Antimicrobial Activity of Aqueous Extracts of *Maytemus putterlickoides*, *Senna spectabilis* and *Olinia usambarensis* on Selected Diarrhea-Causing Bacteria

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Abstract

Diarrhea is one of the leading causes of morbidity and mortality among children under the age of five years in the developing world. Opportunistic bacteria have been identified as the major cause of diarrhea in HIV infected patients. Treatment of these emerging and re-emerging strains of diarrhea causing bacteria has become difficult due to their increased tolerant to the present available antibiotics. There is need to identify and develop alternative drugs, which are effective, affordable and easily accessible to diarrhea patients. However, there is no record in the literature of the antibacterial activity of these plants. The objective of this study was to enhance understanding of the efficacy of ethno-medical materials in the management of diarrhea. Antibacterial activity was evaluated by determination of Minimum Inhibition Concentration and the Minimum Bactericidal Concentration of the plant extracts against the diarrhea causing bacterial. Phytochemical screening for bioactive compounds was undertaken using standard qualitative methods. *Maytenus putterlickoides* (roots) and *Senna spectabilis* (leaves) were active against *Salmonela typhi*, *Shigella flexineriae* and *Shigella Dysenteriae* with zone of inhibition ranging 9.2-15.8 mm. *Olinia usambarensis* (leaves) had antimicrobial activity against several bacterial isolates with zone of inhibition ranging 9-15 mm. Alkaloids, tannins, anthrocyanins, triterpenes and steroids, saponins, flavanoids, coumarins and reducing sugars were present in the three plant extracts. These phytochemicals account for the antibacterial activity of the extracts against the bacterial strains.

Introduction

Diarrhea is one of the leading causes of morbidity and mortality among children under five years in the developing world [1]. During the period from 1950 to 1970s it was estimated that 4.6 million children died annually from diarrhea in developing world [2]. Mortality due to diarrhea declined to approximately 3.3 million annually in the 1980s [1]. Total deaths from diarrhea are estimated at 1.26 million in 2013–down from 2.58 million in 1990. In 2012, it is the second most common cause of death in children younger than five where 0.76 million died [3]. Despite the decline in mortality in most developing countries, diarrhea still remains one of the principal causes of morbidity in the developing world, with each child experiencing an average of three episodes of diarrhea per year [3]. In these countries, diarrheal diseases are the second most common illness of children after acute respiratory illness. Other long term problems that can result include poor intellectual development and stunted growth [4]. Approximately, 80% of children in areas where HIV is more prevalent have been reported to develop diarrhea [5].

A number of studies have focused on bacterial causes of diarrhea in AIDS patients. *Salmonella* spp, *Campylobacter* spp, *Shigella* spp, diarrheagenic *E. coli*, *Clostridium difficile* and *Mycobacterium avium* complex are some of the bacteria that have been isolated in AIDS patients [6]. Among the bacterial causes, diarrheagenic *Escherichia coli* (DEC) is the most important etiologic agent of childhood diarrhea and represents a major public health problem in developing countries [7].

In today's battle against infectious agents, conventional medicine may be effective. However, due to the high cost of drugs and increasing bacterial resistance to these drugs, alternative medicine seems to be the latest and the future weapon. As a result, a growing number of health care consumers are turning to plant medicines which are cheaper, believing that these products are effective and also safe [8]. However, this may not be the case as most of the plant products in use have not been taken through any scientific evaluation. Selected medicinal plants such as *Maytenus putterlickioides loes*, *Boscia angustifolia*, *Lantana trifolia*, *Senna spectabilis* and *Olinia usambarensis* are known to cure diabetes and have antimalarial activity [9,10].

The use of plants and plant products in disease control has persisted despite advances in the modern pharmaceutical products and dominance of synthetic drugs all over the world [11]. The pharmacological effects of these selected plants have not been subjected to rigorous trials and control. Thus these claims remain unsubstantiated. There is therefore lack of credibility, awareness and laboratory data to support most of the reported therapeutic claims. There is also need for continuous development of new antibacterial agents for treatment of diarrhea with known potential traditional herbs to keep in check the growing resistance of bacteria to common conventional drugs. This study was aimed at enhancing understanding of the efficacy of ethno-medical materials in the management of diarrhea through determining the antibacterial potential of the selected medicinal plants on diarrhea-causing bacterial strains.

Materials and Methods

Collection of medicinal plants

The medicinal plants studied included *Maytenus putterlickoides* loes (A. Rich), *Senna spectabilis* (L.) and *Olinia usambarensis* (Gilg.). They were collected from their natural habitats in Mbeere Distrct, Embu county, Kenya. A botanist, Mr. Antony Mutiso from University of Nairobi, Department of Botany assisted in the identification and collection of plant materials with consideration of the bio-conservation aspects.

Processing and extraction

The plant materials were collected when still fresh and dried under shade at room temperature for 14 days to ensure that they were completely dry and avoid decomposition of active compounds. When completely dry, each medicinal plant sample was ground separately using Laboratory Warring Blender (Clarkson LB10G) for leaves and an electric Laboratory mill number 8 for roots and barks. The powdered plant materials were sealed separately in closed plastic containers and were stored at room temperature.

One hundred and twenty five grams of each powdered plant material was later extracted in 1 liter of distilled water at 60°C in a metallic shaker for 6 hours. The mixture was allowed to stand for 6 hours to cool before decantation into a clean dry conical flask and then filtered through folded cotton gauze into another clean dry conical flask. The mixture was then filtered using Whatman No. 1 filter paper under vacuum. The filtrate was then freeze dried in 200 ml portions using a Modulyo Freeze Dryer (Edward England) for 48 hours then further dried in a vacuum desiccator over anhydrous copper sulphate. The fine powder obtained was packed in dry plastic bags and stored at room temperature in desiccators.

Bacterial stocks

Standard microorganism and clinical isolates were obtained from Kenyatta National Hospital (KNH) and Kenya Medical Research Institute (KEMRI) at the Centre for Microbiology Research (CMR) where they were maintained in a lyophilized state. Five diarrhea causing bacteria including known resistant strains of the species were used. In the laboratory, they were sub-cultured in suitable media (Muller Hinton agar) which is suitable for most bacteria, Salmonella Shigella Agar (SSA) for Salmonella and Shigella species and incubated for 24 hr at 37°C to ascertain purity. From these sub-cultures the identities of the organisms was confirmed by colony morphology, gram staining and biochemical tests. The organisms were then sub-cultured into gelatin medium, labeled with code numbers and stored at room temperature. The organisms included *E. coli*, *S. typhi*, *C. jejuni,* and *Shigella dysenteriae*, *S. flexineri*, *Proteus* species and *Pseudomonas aeruginosa*. Standard organisms include *E. coli* ATCC25922, *S. typhi* ATCC19430 and *Pseudomonas aeruginosa* NCTC 10662.

Determination of susceptibility of bacterial isolates to plant extracts

This was carried out using the agar paper disc diffusion method [12-14] which was used to evaluate the antimicrobial activity of plant extracts as a screening preliminary procedure. The media used was Muller Hinton Agar (MHA), also known as Diagnostic Sensitivity Test (DST) Agar. The media was prepared according to the manufacturer's instructions. The media was then sterilized by autoclaving at 15l Psi pressure (121°C) for 15 min. It was cooled to about 55°C and then poured aseptically onto sterile Petri dishes and left to solidify at room temperature for a few minutes. A 24 hr bacteria culture, inoculated the previous day and incubated at 37°C was used.

A McFarland standard of 0.5 was prepared by mixing 0.05 mL of 1.175% barium chloride dehydrate ($BaCl_2\cdot2H_2O$), with 9.95 mL of 1% Sulphuric acid (H_2SO_4) with constant stirring. This was estimated to give a cell density (1.5×10^8 CFU/ml), with optical density (absorbance) of 0.132 at 600 nm wavelength. The standard was put in screw cap tubes which were tightly sealed to prevent loss by evaporation. The tubes were then stored and protected from light at room temperature. The standards were vigorously shaken before use.

A flame sterilized wire loop was used to pick a small part of the 24 hr bacterial colony of each test bacteria and mixed well in sterile 0.89% saline. The turbidity of the bacteria was compared to match the McFarland standard which was assumed to contain 1.5×10^8 CFU/ml. Fifty microlitres (50 μl) of McFarland standard bacteria was introduced on each plate. A sterile L-shaped glass rod was used to spread the prepared bacterial culture on to the dry Muller Hinton Agar media and left to dry completely. Using a paper punch whose diameter was 6 mm, paper discs for bioassay were made from Whatman number one filter paper. These discs were then sterilized by autoclaving at 151 Psi pressures (121°C) for 15 min. in a well-sealed universal bottle.

By this method one gram of each sample was dissolved in four milliliters of sterile saline and kept as stock solution. The stock solution was sterilized by passing it through 0.45 μm pore microfilter. Five microliter containing 1.25 milligram of each stock solution was applied on a sterile paper disk. The paper discs were left to dry at room temperature for a few minutes. The Extract impregnated paper discs were picked by forceps and placed on to the inoculated DST Agar plates in identified areas. To prevent unacceptable overlapping zones of inhibition a 90 mm plate accommodated six discs. The saline was included in the assay as negative controls while chloramphenicol served as the positive controls. The inoculated plates with disc were incubated at 37°C for 24 hr.

The diameters of zones (mm) of inhibitions of the test strain were determined with a ruler. Diameters of 6 mm (equal to the paper disk) in this study represented no inhibition. Zone size ≤ 9 mm was considered resistant while sizes greater than 9 mm were considered sensitive. Each experiment was repeated five (5) times to ensure that results were reproducible. The extracts that showed highest antibacterial activity, was selected for quantitative test such as minimum inhibitory concentration (MIC) and minimum antibacterial concentration (MBC).

Determination of minimum inhibitory concentration (MIC)

A modified agar micro dilution method [15] was used to determine the MIC of extracts of the medicinal plants that produces significant inhibition zones with agar paper disc diffusion bioassay method. McFarland standard of each bacteria strain (1.5×10^8 CFU cells/ml) were made as in disc diffusion method. One micro liter of an overnight culture of each bacterial strain was applied onto MHA supplemented with the medicinal plant extracts. Inoculation of bacteria on MHA plates was done.

One thousand milligrams (1000 mg) of the extracts were weighed and dissolved completely in 1 ml of sterile 0.89% saline to give a concentration of 1000 mg/ml. 1 ml of sterile saline was added to end

up with 500 mg/ml. Serial double dilutions in saline of 500 mg were made to obtain the following concentrations in milligrams: 500, 250, 125, 62.5, 31.25 and 15.6 [16,17]. Then 2 ml of each of these concentrations were added to 18 ml of sterilized MHA and cooled to 50°C to make the following final extract concentrations in milligrams per ml: 50, 25, 12.5, 6.25, 3.125 and 1.5 mg. The MHA mixed with extracts on Petri dish plates were left to solidify at room temperature.

Fifty microlitres (50 μl) of the McFarland standard of an overnight bacterial culture was inoculated onto MHA supplemented with the medicinal plant extracts. Sterile L-shaped glass rods are then used to spread the prepared bacterial culture on to the dry Muller Hinton Agar media and left to dry completely. A control plate to show that bacterial colonies were present and growing accordingly was inoculated and incubated. The plates were incubated at 37°C for 18-24 hr. and observations recorded accordingly. The plates showing no bacterial growth was streaked and inoculated on fresh MHA plates and incubated for 18 hr to confirm that the bacteria were truly inhibited. This procedure was performed repeatedly three times and results expressed as the mean values of the lowest concentration of plant extracts that produced complete suppression of colony growth which was taken as the MIC. An antimicrobial agent with low activity against an organism has a high MIC while a highly active antimicrobial agent gives a low MIC.

Minimum bactericidal concentration (MBC)

The broth dilution MBC method was used to quantitatively measure the in vitro activity of an antimicrobial agent against a bacterial isolate. The minimum bactericidal concentrations (MBC) of plant extracts bioassays were done according to the methods by Andrews, [18]. Briefly, 1000 mg of extracts were weighed and completely dissolved in 1ml of sterile 0.89% saline to give a concentration of 1000 mg/ml. Double serial dilutions of the extracts were made in 0.89% saline to give the following concentrations in mg/ml: 500, 250, 125, 62.5, 31.25 and 15.6. A ten times dilution was made of the extracts by adding 0.2 mL of the extract into 1.8 ml of Muller Hinton Broth (MHB) to make the following final concentrations in milligrams/mL: 50, 25, 12.5, 6.25, 3.125 and 1.5 [19,16].

Nine tubes for each test organism were set at every concentration. A McFarland standard of each bacterial strain of 1.5 x 10^8 CFU was made, and then 50 μℓ McFarland standard of each test organism was added to different extract concentrations. The tubes were then incubated at 37°C for 18 hr. At the end of incubation period, the tubes were examined for turbidity to determine whether there was bacterial growth.

To confirm growth, 50 μℓ from each tube was collected and plated onto nutrient agar and incubated at 37°C for 18 hr. Nutrient agar with no bacterial growth indicated negative score, confirming that the extracts had actually killed the bacteria at that concentration, while plates with bacterial growth were taken as positive, confirming that the extracts had merely inhibited bacteria at that concentration. The lowest concentration of the plant extracts that did not yield any colony growth in Muller Hinton Broth (MHB) after the incubation period was taken as the MBC. The minimum bactericidal concentration was read as the lowest extract concentration which yielded no bacterial growth upon subculture.

This procedure was repeated three times and results expressed as the mean values of the lowest concentration of plant extracts that were

completely bactericidal to the bacteria colony growth. Antimicrobial agents with low activity against an organism had a high MBC while a highly active antimicrobial agent gave a low MBC.

Qualitative phytochemical screening

Phytochemical screening for major constituents was undertaken using standard qualitative methods [20]. The freeze dried extracts were tested for alkaloids, tannins, steroids, triterpenes, flavonoids and anthraquinones [20,21]. The presence or absence of these secondary metabolites was confirmed by color changes of solutions after the addition of the testing reagents. Results were read and indicated as (+ve), showing the presence and (–ve) showing the absence of the phytochemicals.

Data management and statistical analysis

The results were represented in form of Tables and Figures. The antimicrobial activity of the selected medicinal plant extracts was initially tested by Disk Diffusion method. All isolates with zone of inhibition equal or greater than 9 mm were regarded as susceptible to the extracts hence the extracts were selected for quantitative test. For the MIC and MBC values, a regression analysis was used to analyze mean MIC and MBC for each of the selected organisms. The univariate ANOVA was used to determine the relationship between MICs and MBCs of the extracts and Chloramphenicol for the selected organisms. Statistical analysis was done using student T test to compare the means of untreated group of normal mice with group treated with the extracts. Data was expressed as mean ± SD. P<0.05 was considered statistically significant. Stat View ® and Instat ® softwares were used.

Results

Susceptibility of bacterial isolates to plants extract

Table 1 shows the effect of plant extracts against bacterial isolates. *Boscia angustifolia* (Leaves), *Maytenus putterlickoides* (stem), *Boscia angustifolia* (roots), *Lantana trifolia* (roots) and *Olinia usambarensis* (roots) showed inhibition zones ranging from 6.0-7.8 mm. *Lantana trifolia* (Leaves) and *Maytenus putterlickoides* (aerial parts) were slightly active against *S. Flexineriae* with zone of inhibition ranging 7.9-8.1 mm. *Maytenus putterlickoides* (roots) and *Senna spectabilis* (leaves) were active against *S. typhi*, *S. flexineriae* and *S. Dysenteriae* with zone of inhibition ranging 9.2-15.8 mm. *Olinia usambarensis* (leaves) had antimicrobial activity against several bacterial isolates with zone of inhibition ranging 9-15 mm. The most sensitive organism was *Shigella flexineriae* and the least was *Pseudomonas aeruginosa*.

Table 2 shows results of activity of three aqueous plant extracts tested against the various strains of bacterial isolates. The aqueous extract of *Senna spectabilis* produced a MIC range of 12.5 to 50 mg/ml and MBC range of 25 to75 mg/ml for all bacterial species except *C. Jejuni* and *P. aeruginosa*. The aqueous extract of *M. putterlickoides* aqueous produced a MIC range of 6.25-50 mg/ml and MBC range of 12.5-75 mg/ml against the various strains of bacterial isolates except *C. jejuni*. The aqueous extract of *O. usambarensis* gave a MIC range of 6.25 to 50 mg/ml and MBC range of 12.5-75 mg/ml for all bacterial isolates (Table 2).

Bacterial isolates/Medicinal plants	0.89% Normal Saline	*M. putterlickoides* (roots)	*S. spectabilis* (leaves)	*O. usambarensis* (leaves)
Sal. typhi	6	9.2 ± 1.1[*]	9.6 ± 0.6[*]	12.2 ± 0.8[*]
E. coli	6	9.6 ± 0.6[*]	6.0 ± 0.0	14.2 ± 1.6[*]
Sh. dysenteriae	6	11.0 ± 1.0[*]	12.8 ± 0.8[*]	15.0 ± 1.0[*]
Sh. flexineriae	6	12.2 ± 1.9[*]	15.8 ± 1.6[*]	13.8 ± 2.1[*]
Ps. aeruginosa	6	8.0 ± 0.7	7.6 ± 0.5	9.2 ± 1.3[*]
Pr. vulgaris	6	9.8 ± 1.3[*]	9.6 ± 1.1[*]	9.0 ± 0.7[*]
C. jejuni	6	7.0 ± 0.7	6.0 ± 0.0	9.6 ± 0.9[*]
Results are expressed as Mean ± SD of five determinations; values followed by asteric are significant ([*]$p<0.05$).				

Table 1: Zone of inhibition (millimeters) of aqueous extracts of selected medicinal plants against bacterial isolates.

Phytochemical composition of the plant extracts

S. spectabilis (Leaves), *M. putterlickoides* (Roots) and *O. usambarensis* (Leaves) extracts contained alkaloids, tannins, anthrocyanins, triterpenes and steroids, saponins, flavanoids, coumarins and reducing sugars in varying quantities. Tannins, alkaloids, and saponins and flavanoids were most abundant in *O. usambarensis* leaves, while *S. spectabilis* leaves were rich in both coumarins and also alkaloids. Reducing sugars were only found in trace quantities in all plant samples. *S. spectabilis* (Leaves), *M. putterlickoides* (Roots) and *O. usambarensis* (Leaves) showed the presence of alkaloids, tannins, anthraquinones, triterpenes and steroids, saponins, flavonoids, coumarins and reducing sugars at varying intensities (Table 3).

	Chloramphenicol		*M. putterlickoides* (Roots)		*S. spectabilis* (Leaves)		*O. usambarensis* (Leaves)	
Micro-organism	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
S. typhi	0.063	0.143	50	75	25	37.5	50	75
E. coli	0.063	0.113	50	75	-	-	6.25	12.5
Sh. dysenteriae	0.063	0.25	25	37.5	12.5	25	6.25	12.5
S. flexineriae	0.063	0.25	12.5	25	6.25	12.5	6.25	12.5
Ps. aeruginosa	1.2	4.8	-	-	-	-	50	75
Pr. vulgaris	3.9	22.8	50	75	50	75	12.5	25
C. jejuni	0.75	5.0	-	-	-	-	50	75
Results are expressed as Mean of five determinations. Differences between means of the MICs and MBCs were analyzed by student's t-test.								

Table 2: Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs).

Phytochemicals	*M. putterlickoides* (Stem)	*S. spectabilis* (Leaves)	*O. usambarensis* (Leaves)
Alkaloids	+	+	+
Tannins	+	+	+
Anthrocyanins	+	+	+
Triterpenes & steroids	+	+	+
Saponins	+	+	+

Flavonoids	+	+	+
Coumarins	+	+	+
Reducing Sugars	+	+	+
Key: + shows presence of phytochemical			

Table 3: Phytochemical determination of selected medicinal plant.

Discussion

The global perspective in the treatment and control of diseases is slowly changing from the use of convectional drugs to herbal applications due to preference of organic products but also due to their easier accessibility and affordability. This is particularly so in the sub-Sahara Africa where the World Health Organization (WHO) estimates that, 85% of the population relies on herbs for their primary health care needs [22]. Despite the prevalence of herbal extracts in the informal therapy and the many years the extracts have been in use, the use of herbs remains suspicious and most people use herbs as last resort. There is lack of comprehensive laboratory data to support claims and little information on their toxicity and no credibility on their use.

In this study, 100 g of each dried plant materials yielded a range of 5.12 to 9.6 g of fine freeze-dried extract. The study was carried out to evaluate five selected plants for their antibacterial activity against human bacterial pathogen that cause diarrhea. This was accomplished by determining the susceptibility of the organisms to the extracts, its potency and compared with Chloramphenicol, a convectional antibiotic. Chloramphenicol was used in the study as it is a known antibacterial agent with broad spectrum of activity and also due to its bacteriostatic mode of action [23].

Three out of the ten selected medicinal plants extracts tested by disk diffusion technique had inhibitory activity on most bacterial isolates with inhibition diameter ranging from 9mm to18mm. These are *S. spectabilis* (Leaves), *M. putterlickoides* (Roots) and *O. usambarensis* (leaves). This inhibitory activity could be contributed by the phytochemicals present in these plant extracts. The plant extracts contain alkaloids, tannins, saponins, flavonoids, anthrocyanins, triterpenoids, coumarins and reducing sugars in varying amounts. Alkaloids such as ramiflorines A and B, crytolepine, and quinolones have been reported to be active against both gram positive and gram negative bacteria such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Escherichia coli*, *Shigella flexineri* and *Shigella boydii* [24,25]. Flavonoids are reported to be active against both gram positive and gram negative.

Bacillus Subtilis, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* [26]. Coumarins such as cniforin A and edulin are reported to be active against *Staphylococcus aureus* and *Pseudomonas aeruginosa* [27]. Saponin extract have been reported to be active against *Enterococcus faecalis*, *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Proteus miribilis* [28]. Crude tannin fraction has been reported to inhibit the growth of *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli* [29]. Camaric acid is active against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* [30].

The MIC for selected organism ranged from 3.2-50 mg/ml and MBC from 6.25-75 mg/ml. The MIC: MBC ratio of chloramphenicol to the selected bacterial isolates is 1:2. The significant difference in the mean MIC values for chloramphenicol and the plant extracts implies that chloramphenicol has significantly higher potency than the plant extracts. However considering that chloramphenicol is a pure compound and the plant extracts are crude products, then it is prudent to conclude that the plant extracts have considerable effect against bacteria. This implies that in pure form, the plant extracts may actually be more potent against some species of bacteria that cause diarrhea. If a plant extract exhibit similar values for MIC and MBC against the test bacteria, this show that the MIC of the extract indicate the bactericidal activity while if the extract exhibits an MBC greater than MIC, then the MIC of the extract indicate a bacteriostatic activity. The MBC of the plant extracts was higher than MIC; this is similar to Chloramphenicol thus suggesting the mode of action may be closely related. This shows that the plant extracts are bacteriostatic in nature because they could not eliminate bacteria even at a higher MBC values as determined by microtitre method.

Bacteriostatic agents have low potency but increase in concentrations can increase their potency. Low concentrations of bactericidal agents are known to be bacteriostatic [31] and the agents act mainly by inhibiting cell wall, nucleic acid and protein synthesis or inhibition of transcription [32]. The long-term Survival of bacterial strains in the presence of antibiotic even beyond 24 hours is due to the production of osmotically stable L-forms that remain viable, multiply and grow to vegetative forms after the antibiotic is inactivated. The two types of L-forms include unstable L-form which can divide and revert to the original morphology while stable L-forms are unable to revert to original forms. L forms of bacteria are strains of bacteria that lack cell walls [32]. Since bacterial isolates survived even after 24 hours, in this study it shows they are the stable L-form. The L forms are generated in a culture medium that has the same osmolarity as the bacterial cytosol, which prevents cell lysis by osmotic shock [33]. *Pseudomonas aeruginosa* is very adaptable and tolerant to antibiotics [34]. The resistance attributed to Ps. aeruginosa could be capsule related [35].

Bacteriostatic agents are more preferred in treatment of infections other than those requiring emergency attention because they allow the body to develop acquired immunity against the pathogens by delaying the elimination but suppressing multiplication of the same thereby giving the body a chance to fight the agents through the immune mechanism. Therefore, the phytochemicals present in these plant extracts contributed to their antibacterial activity against diarrhea causing bacteria pathogen and thus justifies their continued use in traditional medicine.

Conclusion

The aqueous medicinal plant extracts of *S. spectabilis* (Leaves), *M. putterlickoides* (root) and *O. usambarensis* (leaves) demonstrated bacteriostatic activity and like chloramphenicol does not kill the bacteria but rather inhibits their multiplication. The phytochemicals present in the three medicinal plant extracts contributed to their activity against diarrhea causing bacteria pathogen and thus justifies their continued use in traditional medicine. These three plants are thus potent against diarrhea-causing bacteria and can be used in the management of diarrhea.

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