,* and *S. typhi* all had MIC value of 2.5 mg/mL in the acetone extract. *B. cereus* also had a MIC of 2.5 mg/mL in the methanol extract. All the bacteria tested were, however, highly susceptible to the control drug (ciprofloxacin) with a MIC lesser than the lowest concentration tested (µg/mL).

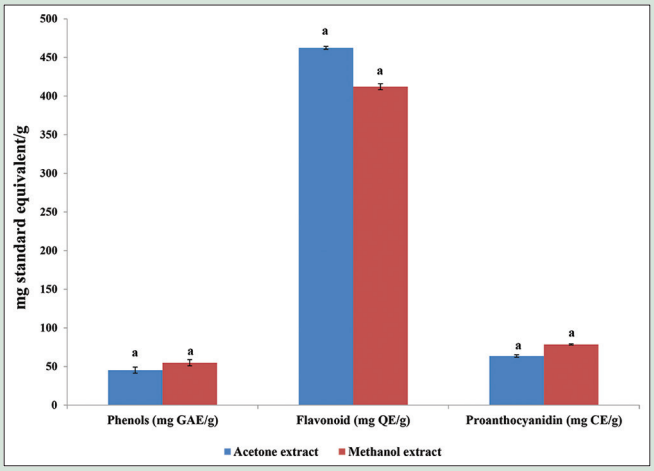

Figure 1: Polyphenolic compositions of the acetone and methanol extracts of *Lauridia tetragona*. Values are means a standard deviation, $n = 3$. Set of bars with the same letter are not significantly different ($P < 0.05$)

Antifungal assay

The results of the MICs of the antifungal screening are shown in Table 2. All the fungi tested were susceptible to the two extracts with a MIC which ranged from 0.625 to 10 mg/mL. The standard drug, nystatin, had a MIC which ranged from 4 to 8 µg/mL. While *T. tonsurans* was more susceptible to both extracts with MIC of 0.625 mg/mL, *T. mucoides* was more resistant or less susceptible with a MIC of 10 mg/mL.

DISCUSSION

Phenolic compounds act as strong antioxidants, preventing oxidative damages to biomolecules such as DNA, lipids, and protein which play a role in chronic diseases such as cancer and cardiovascular diseases.[15] In this study, the phenol was evaluated using gallic acid as the reference standard and was quantified based on the equivalent (GAE) estimated from the gallic acid standard curve. The two solvents, methanol and acetone, had different extraction abilities on the phenol of the plant. The methanol had higher phenol (54.89 ± 4.01 mg GAE/g) as compared to the acetone extract (45.27 ± 3.93 mg GAE/g); however, there was no difference statistically ($P < 0.05$) [Figure 1]. In other related studies on some plants of the *Celastraceae* family, the phenol content of *Cassine orientalis* and *Maytenus pyria* leaf extracts was 24 and <20 mg GAE/g fresh weight (FW), respectively.[23] In the study with *Maytenus dasyclada,* the hexane, ethyl acetate, and ethanol extracts had phenol contents of 1.54 ± 0.68, 43.65 ± 0.80, and 14. 59 ± 0.66 mg GAE/g, respectively.[24]

The flavonoid content was evaluated using quercetin as a reference and was quantified based on the QE estimated from the standard curve. According to Ghasemzadadeh and Ghasemzadadeh,[25] flavonoids are better antioxidants than Vitamins C and E and carotenoids; therefore, it is essential in combating stresses caused by oxidation. Unlike in the

Table 1: Minimum inhibitory concentrations of the acetone and methanol extracts of *Lauridia tetragona (L.F)* R.H. Archer on selected Gram-negative and Gram-positive bacteria

	Acetone extract (mg/mL)	Methanol extract (mg/mL)	Ciprofloxacin (µg/mL)
Enterococcus faecalis (positive)	2.5	5	≤2
Staphylococcus aureus (positive)	>5	>5	≤2
Bacillus subtilis (positive)	5	>5	≤2
Bacillus cereus (positive)	2.5	2.5	≤2
Streptococcus pyogenes (positive)	5	>5	≤2
Vibrio cholerae (negative)	5	5	≤2
Klebsiella pneumoniae (negative)	5	>5	≤2
Pseudomonas aeruginosa (negative)	2.5	>5	≤2
Salmonella typhi (negative)	2.5	5	≤2
Escherichia coli (negative)	0.625	2.5	≤2

Annotations: ">" (value greater than the highest concentration tested) and "≤" (value lesser than or equal to the lowest concentration tested)

Table 2: Minimum inhibitory concentrations of the acetone and methanol extracts of *Lauridia tetragona (L.F)* R.H. Archer on some fungi

	Acetone extract (mg/mL)	Methanol extract (mg/mL)	Nystatin (µg/mL)
Trichophyton mucoides	10	10	4
Trichophyton tonsurans	0.625	0.625	4
Candida albicans	2.5	5	4
Aspergillus niger	2.5	2.5	8

Annotations: ">" (value greater than the highest concentration tested) and "≤" (value lesser than or equal to the lowest concentration tested)

phenol where the methanol extract had more content, the acetone extract had more flavonoid content (462.45 ± 1.93 m QE/g) than the methanol extract with a content of 412.20 ± 3.85 mg QE/g. Statistically, no significant difference ($P < 0.05$) was observed in the flavonoid contents of both solvent extracts [Figure 1]. Proanthocyanidin also known as condensed tannin is a group of polyphenolic compounds that is very important, but often overlooked. Proanthocyanidin as an antioxidant acts by chelating transition metals and as an enzyme inhibitor it inhibits lipid peroxidation and peroxynitrite.[26] The proanthocyanidin content of *L. tetragona* (methanol extract [78.55 ± 0.83 mg CE/g] and acetone extract [63.54 ± 1.67 mg CE/g]) is more than the reported content in both *C. orientalis* and *M. pyria* leaf extracts with 21 ± 0.36 mg/g FW and <10 mg/g FW cyanidin chloride equivalent, respectively.[23] The higher phenolic, flavonoid, and proanthocyanidin contents observed in *L. tetragona* suggested that the plant has the potential in combating oxidative stresses and could be a good source of antioxidant.

In this study, antimicrobial susceptibility test was done to determine how effective *L. tetragona* could be against different human pathogenic micro-organisms. This test is needed to ascertain the usefulness of this species in fighting infections by determining its MIC against selected pathogenic micro-organisms. The results obtained revealed that Gram-positive and Gram-negative bacteria and fungi showed some degrees of susceptibility to the plant extracts. This gives an inclination of broad-spectrum activity exhibited by the crude extracts of this species.

The inhibitory activity of *L. tetragona* was dependent on the solvent of extraction and concentration. While all the bacteria except *S. aureus* were susceptible to the acetone extract, only half of the tested organisms (one Gram-positive and four Gram-negative) were inhibited by the methanol extract. This explains that different solvents extract different bioactive compounds responsible for different activities. Based on this, it is expedient to know the solvent that will extract the right biocompounds responsible for the desired activity (ies).

E. faecalis, which is responsible for several inflammations including urinary tract infections, abdominal and pelvic infections, blood poisoning, stomach cramping, and vomiting,[27] was susceptible to both solvent extracts [Table 1]. This is an indication that the extracts of *L. tetragona* could be useful for the management and treatment of the diseases caused by *E. faecalis*. *B. cereus,* which causes food poisoning, gastro and non-gastrointestinal tract infections which can lead to devastating central nervous system infections and anthrax-like progressive *pneumonia,*[28,29] was also susceptible to both the acetone and methanol extracts in this study. The most susceptible bacterium in this study was *E. coli* with a MIC of 0.625 mg/mL (acetone extract) and 2.5 mg/mL (methanol extract) [Table 1]. *E. coli* causes, among others, urinary tract infections and diarrhea. This suggests the usefulness of this species in fighting these infections. *S. typhi,* which causes typhoid fever, was also susceptible to both solvent extracts of *L. tetragona*. Other pathogenic bacteria inhibited by only the acetone extract of the sample are *P. aeruginosa, K. pneumonia, S. pyrogens,* and *B. subtilis* [Table 1].

The outcome of the study also showed the inhibition of all four fungi by both solvent extracts [Table 2]. The most susceptible of the fungi was *T. tonsurans* with a MIC of 0.625 mg/mL in both solvent extracts. *T. tonsurans* is a dermatophyte which causes *Tinea corporis* (ringworm).[30-32] Therefore, the sample may have potential in the skin care/beauty industries. The extracts of *L. tetragona* inhibited *C. albicans* which is an indication that it could serve a great purpose for the treatment and management of vaginal yeast infections (candidiasis), superficial mucosa, thrush, diaper rash, and even hematogenously disseminated infection caused by the fungus *C. albicans.*[33] The MIC for *A. niger* was 2.5 mg/mL [Table 1]. *A. niger* is believed to be nonpathogenic to humans except plants; however, some early and recent studies have reported its involvement in an invasive and chronic obstructive pulmonary[34,35] and brain[36] aspergillosis; serious infection with *pneumonia* in humans. In both conditions, *A. niger* was resistant to voriconazole medication. This sample, therefore, has potentials in ameliorating the infections/diseases caused by *A. nigers* and may also find application in managing other diseases caused by the *Aspergillus* genera.

Other antimicrobial research on some species of the *Celastraceae* family showed diverse degrees of activities. While the antibacterial activities of the ethanol, water, ethyl acetate, and chloroform leaf extracts of *Pleurostylia capensis* had low to no activities on similar bacteria evaluated in this study,[37] there was mild to strong activities by the methanol bark extract of *Austroplenckia populnea*[38] and very strong inhibitory activity by methanol extracts of *Maytenus polyacantha* (wood extract) and *Mystroxylon aethiopicum* (bark and root extracts).[39] In two of these studies, *E. coli* was resistant to the reported extracts of *A. populnea* and *M. polyacantha.*[38,39] Siddiqui *et al.*[40] also reported the antibacterial and antifungal inhibitory activity of *Cathus edulis* (*Celastraceae*) methanol extract. These reports are indications that species belonging to the different genus of the *Celastraceae* family have a reputation in their use as antimicrobial agents.

Worthy of mention is that *in vitro* evaluation of crude extracts does not necessarily confirm the effectiveness of the plant as a medicine nor indicates its final suitability for drug development; rather, it provides a basic understanding of its efficacy which is a basis for the search for new lead bioactive compounds and to validate its use in ethnomedicine.[41] From the foregoing, the crude extracts of *L. tetragona* present a potential

source for the development of novel therapeutic agents against infections and diseases and to design cheap alternative remedies used in economically less-privileged areas.

CONCLUSION

The outcome of this study is the first report of the phytochemical constituents and antibacterial activity of *L. teragona*, a plant used as a traditional herb in the management and treatment of dysentery, diarrhea, and diabetes in Eastern Cape of South Africa. The high polyphenolic compounds and the antimicrobial activity observed in this study could justify its continued usage in folklore medicine. The acetone extract exhibited more antibacterial activity than the methanol extract. This could be as a result that acetone can extract both lipophilic and hydrophilic compounds. Furthermore, most of the organisms used in this study are associated with causal organisms of gastrointestinal tract and urinary infections and have had reports of some degrees of resistance to conventional medications. Therefore, in the face of increased multidrug resistance, *L. tetragona* could serve as a potential alternative remedy against infections caused by the studied organisms and could present a reservoir for future antibiotic development.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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