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Aqueous Extracts of *Pericopsis angolensis* and *Swartzia madagascariensis* with High Antimicrobial Activities against *Escherichia coli* O157, Shigella spp. and *Salmonella enterica* subsp. enterica (*Serovar typhi*)

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This study determined the potential antidiarrhoeal potential of *Pericopsis angolensis* and *Swartzia madagascariensis* extracts against *Escherichia coli* O157, *Shigella* species and *Salmonella* Typhi. Extracts were obtained using the following methods: (i) hot water extraction (90°C) (LHWE), (ii) cold water extraction (CWED) and (iii) ethanolic extraction (EED). Antimicrobial effects of the extracts were determined using the well diffusion assay. Phytochemical analysis was performed using standard biochemical methods. The LHWE extracts exhibited significantly greater inhibition than CWED and EED extracts as follows: (i) *P. angolensis* bark extract at 0.8 mg/ml against *Shigella* spp. and (ii) *P. angolensis* bark extract at 1.6 mg/ml and *S. madagascariensis* bark extract at 1.6 mg/ml against *S.* Typhi. The aqueous methods largely resulted in *P. angolensis* and *S. madagascariensis* extracts rich in flavonoids, saponins and tannins. The aqueous extraction methods (CWED and LHWE) are therefore suitable to obtain extracts with high antimicrobial effects against *E. coli* O157, *Shigella* species and *S.* Typhi.

Key words: Antidiarrhoeal, phytochemicals, extraction, *Pericopsis angolensis*, *Swartzia madagascariensis*.

INTRODUCTION

The use of plants or their products in traditional medicines has, since historic times, remained significant in the treatment of various medical ailments such as diarrhoea (Maroyi, 2016). Notably, there is a renewed public interest in the use of traditional medicines owing to the high costs of orthodox medicines and the associated side effects, especially antimicrobial resistance (Patwardhan et al., 2005). In African countries, approximately 80% of the population reportedly rely on traditional medicines owing to their low cost and ease of

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access, as well as for cultural reasons (Maroyi, 2016).

Diarrhoeal diseases have remained a global burden and a leading public health threat characterised by high morbidity and mortality especially among children under the age of 5 years (Maroyi, 2016). Diarrhoea is thought to account for between 1 and 9 million deaths among children aged 5 years or younger globally, with the highest rates occurring in low income countries (LIC) especially those in sub Saharan Africa and Asia (Njume et al., 2011).

Plants have maintained their place in traditional medicine owing to their rich composition of healthful bioactive chemical compounds/metabolites (Smith, 2007). Notably, most communities in LIC tend to rely of phytomedicines to manage various forms of diarrhoea including cholera, typhoid and various forms of gastroenteritis. Bioactive compounds are accumulated plant tissues as secondary metabolites (Smith, 2007). These metabolites are often accessed through the use of extraction techniques, with some plant materials being consumed whole to achieve the desired therapeutic or prophylactic effect (Semenya and Maroyi, 2013). The composition and bioactivity of an extract depends on its inherent chemical composition, the solvent used as well as the protocol followed during the extraction process (Muhamad et al., 2017). Traditional medicine has often relied on organic solvents including the use of cold and hot water (steeping) as extractants (Ngarivhume et al., 2015; Palombo, 2011; Wachtel-Galor, 2004). However, the utilisation of organic solvents is often limited by a low extraction yield (Wachtel-Galor, 2004). To enhance the extraction processes, scientists have adopted protocols that use a combination of organic solvents (Pilon et al., 2016). The traditional extraction methods, relying primarily on hot or cold water, utilise fresh or dried plant materials, with the obtained extracts being administered within hours from the time of plant material collection (De Wet and Ngubane, 2014; Odunmbaku et al., 2018). Different extractive strategies reportedly yield products with different clinical efficacies (Odunmbaku et al., 2018). With the availability of more modern extraction methods including maceration, percolation, reflux extraction, super critical fluid extraction (SFC), pressurised liquid extraction (PLE) and microwave assisted extraction (MAE)-with advantages that include enhanced extraction efficiency and improved extract bioactivities (Zhang et al., 2018), their utility compared to that of the traditional hot and cold water extraction methods have remained unappraised.

The current study compared the antidiarrhoeal potential of hot water extraction and cold water extraction methods (LHWE and CWED, respectively) to that of ethanolic extraction method (EED). Specifically, the current study sought to assess the validity and utility of the aqueous extraction methods, which are considered methods of choice in the preparation of traditional antidiarrhoeal medicines. Two plant species with a history of use in the traditional management of diarrhoea in Southern Africa, namely *Pericopsis angolensis* (Baker) Meeuwen and *Swartzia madagascariensis* (Desv.) J.H. Kirkbr. & Wiersama (Table 1), were used for the study. The extracts were tested for antimicrobial activities against selected diarrhoeagenic *Escherichia coli* O157, *Shigella* species and presumptive *Salmonella* Typhi. Additionally, the phytochemical composition of the extracts was determined. This study was considered important as it informs both the traditional and orthodox medicinal practices on the relative utilities of these extraction methods.

MATERIALS AND METHODS

Collection and processing of plant materials

Fresh samples of *P. angolensis* bark, *S. madagascariensis* bark and *S. madagascariensis* leaves were collected in Chiraswa Village in Murehwa, Mashonaland East Province of Zimbabwe (-17°.69′71.55″S, 31°.96′48.90″E) during the months of October-December 2018. Species identification was done by qualified botanists at the National Herbarium and Botanic Garden in Harare (Zimbabwe). Voucher specimens were deposited in the Biological Sciences laboratory for future reference.

The collected plant materials were washed to remove debri and then separated into two batches. Half of each fresh sample was frozen in airtight plastic bags for future use. The other half of each plant sample was air-dried for 72 h, then ground into fine powders with an electric grinder and stored in air tight containers in the dark at room temperature.

Extraction

Cold water (*CWED*) *and ethanolic extraction* (*EED*)

The powdered samples were extracted into cold distilled water (150 ml) and 70% ethanol (150 ml). The plant-solvent mixtures were continuously swirled at 150 rpm on a rotary shaker for 72 h. The extracts were filtered through Whatman No. 41 filter paper (pore size 20-25 µm) and the collected filtrates were evaporated at room temperature. Each dried extract was resuspended into between 1 and 2 ml of sterile Ringers solution. Concentrations of each stock solution were stored at -20°C until further analysis.

Hot water extraction (*THWE*)

Fresh plant samples (10 g) were added to 100 ml of hot boiled water (90°C) and steeped for 30 min. The samples were filtered through Whatman No. 41 filter paper (pore size 20-25 µm) and were stored at -20°C for further tests.

Phytochemical analyses

Qualitative chemical analyses of the extracts were conducted using the following biochemical tests. Ringers solution was used as a negative control for all phytochemical tests.

Test for tannins (*Ferric chloride test*)

A few drops of 0.1 ferric chloride were added to 2 ml of aqueous extracts (CWED, EED and THWE). A blue coloration indicated the

Table 1. Description of selected plant species used in the management of diarrhoea and other ailments.

Plant species	Common name	Distribution in Zimbabwe	Parts of plants used	Extraction method	Bioactive compounds
Pericopsis angolensis (Baker) Meeuwen	Afrormosia (English), Muwanga (Shona), Ubanga (Ndebele)	North, Central and Eastern parts [14]	Roots: abortifacient, aphrodisiac and a tonic, decoctions blood circulation stimulant, diarrhoea, bronchial and chest pains, nausea and eye problems. Dried and powdered root: relieve pain, treat oedema and tumours. Bark: diarrhoea, sore throat, toothache, eye bath. Leaves: vapour for headaches, anthelmintic (http//www.prota.org)	No information found	No information found
Swartzia madagascariensis (Desv.) J.H. Kirkbr. & Wiersama	Snake bean (English), Mucherekese (Shona) [14]	East [14]	Bark: ear treatments, laxative, venereal diseases. Leaves: astringents, mammal and bird poisons, rodenticides. Root: diarrhoea, dysentery, vermifuges, abortifacients, ecbolics, antidotes. Pod: insecticides, arachnicides, leprosy (Royal botanical gardens.	Solvent extraction (ethanol and hexane and ethyl acetate) using Soxhlet apparatus [15]	Hexane extracts (steroids and triterpenes present) [15], Ethanol extracts (flavonoids, saponins, triterpenes, alkaloids. Fruits: glycosides, saponins, steroids. Leaf, root and seeds: tannins

presence of tannins [16].

Test for flavonoids

Dilute ammonia (5 ml) solution was added to 1 ml of each plant extract. Concentrated sulphuric acid (5 ml) was added and a yellow coloration in each plant extract indicated the presence of flavonoids (Zohra et al., 2012).

Test for alkaloids

Aqueous 1% hydrochloric acid (0.2 ml) was added to 2 ml each extract. Each solution was heated in a steam bath for 10 min. The aqueous extract solution was treated with 6 to 10 drops of Dragendoff's reagent. A creamish precipitate indicated the presence of alkaloids (Zohra et al., 2012).

Test for saponins

Aqueous extracts (2 ml) were mixed with distilled water (5 ml) and shaken vigorously for stable persistence froth. The froth was mixed with 3 drops of olive oil and was shaken vigorously. Emulsion indicated the presence of saponins (Zohra et al., 2012).

***Test for reducing sugars* (*Benedict's test*)**

To 1 ml of the plant extract, a few drops of Benedict's reagent (alkaline solution containing cupric citrate solution) were added and boiled in a water bath. A reddish brown precipitate indicated the presence of reducing sugars (Avinash and Waman, 2014).

Bacterial strains

The microorganisms used in determination of the antibacterial activity of the plant extracts' were as follows: presumptive *E. coli* O157, *Shigella* spp. and *S.* Typhi. All bacterial strains were obtained from our in-laboratory stock of environmental isolates. The isolated strains were maintained on Nutrient agar. The bacterial cultures were prepared by transferring a colony of the bacteria into a universal bottle containing 10 ml of nutrient broth and incubated overnight at 37°C. The concentration of the bacterial cultures was standardised to a concentration of 1×10^8 colony forming units per millilitre (CFU/ml) (internal protocol), which is equivalent to an optical density of 0.2 using a Biobase EL 10B Microplate Reader (Jinan, China) at optical density 620 nm. Ringer's solution was used as a negative control for all antimicrobial tests.

Antibacterial screening

Antibacterial tests were performed using standard agar well diffusion assay as described by Soman and Ray (2016). Briefly, agar plates were prepared using sterile HiCrome O157: H7 agar (Sigma-Aldrich, Saint Quentin Fallavier, France) and XLD agar (Sigma-Aldrich, Saint Quentin Fallavier, France) for *E. coli* O157, *Shigella* spp. and *S.* Typhi, respectively. The standardised cultures were evenly spread onto the surface of the agar plates using sterile swabs. Wells were made in each plate with a sterile auger (10 mm diameter). 40 µl of ethanol and aqueous extracts (100 mg/ml) were added in each well, with streptomycin (300 µg, Mast Diagnostics, UK) being used as positive control. The plates were incubated at 37°C for 24 h. Each extract was tested in triplicate.

Antibacterial activity was tested by observing bacterial growth and was indicated as the presence of clear zones around the well

(zones of inhibition). The absence of the zone of inhibition around the wells was interpreted as the absence of activity. The zones of inhibition were measured in millimetres. Only extracts that showed antimicrobial activities were used to determine the minimum inhibition and minimum bactericidal concentration of each preparation.

Determination of minimal inhibitory concentration (MIC)

The minimal inhibitory concentration of the plant extracts/control was determined using the well diffusion assay (Soman and Ray, 2016). Agar plates were prepared using sterile HiCrome 0157: H7 media for *E. coli* 0157 and XLD media for *Salmonella* and *Shigella* spp. The standardised cultures were evenly spread on the surface of the agar plates using sterile swabs under sterile conditions. Wells were made in each plate with a sterile auger (10 mm diameter). 40 µl of plants extracts (two fold concentrations ranging from 0.781 to 100 mg/ml) were added in each triplicate wells. Streptomycin (300 µg, Mast Diagnostics, UK) was used as the positive control. The diffusion of the extracts was allowed at room temperature for 1 h in a sterile laminar flow cabinet and the plates were incubated at 37°C for 24 h. The plates were observed for antimicrobial activity and the zones of inhibition (mm) indicated the minimum concentration at which the extracts inhibited the growth of the test microorganisms. The concentration at which there was no zones of inhibition were recorded as the minimum inhibition concentration.

Determination of minimal bactericidal concentration (MBC)

A modified assay to that described by as modified from the Soman and Ray (2016) method was used to determine the MBC of each extract. Briefly, using the agar plates from the MIC assay, a sterile inoculating loop was used to touch the zone of inhibition of different concentrations of extracts where there was invisible growth. The loops were used to streak labelled and prepared agar plates. The plates were incubated for 24 h and observed for growth at different concentration.

RESULTS

Extracts from plant materials listed in Table 1 were exposed to different solvents and conditions. Briefly, plant materials were exposed to the following: (i) hot water (steeping) for 1 h (LHWE), a method simulating the traditional extraction method; (ii) cold water for 72 h (CWED) followed by evaporation at room temperature and (iii) ethanol for 72 h followed by evaporation at room temperature (EED). Yields per extract (mg) were obtained by weighing the dried samples and subtracting the weight of the containers (Petri dishes). Table 2 provides details of the amount of material used and the yield of extract (mg).

Standard phytochemical analyses were conducted on extracts described in Table 3. Briefly, extracts were exposed to various chemicals in accordance with standard biochemical protocols. Colour and other changes in the extracts were used to show the presence of the target compounds. Relative phytochemical concentration was determined relying on intensities of the extracts. Table 3 provides information on chemical composition of each extract used.

The traditional hot water extraction method (LHWE) yielded extracts that contained the following phytochemicals [flavonoids (*P. angolensis* bark extract and *S. madagascariensis* bark and leaf extracts, saponins (*P. angolensis* bark extract and *S. madagascariensis* bark and leaf extracts) and reducing sugars (*P. angolensis* bark extract and *S. madagascariensis* bark and leaf extracts)] (Table 3). The cold water extraction method (with evaporation) (CWED) yielded extracts that contained the following phytochemicals [flavonoids (*P. angolensis* bark extract and *S. madagascariensis* bark and leaf extracts, saponins (*P. angolensis* bark extract and *S. madagascariensis* bark and leaf extracts), tannins (*P. angolensis* bark extract and *S. madagascariensis* bark and leaf extracts) and reducing sugars (*P. angolensis* bark extract and *S. madagascariensis* bark and leaf extracts)] (Table 3). The ethanolic extraction method (EED) yielded extracts with greater concentrations of reducing sugars (*P. angolensis* bark extract)] (Table 3). All extracts showed no presence of alkaloids.

The traditional hot water extraction method (LHWE) yielded the highest antimicrobial activities in terms of the attainment of the lowest minimum inhibition concentration (MIC)/highest zone of inhibition (ZOI) against *Shigella* spp. in comparison with the other two extraction methods (CWED and EED) as follows: (i) *P. angolensis* bark extract (ZOI = 21 mm) (Figure 3) and (ii) *S. madagascariensis* bark extract (1.56 mg/ml) (Figure 1). The LHWE extract was shown to yield significantly greater ZOI at 100 mg/ml than CWED and EED against *Shigella* spp. (p = 0.001 and p = 0.0003, respectively) (Table A1). Additionally, LHWE extracts of the following extracts attained significantly greater antimicrobial activity (ZOI) against the strain of *Shigella* spp. (at 100 mg/ml) than Streptomycin (300 µg/ml) (ZOI = 20 mm) with the following: (i) *S. madagascariensis* leaf extract (ZOI = 23 mm) (Figure 2) and (ii) *P. angolensis* bark extract (ZOI = 21 mm) (Figure 1). Additionally, the LHWE extract retained the highest activity against *Shigella* spp. compared to the other two (CWED and EED) across all concentrations tested (Figures 1, 2 and 3).

The LHWE extraction method was shown to yield extracts with significantly greater ZOI against *Shigella* spp. than those from the other methods with the following: (i) *P. angolensis* bark extract (LHWE > CWED - p = 0.001) and (ii) *P. angolensis* bark extract (LHWE > EED – p = 0.0003) (Table A1). Additionally, LHWE extracts were shown to have significantly greater ZOI at 100 mg/ml than other against the *S.* Typhi as follows: (i) *S. madagascariensis* bark extract (LHWE > CWED – p < 0.0001); (ii) *S. madagascariensis* bark extract (EED > LHWE – p = 0.002); (iii) *S. madagascariensis* leaf extract (LHWE > CWED – p = 0.0001); (iv) *S. madagascariensis* leaf extract (LHWE > EED – p = 0.0003); (v) *P.*

Table 2. Extract yield per unit weight of plant material used and extraction method (traditional hot water extraction - LHWE, cold water extraction with concentration - CWED and ethanolic extraction with concentration - EED).

Plant species and extract		Weight of plant material used (g)	Yield (mg)
P. angolensis bark	CWED	45.18	510
	EED	45.18	465
	LHWE	10	N.D
S. madagascariensis bark	CWED	45.17	320
	EED	45.17	1 275
	LHWE	10	N.D
S. madagascariensis leaf	CWED	45.17	320
	EED	45.17	720
	LHWE	10	N.D

N.D: Not determined. Extract was used without evaporation; hence its dry weight was not determined. CWED: Cold water extract with desiccation; EED: ethanol extract with desiccation; LHWE: aqueous extract obtained by the traditional extraction method.

Table 3. Qualitative phytochemical composition of extracts obtained using traditional hot water extraction (LHWE), cold water extraction with concentration (CWED) and ethanolic extraction with concentration (EED).

Plant species and extract		Saponins	Alkaloids	Flavonoids	Tannins	Reducing sugars
P. angolensis bark	CWED	+++	-	+++	+++	+
	EED	++	-	+	-	+++
	LHWE	++	-	+	-	+
S. madagascariensis bark	CWED	+++	-	+	-	+
	EED	-	-	+	++	-
	LHWE	+++	-	+	+++	++
S. madagascariensis leaf	CWED	++	-	+	-	+++
	EED	-	-	+	++	-
	LHWE	++	-	+++	-	+++

CWED: Cold water extract with desiccation; EED: ethanol extract with desiccation; LHWE: aqueous extract obtained by the traditional extraction method; + = presence of phytochemical in trace amounts; -: absence of phytochemical; ++: moderate amount of phytochemical; +++: appreciable amounts of phytochemicals.

angolensis bark extract (LHWE > CWED – p = 0.002) and (vi) *P. angolensis* bark extract (LHWE > EED – p = 0.0004) (Table A1, Figure 4 and 5).

The cold water extraction method with desiccation (CWED) yielded the highest antimicrobial activities in terms of the attainment of the lowest minimum inhibition concentration (MIC) / highest zone of inhibition (ZOI) against *S.* Typhi in comparison with the other two extraction methods (LHWE and EED) as follows: (i) *S. madagascariensis* bark extract (MIC = 0.78mg/ml) (Figure 1d) and *S. madagascariensis* leaf extract (MIC = 1.56mg/ml) (Figure 1e). Additionally, the CWED extract retained the highest activity against *S. typhi* compared to the other two (LHWE and EED) across all concentrations of *S. madagascariensis* bark extract (Figure 1d). Additionally, the CWED extract from *P. angolensis* bark was shown to have significantly greater ZOI at 100mg/ml than that of EED extract against the *S. typhi* (CWED > EED – p = 0.02) (Figure 6).

The hot water extraction method (LHWE) yielded extracts that had no antimicrobial activity against *E. coli.* The cold water extraction method with desiccation (CWED) of *S. madagascariensis* leaf extract (ZOI = 21 mm) yielded the higher antimicrobial activities in terms of the attainment of the highest zone of inhibition (ZOI) against *E. coli* than that of extracts from the other two extraction methods (LHWE and EED) (Figure 8). Additionally, CWED extracts attained greater antimicrobial activity against the strain of *E. coli* (at 100 mg/ml) than streptomycin (300 µg/ml) (ZOI = 23 mm) as follows:

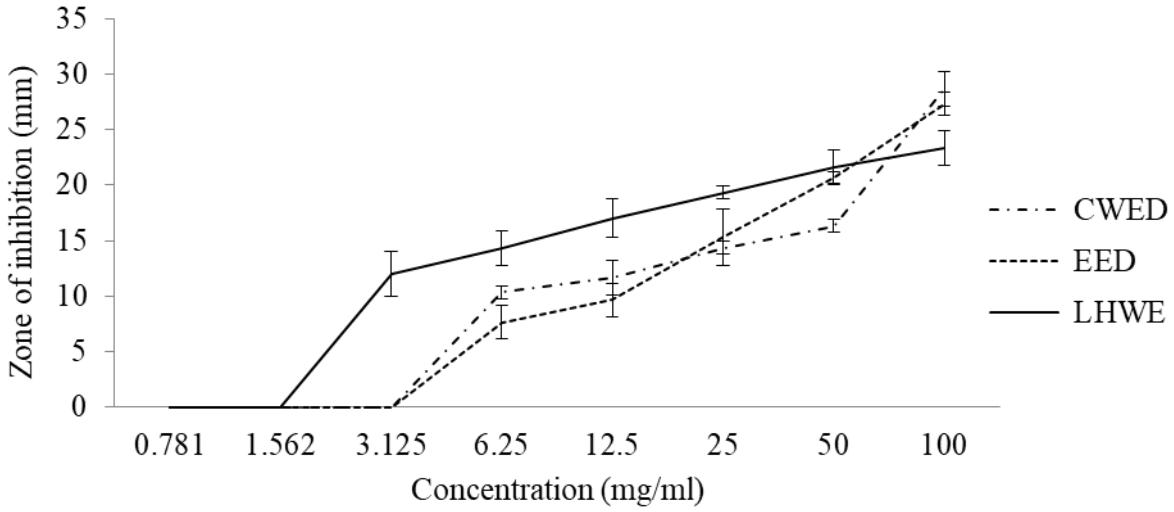

Figure 1. Inhibition of *Shigella* spp. isolate by varying concentrations of *S. madagascariensis* leaf extract.

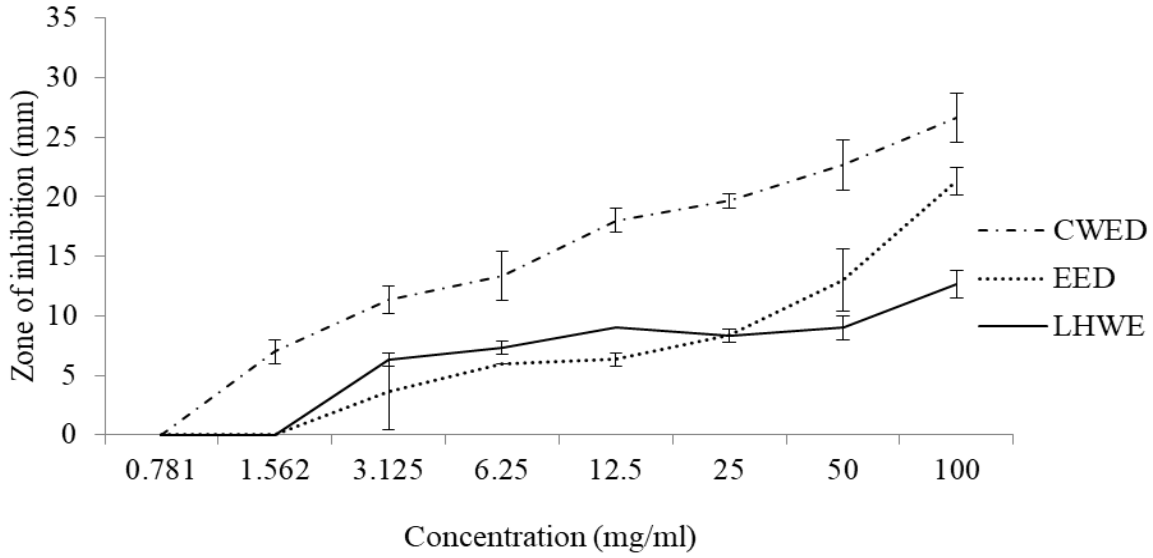

Figure 2. Inhibition of *Shigella* spp. isolate by varying concentrations of *S. madagascariensis* bark extract.

(i) *S. madagascariensis* bark extract (ZOI = 26 mm) (Figure 7), (ii) *S. madagascariensis* leaf extract (ZOI = 34 mm) (Figure 8) and (iii) *P. angolensis* bark extract (ZOI = 30 mm) (Figure 9). Additionally, the CWED extract retained the highest activity against *S. typhi* compared to the other two (LHWE and EED) across all concentrations of *S. madagascariensis* leaf (Figure 8).

The following extracts were shown to have significantly greater ZOI at 100 mg/ml than the other against the *E. coli*: (i) *S. madagascariensis* bark EED extract > CWED extract: $p = 0.02$ and *S. madagascariensis* leaf CWED extract > EED extract: $p < 0.0001$ (Table A1).

The ethanolic extraction with desiccation method yielded extracts with greater antimicrobial activities against *E. coli* O157 as follows: (i) *S. madagascariensis* bark extract (MIC = 0.39 mg/ml / ZOI = 29 mm) (Figure 7) and (ii) *P. angolensis* bark extract (ZOI = 31 mm) (Figure 9). The following extracts achieved greater ZOI than streptomycin against *E. coli* (300 µg/ml) (ZOI = 23 mm): (i) CWED and EED of *S. madagascariensis* bark extracts (26 and 29 mm) (Figure 7), (ii) CWED of *S. madagascariensis* leaf extract (34 mm) (Figure 8) and (iii) CWED and EED of *P. angolensis* bark extracts (30 and 31 mm) (Figure 9).

Overall, the aqueous extraction methods (CWED and LHWE) yielded extracts with greater antimicrobial

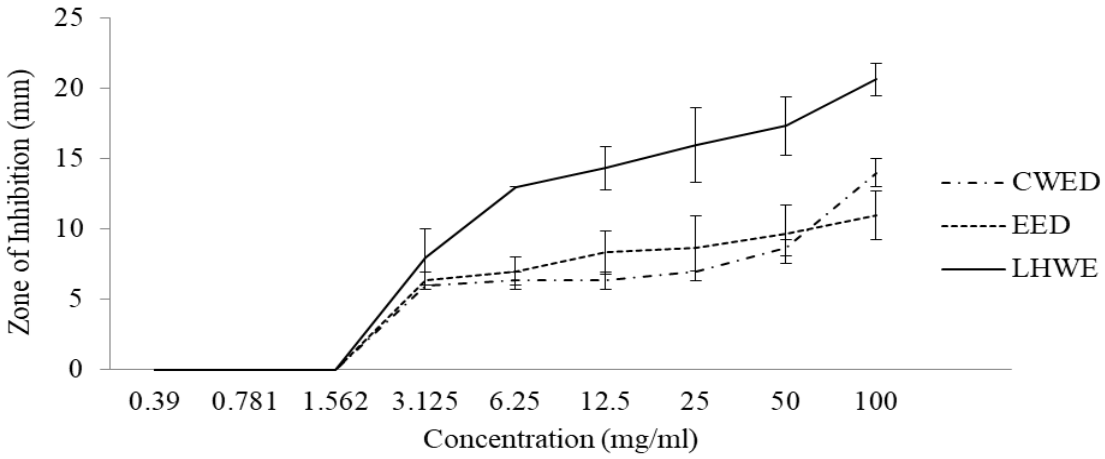

Figure 3. Inhibition of *Shigella* spp. isolate by varying concentrations of *P. angolensis* bark extract.

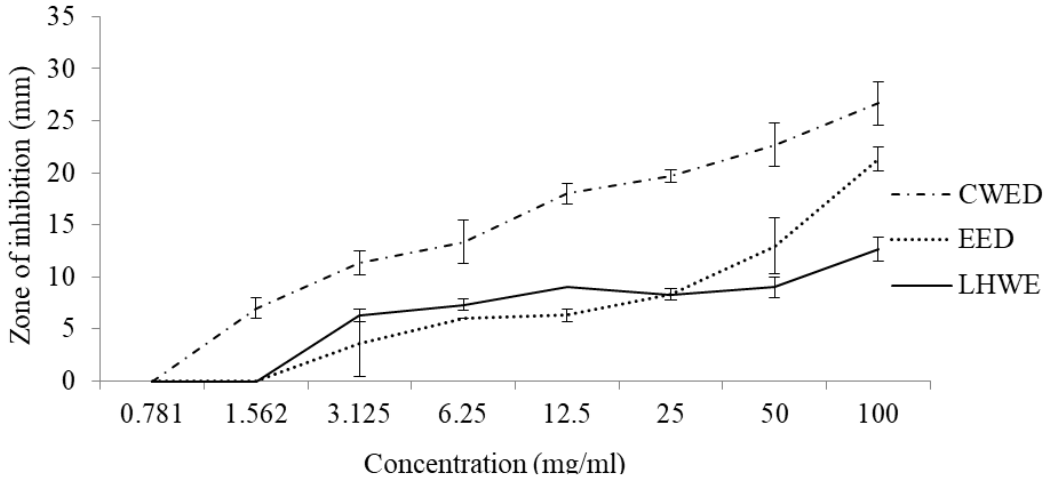

Figure 4. Inhibition of *S.* Typhi isolate by varying concentration of *S. madagascariensis* bark extract.

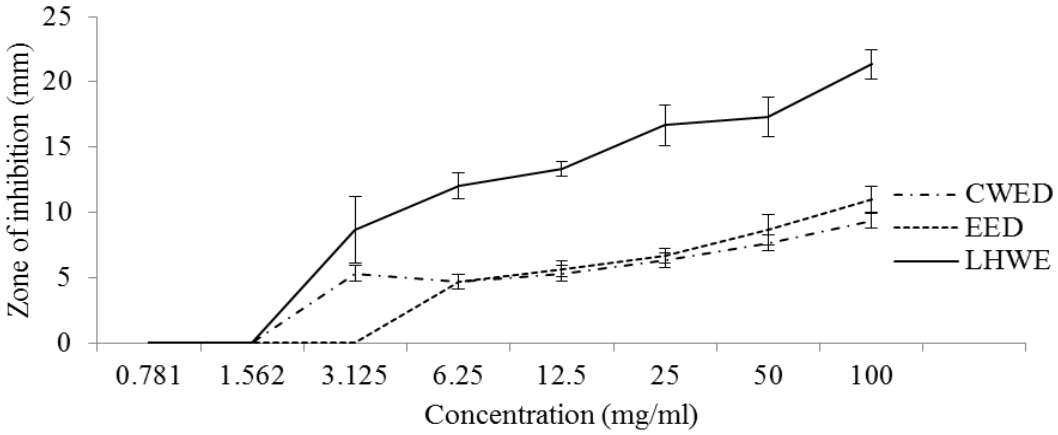

Figure 5. Inhibition of *S.* Typhi isolate by varying concentration of *S. madagascariensis* leaf extract.

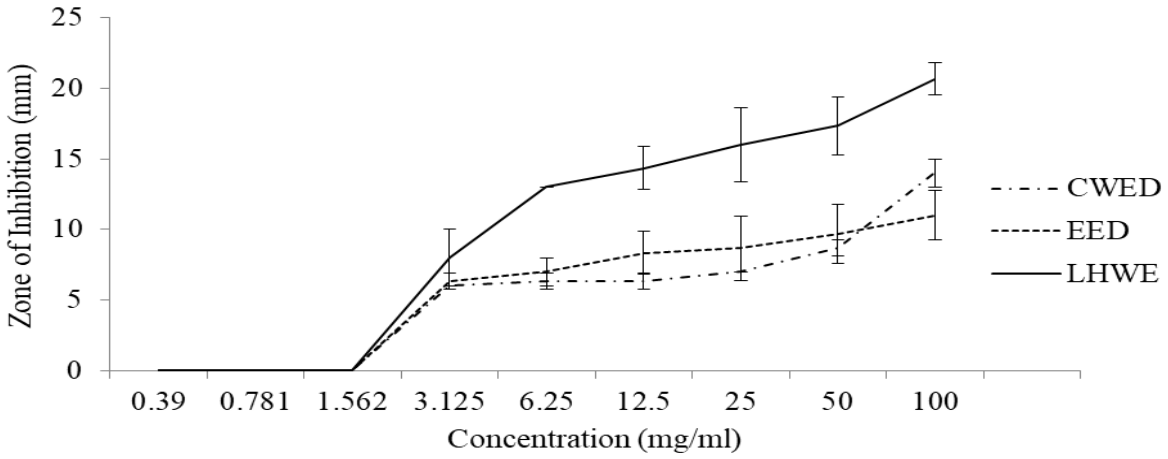

Figure 6. Inhibition of *S.* Typhi isolate by varying concentration of *P. angolensis* bark extract.

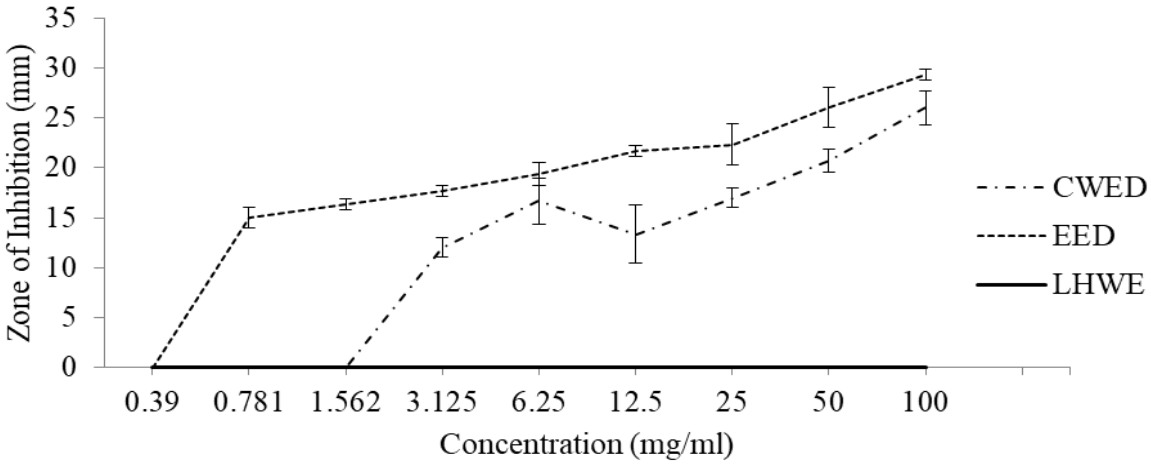

Figure 7. Inhibition of *E. coli* O157:H7 isolate by varying concentration of *S. madagascariensis* bark extract.

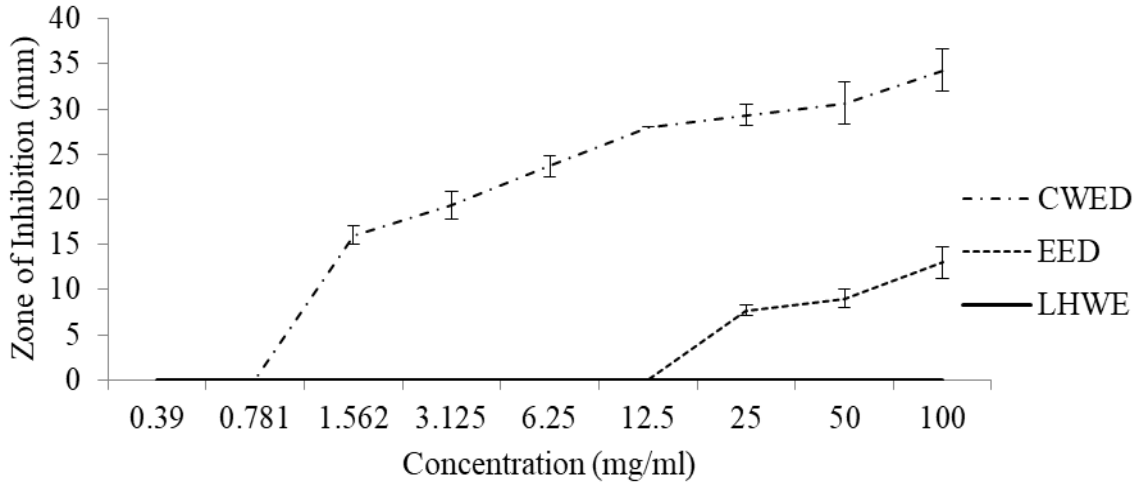

Figure 8. Inhibition of *E. coli* O157:H7 isolate by varying concentration of *S. madagascariensis* leaf extract.

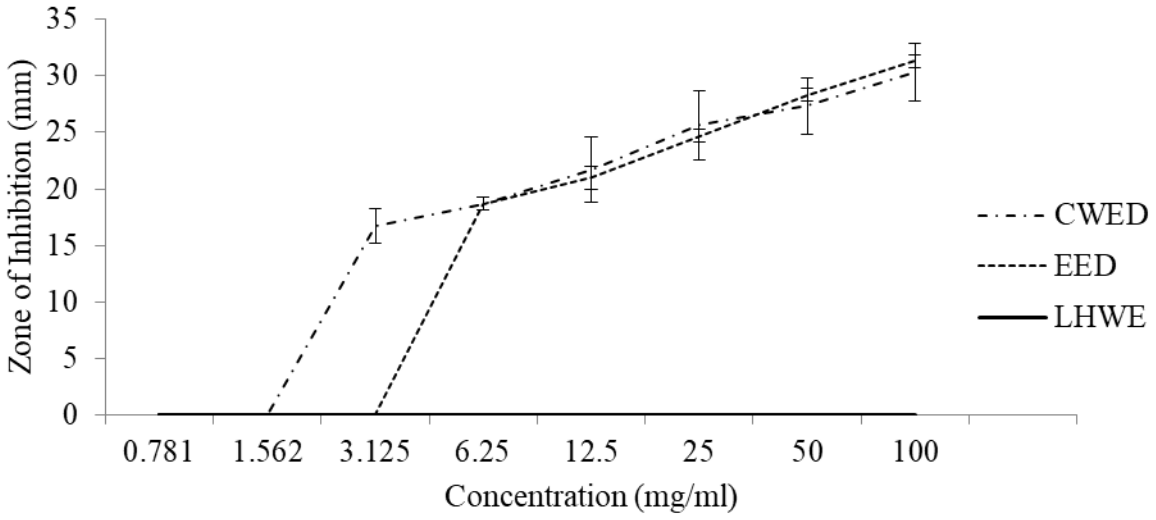

Figure 9. Inhibition of *E. coli* O157:H7 isolate by varying concentration of *P. angolensis* leaf extract.

activities than the other two methods in terms of the attainment of the lowest MIC values and higher ZOI per concentration used depending on plant species or plant part used. Generally, the LHWE method was largely better than the other two methods in (lowest MIC values and higher ZOI against *Shigella* spp. and presumptive *S.* Typhi per concentration used) with different plants. However, the LHWE technique yielded extracts with no antimicrobial activities against *E. coli.* The CWED and EED methods yielded greater antimicrobial activities against the bacteria as follows: (i) CWED with *S. madagascariensis* leaf extract (Figure 8) and (ii) EED with *P. angolensis* bark (Figure 9).

Table 4 shows ZOI of each extract (at the highest concentrations) against *Shigella* spp. that were greater than that for streptomycin (300 µg, Mast Diagnostics, UK) (20 mm). The extract from the traditional hot water extraction method (LHWE) had greater inhibitory activities than that for streptomycin (300 µg) against the following microorganisms with the following extracts (*Shigella* spp.: *S. madagascariensis* bark and leaf extracts and *P. angolensis* bark extract) (Table 4). The cold water extract of *S. madagascariensis* bark showed greater ZOI compared to that for streptomycin against *E. coli* and *S.* Typhi. The cold water extract of *S. madagascariensis* leaf showed greater ZOI compared to that of streptomycin against *E. coli*. Whereas the ethanolic extraction method yielded an extract of *S. madagascariensis* bark and *P. angolensis* bark with greater ZOI than streptomycin.

The traditional hot water extraction method (LHWE), cold water extraction with desiccation (CWED) and the ethanol with desiccation extraction (EED) methods yielded equal or similar MIC values, as seen with *P. angolensis* bark extract (0.78 mg/ml) (Figure 1). The CWED method yielded equal but higher MIC values than LHWE and EED method when used to obtain *S. madagascariensis* bark extract (CWED - 0.2 mg/ml vs. LHWE and EED - 0.78 mg/ml) (Table 5). The LHWE method yielded greater antimicrobial activities (ZOI) at concentrations higher that the respective MIC against presumptive *S. typhi* for *P. angolensis* bark extract and *S. madagascariensis* bark extract (Table 5). Overall, the LHWE method was largely better than the other two methods in (lowest MIC values and higher ZOI per concentration used) for *P. angolensis* bark extract and *S. madagascariensis* bark extract, whereas EED proved inferior (Figure 1).

The traditional hot water extraction method (LHWE) yielded extracts had no antimicrobial effects against the strain of *E. coli* O157 used (results not shown). The CWED method yielded higher MIC values than EED method and LHWE when used to obtain the following: *P. angolensis* (MIC = 0.78 mg/ml, greater activity between 0.78 and 1.56 mg/ml and *S. madagascariensis* (MIC = 0.196 mg/ml, greater activity between 0.196 and 6.25 mg/ml (Figure 1). The EED method yielded higher MIC values than CWED method and LHWE when used to obtain *S. madagascariensis* bark extract (MIC: EED = 0.098 mg/ml, CWED = 0.781 mg/ml).

Minimum inhibition concentration (MIC) of extracts obtained using traditional hot water extraction (LHWE), cold water extraction with concentration (CWED) and ethanolic extraction with concentration (EED) were obtained following the well diffusion protocol described earlier. Table 5 shows MIC values for each extract against each of the microorganisms tested.

The ethanolic extraction (with concentration) method (EED) yielded extracts that had dually greater antimicrobial effect (MIC) against the strains and extracts (than the other two methods): *S.* Typhi and *E. coli* with *P. angolensis* bark extract. The cold water aqueous

Table 4. Extracts with ZOI (at 100 mg/ml) greater than that of Streptomycin against *E. coli, Shigella* spp. and *S.* Typhi.

Microorganism	Plant/Part	Extract	ZOI (100 mg/ml)
***Shigella* spp.**		**Streptomycin**	**20**
	S. madagascariensis bark	LHWE	24
	S. madagascariensis leaf	LHWE	24
	P. angolensis bark	LHWE	24
***S.* Typhi**		**Streptomycin**	**25**
	S. madagascariensis bark	CWED	26
E. coli		**Streptomycin**	**23**
	S. madagascariensis bark	CWED	26
		EED	29
	S. madagascariensis leaf	CWED	34
	P. angolensis bark	EED	31
		CWED	31

CWED: Cold water extract with desiccation; EED: ethanol extract with desiccation; LHWE: aqueous extract obtained by the traditional extraction method.

extraction (with concentration) method (CWED) yielded extracts that had dually greater antimicrobial effect (MIC) against the strains and extracts (than the other two methods): *S.* Typhi and *E. coli* with *S. madagascariensis* bark.

DISCUSSION

In this study, extracts obtained from the extraction LHWE, CWED and EED were analysed for antimicrobial activity against *presumptive E. coli* O157, *Shigella* spp. and *S.* Typhi and were characterised for phytochemical composition. The extraction methods gave yields (dry mass of desiccated extracts) that lied between 711 and 2833 mg (0.07 and 0.25% respectively) per 100 g of plant material used. This means for one to obtain 1 kg of desiccated product, between 35 and 140 kg of dried plant material. Should the plants not be domesticated, harvest for widespread use in the management of diseases would not be sustainable. We recommend the domestication or replanting of such medicinal plants.

Qualitative phytochemical analysis revealed the presence of saponins, flavonoids, tannins and reducing sugars in plant extracts obtained from different extraction methods.

The traditional hot water extraction method (LHWE) yielded greater antimicrobial activities (significantly greater ZOI than the other extracts against *S.* Typhi: (i) *S. madagascariensis* bark extract (LHWE > CWED – $p < 0.0001$), (ii) *S. madagascariensis* bark extract (EED > LHWE – $p = 0.002$), (iii) *S. madagascariensis* leaf extract (LHWE > CWED – $p = 0.0001$), *S. madagascariensis* leaf extract (LHWE > EED – $p = 0.0003$), (v) *P. angolensis* bark extract (LHWE > CWED – $p = 0.002$) and (vi) *P. angolensis* bark extract (LHWE > EED – $p = 0.0004$). Similar dominance of LHWE was shown against *Shigella* spp. as follows: (i) *P. angolensis* bark extract (LHWE > CWED - $p = 0.001$) and (ii) *P. angolensis* bark extract (LHWE > EED – $p = 0.0003$). *Swartzia madagascariensis* has a history of being used in concoctions (mixed with *Isoberlinia doka*, *Annona senegalensis*, *Gardenia ternifolia*, *Terminalia glaucescens* and *Erythrina senegalensis*) that have shown significant antibacterial activities against *Bacillus cereus*, *Mycobacterium fortuitum*, *Staphylococcus aureus*, or *Candida albicans* (Magassouba et al., 2007). No evidence of use of *S. madagascariensis* or *P. angolensis* as sole antimicrobials in the traditional management of diseases was found.

The barks of *P. angolensis* and *S. madagascariensis* were shown to contain a number of phenolic compounds (pterocarpins) (Harper et al., 1969) which could account for the high antimicrobial activities of LHWE against *S.* Typhi and *Shigella* spp. The observed antimicrobial activities in the selected plants may be attributed to high composition of flavonoids and tannins in *S. madagascariensis* leaf extract or pterocarpins in *P. angolensis* (Harper et al., 1969). Flavonoids have been shown to harbour antimicrobial activities against *Salmonella* spp. (Dzoyem et al., 2017), for example quercetin (Wang et al., 2017), rutin (Arima et al., 2002) and others. Generally, no other studies reporting chemical composition of *P. angolensis* were found. Interestingly, all LHWE extracts did not yield antimicrobial

Table 5. Minimum inhibition concentration (MIC) (mg/ml) of extracts obtained using traditional hot water extraction (LHWE), cold water extraction with concentration (CWED) and ethanolic extraction with concentration (EED).

Species	Plant part	Extract	Microbial species	MIC (mg/ml)
P. angolensis	Bark	CWED	*Shigella* spp.	1.562
			S. Typhi	0.781
			E. coli	0.781
		EED	*Shigella* spp.	12.5
			S. Typhi	0.196
			E. coli	0.098
		LHWE	*Shigella* spp.	0.0915
			S. typhi	0.781
			E. coli	0
S. madagascariensis	Bark	CWED	*Shigella* spp.	0
			S. Typhi	0.195
			E. coli	0.098
		EED	*Shigella* spp.	12.5
			S. Typhi	0.781
			E. coli	0.781
		LHWE	*Shigella* spp.	1.562
			S. Typhi	0.781
			E. coli	0
	Leaves	CWED	*Shigella* spp.	1.562
			S. Typhi	0.781
			E. coli	0.196
		EED	*Shigella* spp.	1.562
			S. Typhi	0.781
			E. coli	6.25
		LHWE	*Shigella* spp.	0.781
			S. Typhi	0.781
			E. coli	0

CWED: Cold water extract with desiccation; EED: ethanol extract with desiccation; LHWE: aqueous extract obtained by the traditional extraction method.

effects against *E. coli.*

Ahmed et al. (2014), in a study on the effect of hot versus cold water extraction of *Hibiscus sabdariffa* calyxes revealed greater accumulation of total phenolics, total flavonoids and tannins with short time high temperature extraction process, as well as high antioxidant activity (DPPH assay) than with the cold water extraction method. Yung et al. (2010) demonstrated an increase in phenolics content and antioxidant activities of Pegaga (*Centella asiatica*) extracts with boiling temperature (90°C). The observed high accumulation of the phenolic substances (flavonoids and tannins) as well as saponins may be due to the increased dissolution of these substances with the hot water extraction method in the present study. Saponins are glycosidic secondary metabolites that exert a wide range of pharmacological properties (Podolak et al., 2010).

The LHWE could have attained greater antimicrobial activities due to the short processing time (30 min) that could have prevented antioxidative deterioration of phytochemicals within. Whereas extraction with the EED and CWED methods was done over a period of 72h, plus

a desiccation step that took at least 48 h. The length of exposure to agents of the atmosphere and the time taken could have had deleterious effects on the chemicals.

The aqueous extraction method (with desiccation) (CWED) generally yielded extracts with higher antimicrobial activities against *E. coli* than against *S.* Typhi and *Shigella* spp. where the zones of inhibition were as follows (respectively): *P. angolensis* bark extract (31 mm for *E. coli*). CWED extracts were also shown to have greater concentrations of the following: flavonoids (*P. angolensis* bark extract), tannins (*P. angolensis* bark extract) and saponins (*P. angolensis* bark extract). The cold water extract of *S. madagascariensis* leaves (CWED) was shown to have significantly greater antimicrobial activity against *E. coli* than the ethanolic counterpart (EED) ($p < 0.0001$). The cold water extraction method (with evaporation) (CWED) yielded extracts of *S. madagascariensis* were shown to be rich in the following phytochemicals: flavonoids, saponins and tannins. These components are thought to account for the high antimicrobial activities of the *S. madagascariensis* extracts.

Conclusion

The aqueous extraction methods (CWED and LHWE) were shown to yield extracts with greater antimicrobial activities than the ethanolic extraction method (EED) (significantly lower MIC values or significantly higher ZOI against *Shigella* spp. and *S.* Typhi per concentration used) with the three selected plants. However, the LHWE technique yielded extracts with no antimicrobial activities against *E. coli.* The high antimicrobial activities of CWED and LHWE could be because of the presence of bioactive compounds that exert antimicrobial properties such as flavonoids, saponins, alkaloids and tannins. The hot water extraction method was shown to be an extraction method of choice as it resulted in significantly greater antimicrobial activities against the three diarrhoeagenic microorganisms with the three plant species. The novelty of the hot water extracted preparations is thought to lie with the freshness of such extracts (used within hours from extraction) – meaning reduced oxidative degradation of their phytochemistry. The current study therefore validates the widespread use of aqueous extraction methods in traditional medicinal practices.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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APPENDIX A

Analysis of variance: ZOI at highest concentration of extracts used (*100 mg/ml*)

The inhibitory activities of the extracts obtained using the different extraction methods (traditional African hot water extraction - LHWE, cold water extraction with concentration - CWED and ethanolic extraction with concentration - EED) were analysed using One Way Analysis of Variance (ANOVA) statistical tool. Table A1 shows that there was no significant difference in zones of inhibition at 100mg/ml of each extract ($p > 0.005$).However, the LHWE method was shown to yield extracts with greater antimicrobial activities than the other two methods in (lowest MIC values and higher ZOI against *Shigella* spp. and *S. typhi* per concentration used) with different plants. Notably, the LHWE technique yielded extracts with no antimicrobial activities against *E. coli.* The CWED and EED methods yielded greater antimicrobial activities against the bacteria as follows: (i) CWED with *P. angolensis* and *S. madagascariensis* bark extracts, and (ii) EED with: *S. madagascariensis* bark extract.

Table A1. Analysis of Variance of antimicrobial activities (zone of inhibition) of extracts from extraction methods (traditional African hot water extraction - LHWE, cold water extraction with concentration - CWED and ethanolic extraction with concentration - EED) against *E. coli, S. typhi* and *Shigella* spp.

Microorganism	Pair of extracts compared	F value	P value	Comment
Shigella spp.	*S. madagascariensis* bark (CWED vs LHWE)	294	< 0.0001	Significance (CWED > LHWE)
	S. madagascariensis bark (CWED vs EED)	54	0.002	Significance (CWED > EED)
	S. madagascariensis bark (EED vs LHWE)	96	0.0006	Significance (EED >LHWE)
	S. madagascariensis leaf (CWED vs LHWE)	37.5	0.004	Significance (CWED > LHWE)
	S. madagascariensis leaf (CWED vs EED)	1.5	0.30	No significance (CWED vs EED)
	S. madagascariensis leaf (LHWE vs EED)	24	0.008	Significance (EED > LHWE)
	P. angolensis bark (CWED vs LHWE)	73.5	0.001	Significance (LHWE > CWED)
	P. angolensis bark (CWED vs EED)	13.5	0.02	Significance (CWED > EED)
	P. angolensis bark (LHWE vs EED)	150	0.0003	Significance (LHWE > CWED)
S. typhi	*S. madagascariensis* bark (CWED vs LHWE)	294	< 0.0001	Significance (LHWE > CWED)
	S. madagascariensis bark (CWED vs EED)	96	0.0006	Significance (EED > CWED)
	S. madagascariensis bark (EED vs LHWE)	54	0.002	Significance (LHWE > EED)
	S. madagascariensis leaf (CWED vs LHWE)	216	0.0001	Significance (LHWE > CWED)
	S. madagascariensis leaf (CWED vs EED)	6	0.07	No significance (CWED vs EED)
	S. madagascariensis leaf (LHWE vs EED)	150	0.0003	Significance (LHWE > EED)
	P. angolensis bark (CWED vs LHWE)	54	0.002	Significance (LHWE > CWED)
	P. angolensis bark (CWED vs EED)	13.5	0.02	Significance (CWED > EED)
	P. angolensis bark (LHWE vs EED)	121.5	0.0004	Significance (LHWE > EED)
E. coli	*S. madagascariensis* bark (CWED vs EED)	13.5	0.02	Significance (EED > CWED)
	S. madagascariensis leaf (CWED vs EED)	726	< 0.0001	Significance (CWED > EED)
	P. angolensis bark (CWED vs EED)	1.5	0.290	No significance (CWED vs EED)