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Research Article
Antimicrobial and Antidiarrheal Activities of *Pelargonium luridum* (Andrews) Sweet Root Extracts

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Abstract

Background: *Pelargonium luridum* is one of the commonly used plants in the Basotho traditional system of medicine for the management of many diseases including diarrhea. This study investigated antimicrobial and antidiarrheal potentials of its root extracts. **Methodology:** The antimicrobial assays were performed against bacteria and Candida isolates using 96 wells microtitre dilution method, while Wistar rats were employed in the castor oil-induced diarrheal and enteropooling. **Results:** The ethanol and water extracts inhibited all bacterial and Candida strains at MIC ranging from 0.78-3.13 mg mL^{-1}. The aqueous extract at 75 mg kg^{-1} b.wt., significantly ($p<0.05$) reduced the severity of characteristic wet faeces in castor oil-induced diarrheal in Wistar rats by 85% inhibitory activity. The intestinal activity of Na^+-K^+ATPase was dose-dependently improved, while the level of nitric oxide was markedly attenuated. Similarly, the intestinal propulsion, volume and content were reduced following administration of the extract. The presence of tannins, saponins, flavonoids, steroids and cardiac glycosides could be responsible for the observed activities elicited by the extracts. **Conclusion:** The results from the present study have shown *P. luridum* root to be strongly antimicrobial with antidiarrheal activity and therefore, validate its application in the management of infectious diseases by the Basotho traditional medicine practitioners.

Key words: Basotho, castor oil, enteropooling, infectious diseases, intestinal propulsion, Na^+-K^+ATPase

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diarrhea is one of the main causes of high mortality rate in developing countries, where over five million children under the age of five die annually from its complications[1]. It is mostly common in crowdy living conditions coupled with poor hygiene. In spite of government's efforts and various international interventions to stem the progressive incidences of the disease, it still remains an important health issue requiring concerted attention[2]. It is therefore important to identify and evaluate available natural drugs as alternatives to presently used orthodox antidiarrheal drugs, which are not always toxicity-free[3,4]. Though, several medicinal plants with anti-diarrheal properties have been exploited in folkloric medicine, the pharmacological and therapeutic significance of many of them is yet to be scientifically substantiated.

Pelargonium luridum (Andrews) sweet belongs to the Geraniaceae family and is popular for its scented leaves and different medicinal applications. *Pelargonium luridum* also known as wild geranum (English), nafiga (Nyika TZ), umsongelo, ishwaqa (Local South African names), is a perennial herb with round leaves when young, but more dissected into lobes at maturity. The stem is slender, brownish in colour and bears white or tinged pink night scented flowers blooming between November and February. The species usually grows up to 0.8-1 m tall and commonly found in open areas, grassland and burnt fields. Ecologically, it is widely distributed in South Africa and some other parts of Southern Africa[5]. In South Africa folkloric medicine, the scented flower is used among the Zulu young men as a charm for opposite sex during courtship. The leaves are eaten raw as vegetable to treat dysentery, relieve nausea, fevers and to cease vomiting. Its crushed leaf paste is applied on to wounds for speedy healing, while decoction from its roots is mixed with food and taken to treat dysentery/diarrhea[6]. The commonly used *Pelargonium* species in South Africa are *P. luridum* Andr., *P. antidysentericum* (Eckl. and Zeyh.) Kostel., *P. rapaceum* (L.) L'Hér., *P. reniforme* Curt., *P. sidoides* DC. and *P. triste* (L.) L'Hér[7]. *Pelargonium sidoides* have been investigated and reported to possess antibacterial, antifungal and antitubercular activities[8-10]. The concern of the present study was to investigate *in vivo* antidiarrheal potential of *P. luridum* since it has not been reported in any scientific literature till date. Its potency against some of the microbial isolates implicated in diarrhea diseases was also evaluated.

MATERIALS AND METHODS

Chemicals and reagents: Loperamide hydrochloride was purchased from Sigma Aldrich (South Africa). Castor (Hercules) oil and activated charcoal were products of Marico South Africa (Pty) Ltd and Ranbaxy Laboratories, India, respectively. While, water used was glass-distilled, other chemicals and reagents were of analytical grade.

Test organisms: The microbial cultures and isolates used were obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, South Africa. The isolates are: Three Gram-positive bacteria (*Staphylococcus aureus* (ATCC6538), *Staphylococcus aureus* (OK2a), *Staphylococcus aureus* (OK2b)), five Gram-negative bacteria (*Escherichia coli* (ATCC8739), *Shigella flexneri* (KZN), *Shigella sonnei* (ATCC29930), *Salmonella typhi*, *Salmonella typhimurium*) and three *Candida* species (*Candida rugosa*, *C. neoformans* and *C. albicans*) and *Trychophyton mucoides*). The organisms were maintained on nutrient agar plates and were revived for bioassay by sub culturing in fresh nutrient broth (Oxoid Ltd., Basingstoke, Hampshire, England) for 24 h before being used.

Plant collection, identification, pulverization and preparation of extracts: Whole plants of *Pelargonium luridum* were collected in January, 2013 within the premises of the University of the Free State, QwaQwa Campus. The annual rainfall of the area is about 750-1300 mm with a temperature range of 17-22°C. The species was authenticated by Dr. Buwa of the Department of Plant Sciences, University of the Free State, QwaQwa Campus. Voucher specimen (AshMed/01/2013/QwaHb) was prepared and deposited at the University's herbarium. The root was sliced into small bits and dried in the oven at 40°C to a constant weight and then pulverized using Waring Commercial Laboratory (Labcon PTY, Durban, South Africa) electric blender.

Fifteen grams each of the powdered root material was suspended in acetone, ethanol, methanol and distilled water, respectively and placed on Labcon Platform shaker (Laboratory Consumables, PTY, Durban, South Africa) for 24 h. The resulting infusions were filtered using Whatman number 1 filter paper. While the filtrates from acetone, ethanol and methanol were concentrated under reduced pressure (40°C) using rotary evaporator (Cole Parmer SB 1100, Shangai, China), the water extract was freeze dried using Virtis Bench Top (SP Scientific Series, USA) freeze dryer. The individual extract was reconstituted in distilled water to give a stock solution of 50 mg mL^{-1} used for the antimicrobial assays[11].

Another portion (85 g) of the powdered plant material was boiled in distilled water for 15 min and placed on Labcon

platform shaker (Laboratory Consumables, PTY, Durban, South Africa) for 24 h. The decoction obtained was then filtered (using Whatman No. 1 filter paper) and the resulting filtrate was freeze dried. The yield obtained (12.12 g) was thereafter reconstituted to final concentrations of 75, 150, 300 and 600 mg kg^{-1} b.wt., used in the *in vivo* bioassay. The choice of the aqueous root decoction for the *in vivo* studies was influenced in part by the results of the preliminary phytochemical and antimicrobial analyses and largely to its real ethnomedicinal applications[6].

Phytochemical screening: Qualitative phytochemical constituents of *P. luridum* root was determined in the acetone, methanol, ethanol and water extracts adopting standard methods[12-15]. Screenings for alkaloids, tannins, phlobatannins, saponin, flavonoids, steroids, terpenoids and cardiac glycosides were conducted.

Microplate (96 wells) antibacterial assay: Minimum Inhibitory Concentration (MIC) values of the extracts on each organism were determined using microplate dilution method[16], with slight modifications. Briefly, bacterial strains were cultured overnight (24 h) in autoclaved nutrient broth (Oxoid Ltd., Basingstoke, Hampshire, England) and was adjusted to a final density of 10^6 CFU mL^{-1}. This was used to inoculate 96-well microtitre plates containing serial two fold dilutions of extracts (12.50-0.08 mg mL^{-1}) under aseptic condition. The plates were incubated under aerobic conditions at 37°C and examined after 24 h. As an indicator of bacterial growth, 40 µL of 0.2 mg mL^{-1} p-iodonitrotetrazolium (97% purity, Sigma, South Africa) solution was added to each well and incubated for 30 min at 37°C. The colourless tetrazolium salt was reduced to a red-coloured product by the biological activity of the organisms. Each treatment was performed in triplicate and complete suppression of growth at a specific concentration of the extract was required for it to be declared active[16]. Ciprofloxacin (Geltec, Bangalore, India) and fluconazole (Austell Laboratory, Johannesburg, South Africa) were used as positive antibacterial/antifungal controls, respectively. For a good comparative study, the concentration range for the extracts and antibiotics was 12.50-0.08 mg mL^{-1}. Pure solvent and sample free solutions were used as blank controls. For the anticandida testing, the plates were incubated under aerobic conditions at 25°C and examined after 24 h, while other protocols remain the same as performed in antibacterial assay. The MIC in each case was taken as the lowest concentration that prevented the growth of the microbes.

Experimental animals: Female albino rats of Wistar strains weighing 180-220 g were used for the study. The animals were obtained from the animal facility of the University of the Free State, Bloemfontein, Free State Province, South Africa. All experimental animals were allowed to acclimatize to the animal facility condition for 7 days and were kept in clean metallic cages placed in a well ventilated house with optimum condition (temperature: 25±2°C; photoperiod: 12 h natural light and 12 h dark; humidity: 40-45%). They were allowed free access to commercial pelleted rat chow and water *ad libitum*. The floors of the cages were filled with autoclaved saw dusts, while the cleaning was done on regular basis. The study was carried out following approval from the Inter-Faculty Animal Ethics Committee of the University of the Free State with approval number NR02/2013.

Castor oil-induced diarrhea in rats: The method described by Ezeja *et al.*[17] was adopted for this experimentation. Forty nine rats were randomly divided into seven groups (A-G) of seven animals each. They were fasted for 18 h prior to the test but had free access to water. Animals in groups A (normal control) and B (diarrheal control) received 1 mL each of normal saline, while those in group C received loperamide (3 mg kg^{-1} b. wt.). Groups D, E, F and G received 75, 150, 300 and 600 mg kg^{-1} b.wt., of the extract, respectively. All administrations were done via oral intubation. The animals were then housed singly in cages underlaid with weighed A4 papers. One hour after pre-treatment with the extract and loperamide, the animals (except those in group A) were orally challenged with 1 mL of castor oil. Subsequently, they were observed for 6 h (at 2 h intervals) for the presence of diarrhea defined as watery (wet), unformed stool. The observation for total number of faeces and the number of wet feaces passed were recorded and the percentage inhibition of diarrhea was calculated with wet feaces using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Control-test}}{\text{Control}} \times 100$$

Small intestine supernatants preparation: Adopting the method of Akanji and Yakubu[18], the small intestine supernatant was prepared. In brief, under halothane euthanasia, the animals were dissected and small intestine was excised. Its contents were thereafter drained, blotted and homogenized in 0.25 pM sucrose solution (1:4 w/v) using teflon homogenizer. The homogenate was centrifuged at 1500x g for 5 min and the resulting supernatant was used to evaluate intestinal concentrations of protein and nitric oxide as well as the activity of sodium potassium ATPase.

Evaluation of protein content of the supernatant and Na^+-K^+ATPase activity: The method of Gornall *et al.*[19] using biuret reagent was employed in evaluating the intestinal protein concentrations of the animals. The estimated protein concentration was obtained from the calibration curve for bovine serum albumin using the expression:

$$\text{Concentration of protein (mg mL}^{-1}) = Cs \times F$$

Where:

Cs = Corresponding protein concentration from the calibration curve

F = Dilution factor

The values thus obtained were subsequently used in assaying for Na^+-K^+ATPase activity as reported by Bewaji *et al.*[20] using the expression:

$$\text{Specific activity of } Na^+\text{-}K^+\text{ATPase } (\mu\text{mole Pi mg}^{-1}\text{ protein h}^{-1}) = \frac{(Pi)\times 2\times \text{dilution factor}}{1000\times \text{protein concentration (mg mL}^{-1})}$$

where, (P) is the concentration of inorganic phosphate in nmoles (obtained from the calibration curve), 2 is the factor introduced to obtain the amount of Pi released per hour and 1000 is the factor introduced to convert the Pi released to µmoles.

Evaluation of nitric oxide concentration: The procedure described by Wo *et al.*[21] was used in the determination of nitric oxide concentration in the small intestine supernatants of the rats.

Gastrointestinal motility study: The effect of *P. luridum* aqueous root extract on gastrointestinal transit of rats was evaluated adopting previously reported procedure[17], with slight modifications. Briefly, 35 female albino rats were randomized into seven groups (A-G) of five animals per group and fasted overnight. The animals were thereafter treated as highlighted in the castor oil-induced diarrhea model. Exactly 30 min after the treatments, the animals were challenged with 1 mL of charcoal diet and in another 30 min, they were humanely sacrificed under halothane euthanasia. The animals were dissected and the total length travelled by the charcoal from the pylorus to the caecum was measured and recorded. Percentage inhibition was calculated thus:

$$\text{Inhibition (\%)} = \frac{\text{Distance travelled by charcoal}}{\text{Total length of intestine}} \times 100$$

Castor oil-induced enteropooling: Adopting the method of Ezeja *et al.*[17] the effect of the extract on inhibition of intraluminal fluid accumulation was determined by measuring the volume of fluid accumulated in the intestine of rats over time. Briefly, 35 female albino rats were randomly divided into seven groups (A-G) of five animals per group and treated as highlighted in the castor oil-induced diarrhea model above. Thirty minutes post treatments, the animals were challenged with 1 mL of castor and in another 30 min, they were humanely sacrificed under halothane euthanasia. The animals were dissected and the total intestine from the pylorus to the caecum was removed and the content emptied into a measuring cylinder. The volume of the intestinal contents was measured, recorded and percentage inhibition calculated.

Statistical analysis: Results were presented as Mean of determination±SEM and were analyzed using one way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test and data were considered statistically significant at $p<0.05$.

RESULTS

Phytochemical screening: The qualitative phytochemical screening of the acetone, methanol, ethanol and water extracts of *Pelargonium luridun* revealed the presence of tannins, saponins, flavonoids, steroids and cardiac glycosides in all the extracts. Terpenoids were absent in acetone and methanol extracts but were observed in the ethanol and water extracts. Alkaloid was conspicuously absent in all the extracts studied (Table 1).

Antibacterial activity: The results from the antibacterial assay showing Minimum Inhibitory Concentrations (MIC) of the extracts against respective bacteria strains are presented in Table 2. The acetone and methanol extracts inhibited *Escherichia coli*, *Shigella sonnei*, *Staphylococcus aureus*

Table 1: Qualitative phytochemical screening of *Pelargonium luridum* root extracts

	Extracts			
Compound	Acetone	Methanol	Ethanol	Water
Alkaloid	-	-	-	-
Tannins	+	+	+	+
Phlobatannins	-	-	-	-
Saponin	+	+	+	+
Flavonoids	+	+	+	+
Steroids	+	+	+	+
Terpenoids	-	-	+	+
Cardiac glycosides	+	+	+	+

+: Detected and -: Not detected

Table 2: Minimum Inhibitory Concentrations (MICs) (antidiarrheal activity) of root extracts of *Pelargonium luridum* and standard antibiotic against susceptible bacterial

		Extracts/Drug (mg mL^{-1})				
Organism	Gram+/-	Acetone	Methanol	Ethanol	Water	Ciprofloxacin
Escherichia coli ATCC 8739	-	0.39	0.39	0.78	1.56	0.08
Salmonella typhi	-	0.78	0.78	0.78	3.13	0.08
Salmonella typhimurium	-	0.78	0.78	0.78	1.56	0.08
Shigella flexneri KZN	-	0.78	0.78	0.78	0.78	0.08
Shigella sonnei ATCC 29930	-	0.39	0.39	0.08	0.78	0.08
Staphylococcus aureus OK2a	+	0.39	0.39	0.78	0.78	0.08
Staphylococcus aureus ATCC 6538	+	0.39	0.39	0.78	0.78	0.08
Staphylococcus aureus OK2b	+	0.78	0.78	0.78	0.78	0.08

ATCC: American type culture collection and KZN: Kwazulu natal

Table 3: Anticandida activity or the Minimum Inhibitory Concentrations (MICs) of root extracts of *Pelargonium luridum* and standard antifungal against susceptible isolates responsible for diarrhea in humans

	Extracts (mg mL^{-1})				
Organism	Acetone	Methanol	Ethanol	Water	Fluconazole
Candida rugosa	1.56	1.56	1.56	1.56	3.13
Candida neoformans	1.56	0.78	1.56	1.56	12.50
Candida albicans	1.56	1.56	1.56	1.56	12.50
Trichophyton mucoides	1.56	1.56	1.56	1.56	6.25

Table 4: Effect of *Pelargonium luridum* aqueous root extract on castor oil-induced diarrhea in female Wistar rats

Treatment	Mean defecation in 4 h	Inhibition (%)
Castor oil	10.00±0.05^{a}	-
Loperamide (3 mg kg^{-1})+castor oil	3.00±0.01^{b}	70
75 mg kg^{-1}+castor oil	1.50±0.04^{c}	85
150 mg kg^{-1}+castor oil	2.00±0.01^{c}	80
300 mg kg^{-1}+castor oil	2.50±0.02^{c}	75
600 mg kg^{-1}+castor oil	4.50±0.05^{b}	55

Data are expressed as Mean±SEM, Values carrying different alphabetical superscript for the parameter are significantly different (p<0.05)

OK2a and *S. aureus* ATCC6538 at MIC of 0.39 mg mL^{-1}, whereas, it was 0.78 mg mL^{-1} for *Salmonella typhi*, *S. typhimurium*, *Shigella flexneri* and *Staphylococcus aureus* OK2b. The ethanol extract on the other hand could only inhibit all the organisms at 0.78 mg mL^{-1} except *Shigella sonnei* that was inhibited at 0.08 mg mL^{-1}. Similarly, the water extract was able to suppress the growth of all bacterial strains at MIC of 0.78 mg mL^{-1} except *E. coli*, *Salmonella typhimurium* and *S. typhi* that were inhibited at 1.56 and 3.13 mg mL^{-1}, respectively. The over-the-counter standard antibiotic (ciprofloxacin) inhibited all bacterial strains at 0.08 mg mL^{-1}, although this concentration is less than that of the extracts but it should be note that extracts are mixture of many compounds while antibiotics are generally pure compounds.

Anticandida activity assay: The acetone, methanol, ethanol and water extracts displayed similar inhibitory capability by suppressing the growth of all Candida species and *Trichophyton mucoides* at MIC of 1.56 mg mL^{-1} (Table 3). All the extracts displayed better anticandida activity than fluconazole (standard antifungal), which could only inhibit all fungi tested at MIC of 3.13-12.50 mg mL^{-1}.

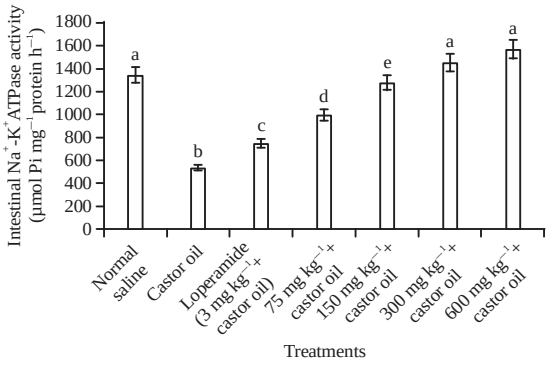

Fig. 1: Effects of *Pelargonium luridum* aqueous root extract on intestinal Na^+-K^+ATPase activity of castor oil-treated Wistar rats (n = 7, X±SEM). Bars with different superscripts are significantly different (p<0.05)

Castor oil-induced diarrhea: The administration of the aqueous root extract of *Pelargonium luridum* to castor oil-induced diarrheal rats significantly (p<0.05) reduced the severity of characteristic wet faeces in all the doses tested compared to both castor oil and loperamide-treated groups. At 75 mg kg^{-1} b.wt., of the extract, the inhibition was 85% compared to 70% inhibition obtained from the loperamide-treated animals. The best result was observed in the 75 mg kg^{-1} body of the extract, when compared to other concentrations tested in this study (Table 4). While, the activity of Na^+-K^+ATPase in the small intestine improved significantly (p<0.05) in a dose-dependent manner in the extract-treated animals when compared with both the control and standard drug used (Fig. 1), a sharp contrast was observed in the concentration of nitric oxide, where there was a marked (p<0.05) and dose-dependent attenuation (Fig. 2). In fact, the effect elicited by the 75 and 150 mg kg^{-1} b.wt., doses of the extract competed favourably well with loperamide (Fig. 2).

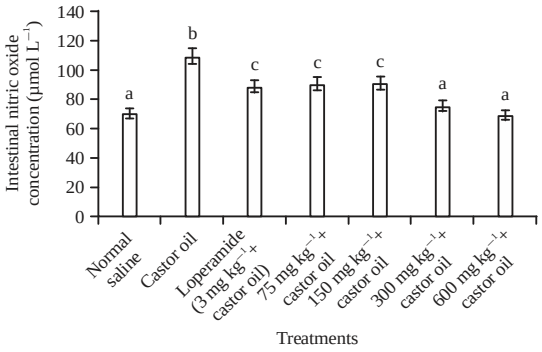

Fig. 2: Effects of *Pelargonium luridum* aqueous root extract on intestinal nitric oxide concentration of castor oil-treated Wistar rats (n = 7, X±SEM). Bars with different superscripts are significantly different (p<0.05)

Table 5: Effect of *Pelargonium luridum* aqueous root extract on gastrointestinal transit of female Wistar rats

Treatments	Intestine length (cm)	Distance travelled by agent (cm)	Inhibition (%)
Castor oil	96.50±0.33	63.00±0.97[a]	-
Loperamide+castor oil	106.00±0.10	53.75±0.08[b]	50.71
75 mg kg^{-1}+castor oil	107.00±0.31	72.50±0.78[c]	67.76
150 mg kg^{-1}+castor oil	109.50±0.36	69.25±0.59[a]	63.24
300 mg kg^{-1}+castor oil	106.00±0.00	75.50±0.44[c]	71.23
600 mg kg^{-1}+castor oil	116.25±0.48	83.00±0.49[c]	71.55

Data are expressed as Mean±SEM. Values carrying different alphabetical superscript for the parameter are significantly different (p<0.05)

Table 6: Effect of *Pelargonium luridum* aqueous root extract on castor oil-induced enteropooling of female Wistar rats

Treatments	Intestine length (cm)	Intestinal content (mL)	Inhibition (%)
Castor oil	108.50±0.71	3.40±0.57[a]	-
Loperamide+castor oil	105.00±1.41	1.80±0.85[b]	47.06
75 mg kg^{-1}+castor oil	107.50±0.26	2.00±0.21[c]	41.17
150 mg kg^{-1}+castor oil	104.00±1.41	2.33±0.98[c]	31.47
300 mg kg^{-1}+castor oil	103.00±0.24	2.05±0.92[c]	39.71
600 mg kg^{-1}+castor oil	93.00±0.79	1.69±0.14[b]	50.30

Data are expressed as Mean±SEM. Values carrying different alphabetical superscript for the parameter are significantly different (p<0.05)

Gastrointestinal motility: Compared with loperamide-treated animals, higher percentage inhibition of intestinal motility transit was observed in the extract-treated animals with inhibitory potentials ranging from 67.76-71.55% (Table 5).

Castor oil-induced enteropooling: Data obtained with respect to intestinal content volume of the animals treated with graded doses of *P. luridum* aqueous root extract are presented in Table 6. The effect elicited by the extract on this parameter were remarkable and compared well (especially at 75 and 600 mg kg^{-1}) with the reference drug (loperamide) used in this study.

DISCUSSION

The major causative agents of diarrhea in human beings include various enteropathogens like *Shigella flexneri*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Candida albicans*[22,23]. For the treatment and management of diarrhea in developing countries, the World Health Organization (WHO) has constituted a Diarrhea Disease Control (DDC) programme, which includes studies of traditional medicine practices together with the evaluation of health education and prevention approaches[24].

The results from the present study have shown that crude extracts from the subterranean parts of *P. luridum* could inhibit *Shigella flexneri, Shigella sonnei* and strains of *Staphylococcus aureus* at MIC less than 1.0 mg mL^{-1}. Majority of plant extracts have been reported to be more active against Gram-positive bacteria than the Gram-negative bacteria strains[25,26]. In this study, extracts from the root of *P. luridum* were very active against both the Gram-negative and the Gram-positive bacteria strains. Water is known to extract almost all the active ingredients from plant samples, although, its activity at low concentration is very rare[27]. Seemingly, it becomes efficacious due to dosage administration by traditional healers. In the present study, the water extract was able to inhibit all bacterial strains at MIC ranging from 0.78-3.13 mg mL^{-1}, indicating that the

antibacterial bioactive phytochemicals in this plant are likely more polar in nature. The susceptibility of these nosocomial opportunistic pathogens to the extracts from *P. luridum* is interesting as some of them have been implicated in cases of immuno-compromised patients[28]. Standard antibiotics are pure compounds synthesized from their isolated prototypes and therefore act as individual compound and not in synergy with other compounds. Although, ciprofloxacin inhibited all bacterial strains at 0.08 mg mL^{-1}, the fact remains that plant extracts are crude product composed of many compounds. The ability of the extracts from *P. luridum* root to inhibit many of the bacteria tested at concentration less than 1.00 mg mL^{-1}, is an indication that further fractionation of the acetone and methanol or ethanol extracts could give results that will compete favourably with ciprofloxacin and other antibiotics. *Candida* species are known to cause candidiasis, which encompasses infections that range from superficial, such as oral thrush and vaginitis, to systemic and potentially life-threatening diseases such as diarrhea. The capability of the extracts to suppress *Candida* strains at MIC ranging from 0.78-1.56 mg mL^{-1} was encouraging as the organisms are rarely susceptible to plant extracts action especially at low concentrations. All the extracts exhibited better antifungal activity against *Candida* species and *Trichophyton mucoides*. Basotho tribe uses infusion or decoction to manage infectious diseases. The fact that the water extract displayed potent anticandida activity is indicative that the antifungal bioactive compounds in *P. luridum* root are polar/water soluble in nature. The inhibitory effect of the root extracts from *P. luridum* against arrays of bacteria and fungi strains could partly account for the use of the plant in the management of microbial infections including those responsible for complications and disorders associated with diarrhea. This further validates the use of this plant in the Basotho traditional medicine in the management of infectious diseases.

The use of castor oil-induced diarrhea in experimental animals is a common model because of its ability to increase intestinal content by preventing the re-absorption of water and the liberation of ricinoleic acid from the oil. This subsequently results in irritation and inflammation of the intestinal mucosal leading to release of prostaglandins, which enhances motility stimulation and secretion as well as prevention of NaCl and water re-absorption[17,29]. The consequential effect of this is increase in the secretion of water and electrolytes as well as corresponding increases in the number of wet faeces and intestinal transit time[30].

The aqueous root extract of *P. luridum* exhibited significant antidiarrheal activity better than the reference drug (loperamide) by significantly reducing the castor oil-induced diarrhea in rats, delaying the onset of copious diarrhea, decreased the frequency of purging, reduced the number and weight of wet stools and inhibited the severity of diarrhea generally. Similar results were observed when *Pyrenacantha staudtii* was investigated for its antidiarrheal potential[31]. Furthermore, the specific activity of Na^+-K^+ATPase in the absorption of sodium and fluids in the intestine of animals was significant. Its inhibition may facilitate intestinal fluid accumulation which, consequently predisposes animals to diarrheal[32]. The dose-dependent increase in the activity of Na^+-K^+ATPase by the aqueous root extract of *P. luridum* is indicative of intestinal fluid normalization which buttresses antidiarrheal potential of *P. luridum*. The attenuation in the concentrations of intestinal nitric oxide by the extract might also be suggestive of its capability to either moderate electrolyte metabolism or palliate luminal osmolarity across the rat's intestinal mucosal and thus further substantiating its antidiarrheal activity. This submissions are consistent with previous findings[33-35], where, similar observations were reported in plant extracts-administered animals exposed to castor oil treatment. Although, in the present study, the activities were dose specific, the best observation was made in the 75 mg kg^{-1} b.wt., of the extract, which suggests that the anti-diarrhoic activity revolves around this dose. It is also interesting to note that the remarkable moderations in fecal indices, Na^+-K^+ ATPase activity and nitric oxide concentration by aqueous root extract of *P. luridum* may substantiate and be informative of the probable mechanism of action of the antidiarrheal bioactive principles in the extract. This could either be that the extract was able to stimulate the re-absorption of water from the intestinal lumen or by anti-prostaglandin activities that contribute to the patho-physiological functions in the gastrointestinal tract.

The administration of castor oil to the experimental animals stimulated small intestinal transit as shown by over 80% of the small intestine travelled by the charcoal plug in normal saline treated rats. Oral administration of *P. luridum* aqueous root extract brought about dose specific and significant reduction in the percentage of the intestinal transit with the extract at 75 mg kg^{-1} b.wt., being more effective than the reference drug. The observed inhibition of the intestinal transit by this extract can be used to establish that the extract possessed the ability to relax intestinal smooth muscles. Most antidiarrheal drugs have been reported to possess the ability to reduce intestinal contraction and transit[17,36] and this was observed in the *P. luridum* treated animals, which further confirm the antidiarrheal property of this species.

In the enteropooling study, *P. luridum* extract at all the investigated doses significantly reduced the volume of the

animal intestinal contents, in which case the intraluminal fluid accumulation induced by castor oil was blocked by the extract in a dose specific manner. The prevention of intraluminal fluid secretion by *P. luridum* aqueous root extract in this study may be due to inhibition of prostaglandins biosynthesis with resultant reduction in the secretion of fluid into the lumen or could be due to the promotion of absorption of water and electrolytes in the gut[17].

The phytochemical screening of the root extracts revealed the presence of tannins, flavonoids, saponins and steroids. Some of these compounds have been reported to possess strong antidiarrhea activity[37,38]. The antidiarrheal potential of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretions[39]. Tannins have also been implicated in the denaturation of protein thereby preventing the formation of protein tannate. This makes the intestinal mucosa more resistant and reduces secretion[40].

CONCLUSION

Overall, the remarkable antidiarrheal effect of *Pelargonium luridum* root extracts against various pathogenic organisms and castor oil-induced diarrhea models attest to a wide range of utility in secretory and functional diarrhea management. Although, the mechanism of action of *P. luridum* extract is still poorly understood at this stage, yet it is clear that the root of this plant could be an important raw material for antidiarrheal bioprospecting for South and Southern African population.

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