

Antibacterial Activities of Some Antidiarrheal Herbal Concoctions Locally Prepared in Ilorin, Nigeria, on Fecal Isolates

B. K. Saliu*, I. I. Anibijuwon, R. F. Zakariyah
bks3264@gmail.com; saliu.bk@unilorin.edu.ng

Department of Microbiology, University of Ilorin, Ilorin Nigeria

Abstract

Antibacterial activity of herbal concoctions commonly used to treat diarrhea in Ilorin, Nigeria was determined against fecal isolates. Aqueous extracts of different combinations of the herbs used were prepared by soaking for eighteen days. Samples were taken daily for four days and weekly thereafter and used to challenge bacteria isolated from human feces. The efficacy of the herbal extracts was compared with a standard antibiotic, streptomycin. Most of the isolates were inhibited by the 48 hours herbal extracts. Inhibition zone ranged from 11.4 to 20.0 mm and 4.6 to 19.8 mm for the adult men and women extracts; and 9.6 to 32.0 mm for the children extract. The minimum inhibitory concentration ranged from 25 to 100%. Apart from *Enterococcus faecalis* and *Staphylococcus aureus*, susceptibility to the herbal extracts compared favorably with reference drug, streptomycin with percentage inhibition reaching 100, 120, 150 and 160 respectively for *Proteus vulgaris*, *Salmonella enteritica*, *Escherichia coli*, and *Klebsiella* sp. Aqueous extract of a combination of herbs used for treatment of diarrhea in Ilorin has antibacterial activity comparable with streptomycin and can therefore be recommended for use after determining its toxicity.

Keywords: Antibacterial, herbal concoctions, fecal isolates, diarrhea

Introduction

Fresh human feces contain a variety of microorganisms mainly bacteria from the gastrointestinal tracts (GIT). The organisms comprise of the normal flora of the GIT which are mostly beneficial, and contaminants of foods and water which may include pathogens. Infections of the GIT cause diarrhea, a disease of the gastrointestinal tracts, characterized by frequent and watery stools, cramps and general body weakness. Diarrhea is an endemic disease in Nigeria affecting people of all ages but most prevalent among the young ones. It is hardly reported in hospitals and clinics as most homes use herbal remedies{1,2}. Studies have shown that inaccessibility to health facilities, illiteracy and poverty are some of the underlying factors {3,4}.

Variety of herbs are combined in no standard ratio and suspended in cold water in a bottle or bowl for extraction of phytochemicals which are deemed useful in the treatment/prevention of diarrhea. Concoctions are usually administered also in no standard measures on daily basis before breakfast as prophylaxis or used for treatment as required until the diarrhea subsides. The herbs commonly used to prepare concoction against diarrhea in Ilorin, Nigeria include the bark of *Nauclea latifolia*, *Anogeissus leiocarpa*, *Khaya grandifoliola*, *Brassica juncea* and *Rauvolfia vomitoria*; the leaves of *Allium ascalonicum*; leaves and root of *Aristolochia albida*; fruits of *Picralima nitida* and *Eugenia caryophyllus* and bulbs of *Allium salivum* among others. The antibiotic activities of some of these herbs have been documented {5,6,7,8,9,10,11,12}.

In this era of antibiotic resistance, herbal remedies are gaining popularity in the treatment of infections. Being from natural sources, herbal medicines reduce the risks associated with the use of synthetic drugs. Herbs are cheap and readily available especially in rural settings. The antibacterial activities of the decoctions and concoctions of herbs commonly used in treating diarrhea have been studied. While some of the herbs have proven successful against most of the organisms that cause the disease {13,14}, others have no antibacterial effect but serve other medicinal functions in the body. Herbs combined in preparing concoctions vary from one region to another depending on the types available in the locality. Documentation on the specific combinations used for concoction against diarrhea in Ilorin, Nigeria is scanty. This study therefore assessed the effect of a concoction of local herbs used against diarrhea in Ilorin, on enteric organisms with a view to come up with document about its benefits.

Methodology

Herbs used

A total of thirteen herbs and edible camphor were used in this study. The herbs were purchased from a retailer at a popular herbs market known as “Ojatitun” in Ilorin, Kwara State, Nigeria. The herbs were identified at the Herbarium of the Department of Plant Biology, University of Ilorin. A combination of six herbs each was used for the adult concoctions while the children extract was prepared from eight herbs and the edible camphor (Table 1).

Preparation of herbal concoction

The herbs were combined following the retailer’s advice in order to mimic the local method of preparation. Aqueous extracts of the different combinations were prepared by placing in 1 litre bottles and filling with cold water to soak the entire herbs. Bottles were covered and allowed to stand for 18 days during which samples were taken for analysis daily for the first four days and weekly thereafter.

Isolation and characterization of organisms from fecal materials

Fecal materials were collected from healthy human volunteers first thing in the morning into sterile specimen bottles. A serial dilution up to 10^{-6} was prepared after suspending 1.0 g of fecal sample in 9.0 ml sterile distilled water. Aliquots (0.1 ml each) of the last two diluents were used to inoculate Nutrient Agar, Eosin Methylene Blue Agar and Salmonella-Shigella Agar. All the plates were incubated at 37°C. Isolates were purified and maintained on slants using the respective media. All isolates were characterized based on conventional laboratory methods.

Susceptibility test of the herbal concoction on fecal isolates

The herbal extracts were diluted with sterile distilled water to obtain 50, 25, 12.5 and 5% concentrations. A suspension of each of the isolates was prepared in normal saline and standardized to 0.5 McFarland. This was inoculated onto Mueller Hinton agar using spread plate technique. Holes measuring 6 mm diameter were made on the seeded agar using flame sterilized metal borer. Aliquots (0.1 ml) of the herbal extracts were placed in holes and the plates were incubated at 37°C in upright positions for 24 hours. Controls were set up with streptomycin (1%) and sterile distilled water. After incubation, plates were observed for bacterial growth and clear zones around the holes to show inhibition by the herbal concoction.

Inhibition zones were measured at five planes around each hole and statistical mean used to represent antibacterial activity of the herbal concoction. The lowest concentration of the herbal extract at which inhibition zones occurred for individual concoction was taken as the minimum inhibitory concentration.

Table: 1: List of herbs used

S/N	Common name	Botanical names	Family	Local name (Yoruba)	Parts Used	Extract
1	African peach	<i>Nauclea latifolia</i>	Rubiaceae	Egbesi	Bark	Adult
2	Dutchman's pipe/Pipevine	<i>Aristolochia albida</i>	Aristolochiaceae	Paranfunfun	Root and leaves	Adult and children
3	Shallot	<i>Allium ascolanicum</i>	Liliaceae	Alubosaelewe	Leaves	Adult
4	Ghanaian quinine	<i>Cryptolepis anguinolenta</i>	Apocynaceae	Paran pupa	Root	Adult
5	Not available	<i>Pteleiopsis suberosa</i>	Combretaceae	Okuku	Stem	Adult
6	African mahogany	<i>Khaya grandifoliola</i>	Meliaceae	Oganwo	Bark	Adult
7	African birch	<i>Angeissus leiocarpus</i>	Combretaceae	Epoayin	Bark	Children
8	<u>Milkweeds</u>	<i>Grongonema latifolia</i>	Apocynaceae	Modunmoro	Stem	Children
9	Akuamma	<i>Picralima nitida</i>	Apocynaceae	Abere	Fruits	Children
10	Mustard	<i>Brassica juncea</i>	Brassicaceae	Agano	Bark	Children
11	Clove	<i>Eugenia caryophyllus</i>	Myrtaceae	Kanafuru	Fruits	Children
12	Poison devil's-pepper	<i>Rauvolfia vomitoria</i>	Apocynaceae	Epoorira	Bark	Children
13	Garlic	<i>Allium salivum</i>	Liliaceae	Ayu	Bulb	Children
14	Camphor			Kafura	Tablet	Children

Results and Discussion

Bacterial isolates

Eight bacteria were isolated and characterised (Table 2). Most of the isolates are harmless flora of human gastrointestinal tracts which may become opportunistic pathogen when the condition arises. Enterococci are part of the normal intestinal flora of humans and animals and cause a wide variety of diseases in humans, infecting the urinary tract, bloodstream,

endocardium, abdomen, biliary tract, burn wounds, and indwelling foreign devices {15}. The presence of *Staphylococcus aureus* which is a normal flora of the skin and nose of humans in the fecal sample is indicative of intestinal colonization and may increase the risk of infecting other tissues due to the added advantage of proliferating in the nutrients rich gastrointestinal tracts {16}. *Staphylococcus aureus* is implicated in a number of skin diseases, breast and lung infections, bacteremia, endocarditis, osteomyelitis and food poisoning {17}. *S. aureus* produces a heat stable toxin called enterotoxin which causes diarrhea, nausea, vomiting, abdominal cramps and other associated symptoms {18}.

Proteus, *Salmonella*, *Shigella*, *Escherichia* and *Klebsiella* belong to the Enterobacteriaceae, occurring mainly in the intestinal tracts as normal flora. *Escherichia coli* is an opportunistic pathogen of the urinary tract and the pathogenic strains are involved in serious gastrointestinal diseases. Although *Proteus mirabilis* is implicated in 90% of *Proteus* infections especially of the urinary tracts, the importance of *P. vulgaris* in nosocomial infections and particularly of individuals with compromised immunity cannot be over emphasized {19}. *Salmonella enterica* causes salmonellosis (a foodborne gastrointestinal disease), bacteremia and fever {20}. *Shigella*, the causative agent of human shigellosis, is one of the leading bacterial causes of diarrhea worldwide. As of 2006, the WHO reported that *Shigella* causes about 165 million cases of severe dysentery, with a million resulting in death each year and mostly among children in the developing world. Among the three *Shigella* spp. (*S. sonnei*, *S. flexneri* and *S. dysenteriae*) that are commonly associated with shigellosis, *S. flexneri* is the most frequently isolated worldwide and is endemic in developing countries {21}.

Antibacterial Activities

All the three herbal preparations showed activity against the isolates. In the adult men's extracts, activity was delayed till 48 hours of extraction in *S. aureus* and *P. vulgaris* but kicked off in the first 24 hours for the other isolates (Table 2). *S. aureus* inactivates penicillin by β lactamase mechanism. Strains that are resistant to methicillin are also resistant to other

penicillins and cephalosporins. Vancomycin-resistant *S. aureus* strains are envisaged {22} and this corroborates the need for alternative medicines for its treatment. *Enterococcus faecalis* and *P. aeruginosa* were the late responders in the women's extracts (Table 3). In the case of the children's extract, *Enterococcus faecalis* was not susceptible, a prolonged delay in activity to the 6th day occurred with *S. aureus*, while *Salmonella enteritica*, *Shigella* sp. *Klebsiella* sp. and *P. aeruginosa* also had delayed activity (Table 4). This may be a result of low level of phytochemicals in the herbs used considering the tolerance of the target users. Moreover, the resistance of *Enterococcus faecalis* to antibiotics such as penicillinase-susceptible penicillin (low level), penicillinase-resistant penicillins, cephalosporins, nalidixic acid, aztreonam, macrolides, and low levels of clindamycin and aminoglycosides{23}and penicillin by beta-lactamases, chloramphenicol, tetracyclines, rifampin, fluoroquinolones, aminoglycosides (high levels), and vancomycin{14} has been reported. Although the herbal extract, in this study had delayed, activities against *E. faecalis* in the women extract and none in the children's extracts, it showed promise as alternative to conventional drugs in treating diseases of the gastrointestinal tracts, caused by the organism.

Strains of *Salmonella* resistant to chloramphenicol, ampicillin, cotrimoxazole and ciprofloxacin are prevalent in many clinical samples in several areas of the world {21}. *Salmonella enteritica* showed a susceptibility pattern that compares favorably with the reference drug, streptomycin with all the herbal preparations used. Similarly, sensitivity of the *Shigella* sp. used in this study compares favorably with that of streptomycin. Although diarrhea caused by *Shigella* can be treated with only fluids and rest, severe cases may require the use of antibiotics. Meanwhile resistance to antibiotics is common among members of the genus *Shigella* (CDC) which brings to the fore the importance of herbal remedies such as was used in this study. Comparative inhibition ranges of 80 to 100%; 85 to 150% and 90 to 160% in reference to streptomycin, was obtained for *Proteus vulgaris*, *E. coli* and *Klebsiella* sp. respectively (Figure 1). Control of diseases caused by these coliforms has been achieved with the use of

antibiotics such as Ampicillin, sulfonamides, cephalosporins, tetracycline, trimethoprim-sulfamethoxazole, nalidixic acid, ciprofloxacin, and nitrofurantoin, Gentamicin, amikacin, tobramycin, ticarcillin/clavulate, imipenem, aztreonam, and a variety of third-generation cephalosporins. However, resistance to ampicillin, and first generation cephalosporins and the emergence of coliforms carrying chromosomal or plasmid-encoded extended spectrum B-lactamase activity with resistance to third generation cephalosporins is narrowing the range of antibiotics for treatment {24}. Alternative remedies can therefore be provided with the various herbal preparations used in this study.

The minimum inhibitory concentration of the herbal extracts ranged from 25 to 100% (Table 5). All the organisms, except *S. aureus* was inhibited by the adult men extract with minimum inhibitory concentration at 100% after 24 hours of extraction. The women extract was a little stronger with MIC values ranging from 25 to 50% in most of the organisms. Though inhibition was not observed with the children extract before 48 hours of extraction, the MIC for most of the organisms ranged between 25 to 50%.

The effectiveness of the herbal preparation in controlling microorganisms of the gastrointestinal tract is a result of the antibacterial activities of the phytochemical components of the various herbs used. *Nauclea latifolia* is a rich source of monoterpene indol alkaloids. Extracts of the plant have exhibited activity against *E. coli*, *Shigella* spp., *Salmonella* spp. and *S. aureus*, *Klebsiella* spp., *P. aeruginosa*{7,9}. In Northern Nigeria the plant is used to deworm cattle and sheep {5,6}. No record of antibiotic effect was found for *Aristolochia albida*. Its inclusion in the traditional herbal preparation can however be attributed to its antihelminthic property. Members of the family *Alliaceae* to which belongs *Allium ascolanicum*, (shallot) have demonstrated activities against bacteria of the gastrointestinal tracts. *Allium sativum* was active against *E. coli*, *P. aeruginosa* and *K. pneumonia* {25}. The major phytochemical compound of *Cryptolepsiss anguinolenta* is cryptolepine which may be responsible for its antimicrobial activities as reported against *Salmonella typhi*, *S. aureus*, *Klebsiella*

pneumonia and *P. aeruginosa*{26}.*Pteleopsis suberosa* Engl. et Diels decoction were active against all the bacterial strains tested{27}. *Khaya grandifoliola* containing alkaloids, tannins, saponins and flavonoids were found to be active against *S. aureus*, *K. pneumonia* and *Proteus mirabilis* {30}.

The stem bark of *Anogeissus leiocarpa* (African birch) contains castalagin and flavogallonic acid dilactone {29,30}.The root bark extracts contained alkaloids, tannins, flavonoids, phenolics, saponins, phlobatannins, and terpenes{11}. The inner bark is used as a chewing stick in *Nigeria* and extracts of the bark show *antibacterial* properties {8}. *Gongronema latifolium* contains the essential oils, saponins and pregnanes among others{31,32}. Though, no report of its antimicrobial activity was found, aqueous and ethanolic extracts had anti-inflammatory, hypoglycemic, hypolipidemic and antioxidative properties {31,33,34,35}. *Picralimanitida* which produces the alkaloids pericine and akuammine demonstrated antibacterial activity against *S. aureus*, *P. aeruginosa*, *Escherichia coli*, *Bacillus subtilis* and *Salmonella* {36}. *Brassica juncea* produces several classes of bioactive phyto-chemicals majorly Allyl isothiocyanate and others including glycosides, flavonoids, phenolic compounds, sterols and triterpene alcohols{37,38,39}, which may account for its antimicrobial activity {40,41}. *Eugenol*, the major chemical component of clove is a known antimicrobial agent whose activity has been demonstrated against Gram positive and Gram negative bacteria {10,42}.

Conclusion

Findings in this study show that the herbal concoctions used to treat/prevent diarrhea in Ilorin, Nigeria has antibacterial activity that compares favorably with streptomycin which is a broad spectrum antibiotic. This corroborates claims by retailers of the herbs and forms the basis for recommending its use after determining its toxicity effect.

Table 1: Morphological and biochemical characteristics of bacterial isolates

Characters	Isolates							
	A	B	C	D	E	F	G	H
Colony Characteristics								
Shape	Circular	Circular	Swarm	Circular	Circular	Circular	Circular	Circular
Margin	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Undulate
Elevation	Convex	Convex	Flat	Convex	Convex	Raised	Convex	Raised
Size	Small	Large	Spreading	Medium	Small	Small	Small	Large
Texture	Smooth	Rough	Smooth	Smooth	Smooth	Smooth	Smooth	Rough
Appearance	Glistening	Glistening	Glistening	Glistening	Glistening	Glistening	Glistening	Glistening
Pigmentation	Cream	Cream	Greyish	Greyish	Greyish	Colorless	Greyish	Blue-Green
Optical Char.	Opaque	Opaque	Opaque	Translucent	Translucent	Translucent	Opaque	Opaque
Cellular Morphology								
Gram rxn	+	+	-	-	-	-	-	-
Capsule	-	-	-	-	-	-	+	-
Shape of cell	Coccus	Coccus	Rod	Rod	Rod	Rod	Rod	Rod
Arrangement	S, P, C	S, Cl	S	S	S	S	S	S
Motility	-	-	+	+	-	+	-	+
Sporulation	-	-	-	-	-	-	-	-
Biochemical Characteristics								
Urease	+	+	+	-	-	-	+	-
Catalase	-	+	+	+	+	+	+	+
Starch	+	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	+
Citrate	+	-	-	+	-	-	+	+
Litmus milk	Acid	Acid	Acid	Alkaline	Acid	Acid	Acid	Alkaline
Gelatin liq	-	+	+	-	-	-	-	+
Indole	-	-	+	-	-	+	-	-
MR	+	+	+	+	+	+	-	-
VP	+	+	-	-	-	-	+	-
Carbohydrate Utilization								
Mannitol	+	+	-	+	+	+	+	-
Glu	+	+	+	+	+	+	+	-
Suc	+	+	+	-	-	+	+	-
Lac	+	+	-	-	-	+	+	-

Gas	-	-	+	+	-	+	+	-
H ₂ S	-	-	+	+	-	-	-	-
Growth Characteristics								
O ₂ Rq	FA	FA	FA	FA	FA	FA	FA	AE
6.5% NaCl	+	+	ND	ND	ND	ND	ND	+
Blood Agar	Heamolysis	Heamolysis	Heamolysis	No Heamolysis	No Heamolysis	No Heamolysis	No Heamolysis	No Heamolysis
MacConkey	-	-	No Clearance	No Clearance	No Clearance	Clearance	Clearance	No Clearance
Growth at 10°C	+	+	+	+	+	+	+	+
Growth at 42°C	+	+	-	+	+	+	+	+
Organisms	<i>Enterococcus faecalis</i>	<i>Staphylococcus aureus</i>	<i>Proteus vulgaris</i>	<i>Salmonella enteritica</i>	<i>Shigella</i> sp.	<i>Escherichia coli</i>	<i>Klebsiella</i> sp.	<i>Pseudomonas aeruginosa</i>

Legend: '+' denotes positivity while '-' denotes negativity i.e. gram positive/gram negative; presence/absence of capsule; motile/non motile; spore forming/non spore forming; urease, catalase, oxidase and indole production/ non production; starch and citrate hydrolysis/non hydrolysis; gelatin liquefaction/non liquefaction; methyl red and Voges Proskuear reactions; ability to utilize carbohydrate; gas and hydrogen sulfide production; growth in 6.5% sodium chloride, at 10 and 45°C, or otherwise. S = single, P = pairs, C = chain, Cl = cluster, FA = facultative anaerobes, AE = aerobes, ND = not determined.

Table 2: Effects of period of extraction on the activity of adult herbal extract for men on fecal isolates

Isolates	Zone of inhibition (mm)							
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Strept D/W
<i>E. faecalis</i>	14.2±1.5 ^b	16.8±1.3 ^c	17.8±1.6 ^{c,d,e}	19.8±1.6 ^e	17.4±1.5 ^{c,d}	19.0±1.6 ^{d,e}	18.8±1.5 ^{c,d,e}	21.0±0.7 ^f 0.0 ^a
<i>S. aureus</i>	0.0 ^a	11.8±1.3 ^b	11.4±0.9 ^b	14.0±0.7 ^c	13.8±1.5 ^c	14.0±0.7 ^c	17.0±1.6 ^d	20.2±1.3 ^e 0.0 ^a
<i>P. vulgaris</i>	0.0 ^a	12.0±0.7 ^b	12.0±1.2 ^b	13.2±1.5 ^b	15.2±0.8 ^c	16.4±1.1 ^c	15.2±0.8 ^c	20.8±0.8 ^d 0.0 ^a
<i>S. enteritica</i>	11.6±1.5 ^b	13.0±1.0 ^{b,c}	14.2±1.5 ^c	18.6±1.8 ^{d,e}	17.6±1.1 ^d	18.2±1.9 ^{d,e}	20.0±1.6 ^e	19.0±0.7 ^f 0.0 ^a
<i>Shigella</i> sp.	16.2±0.8 ^c	13.8±0.8 ^b	15.2±1.5 ^{b,c}	19.0±1.9 ^d	14.8±1.1 ^{b,c}	15.6±1.5 ^{b,c}	19.2±2.3 ^d	21.4±0.5 ^e 0.0 ^a
<i>E. coli</i>	11.6±1.1 ^b	13.8±1.5 ^c	16.2±1.6 ^{d,e}	16.0±2.6 ^{c,d,e}	17.0±0.7 ^e	14.4±1.1 ^{c,d}	15.8±1.6 ^{c,d,e}	18.8±0.8 ^f 0.0 ^a
<i>Klebsiella</i> sp.	15.6±1.5 ^b	14.8±0.8 ^b	17.8±1.1 ^c	17.8±2.4 ^c	14.8±0.8 ^b	15.0±1.6 ^b	18.0±0.7 ^c	19.2±0.8 ^d 0.0 ^a
<i>P. aeruginosa</i>	13.4±1.3 ^{b,c}	15.2±0.8 ^d	12.2±0.8 ^b	13.6±1.1 ^c	14.2±0.5 ^{c,d}	17.4±1.1 ^e	19.2±0.8 ^f	14.4±1.1 ^g 0.0 ^a

Legend: Each value represents mean of five independent readings ± standard deviation. Means displayed with homogenous superscript within the same row are insignificantly different P = 0.05.

Table 3: Effects of period of extraction on the activity of adult herbal extract for women on fecal isolates

Isolates	Zone of Inhibition								D/W
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Strept	
<i>E.faecalis</i>	0.0 ^a	13.2±0.8 ^b	12.0±0.7 ^c	14.0±1.2 ^{c,d}	14.6±0.9 ^d	14.0±0.7 ^{c,d}	15.8±1.4 ^e	21.0±0.7 ^f	0.0 ^a
<i>S.aureus</i>	12.4±1.1 ^b	15.2±1.1 ^{d,e}	12.8±0.8 ^{b,c}	15.6±0.9 ^{e,f}	14.2±1.1 ^{c,d}	13.8±1.5 ^{b,c}	16.4±1.3 ^f	20.2±1.3 ^g	0.0 ^a
<i>P.vulgaris</i>	14.2±0.8 ^b	15.0±0.7 ^c	17.6±1.1 ^e	16.6±1.1 ^{d,e}	16.6±0.9 ^{d,e}	15.6±1.5 ^{c,d}	13.0±1.5 ^b	20.8±0.8 ^f	0.0 ^{a,c}
<i>S.enteritica</i>	12.4±1.1 ^b	12.6±0.9 ^b	14.2±1.3 ^c	14.2±1.3 ^c	17.0±1.6 ^d	17.8±1.1 ^{d,e}	20.4±1.1 ^f	19.0±0.7 ^{e,f}	0.0 ^a
<i>Shigella</i> sp.	4.6±1.1 ^b	11.2±4.1 ^c	15.2±2.6 ^d	18.4±1.5 ^e	17.8±0.8 ^e	18.2±1.5 ^e	19.8±1.1 ^{e,f}	21.4±0.5 ^f	0.0 ^a
<i>E.coli</i>	12.8±0.8 ^b	15.2±1.4 ^c	15.6±1.1 ^d	18.2±0.5 ^d	17.8±1.6 ^d	18.8±1.9 ^d	18.6±1.8 ^d	18.8±0.8 ^d	0.0 ^a
<i>Klebsiella</i> sp.	14.8±1.1 ^c	15.8±1.9 ^{d,e}	14.6±0.6 ^{b,d}	12.8±0.8 ^{b,c}	13.6±0.9 ^b	16.4±1.7 ^{e,f}	17.6±1.5 ^{f,g}	19.2±0.8 ^g	0.0 ^{a,d}
<i>P.aeruginosa</i>	0.0 ^a	15.0±1.8 ^{b,c}	16.6±2.1 ^c	16.6±1.1 ^c	16.6±2.3 ^c	15.0±0.7 ^{b,c}	14.6±1.1 ^{b,c}	14.4±1.1 ^b	0.0 ^a

Legend: Each value represents mean of five independent readings ± standard deviation. Means displayed with homogenous superscript within the same row are insignificantly different P = 0.05.

Table 4: Effects of period of extraction on the activity of herbal extract for children on fecal isolates

Isolates	Zone of Inhibition (mm)							Strept	D/W
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7		
<i>E. faecalis</i>	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	21.0±0.7 ^b	0.0 ^a
<i>S. aureus</i>	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	14.0±0.8 ^b	15.2±1.1 ^b	20.2±1.3 ^c	0.0 ^a
<i>P. vulgaris</i>	9.6±1.1	12.2±1.1 ^c	14.0±0.7 ^d	19.8±0.5 ^e	24.6±1.1 ^g	21.6±1.1 ^f	24.2±1.5 ^g	20.8±0.8 ^{e,f}	0.0 ^{a,b}
<i>S. enteritica</i>	0.0 ^a	10.2±2.4 ^b	21.8±1.5 ^d	30.2±1.5 ^e	21.0±2.5 ^{c,d}	29.2±1.6 ^e	23.2±1.8 ^d	19.0±0.7 ^c	0.0 ^a
<i>Shigella</i> sp.	0.0 ^a	0.0 ^a	9.4±2.4 ^b	24.4±1.1 ^d	24.6±1.1 ^d	20.2±1.5 ^c	23.8±1.3 ^d	21.4±0.5 ^c	0.0 ^a
<i>E. coli</i>	0.0 ^a	10.0±1.9 ^b	9.6±2.3 ^b	15.8±1.5 ^b	22.4±1.1 ^e	25.0±0.7 ^f	19.8±1.5 ^d	18.8±0.8 ^d	0.0 ^a
<i>Klebsiella</i>	0.0 ^a	30.2±1.5 ^e	32.0±1.5 ^f	26.8±1.5 ^d	21.4±1.1 ^c	30.8±0.8 ^{e,f}	25.4±1.1 ^d	19.2±0.8 ^b	0.0 ^a
sp.									
<i>P. aeruginosa</i>	0.0 ^a	0.0 ^a	10.0±1.9 ^b	12.6±1.1 ^c	19.6±2.1 ^d	21.0±2.5 ^{d,e}	22.2±0.8 ^e	14.4±1.1 ^c	0.0 ^a

Legend: Each value represents mean of five independent readings ± standard deviation. Means displayed with homogenous superscript within the same row are insignificantly different P = 0.05.

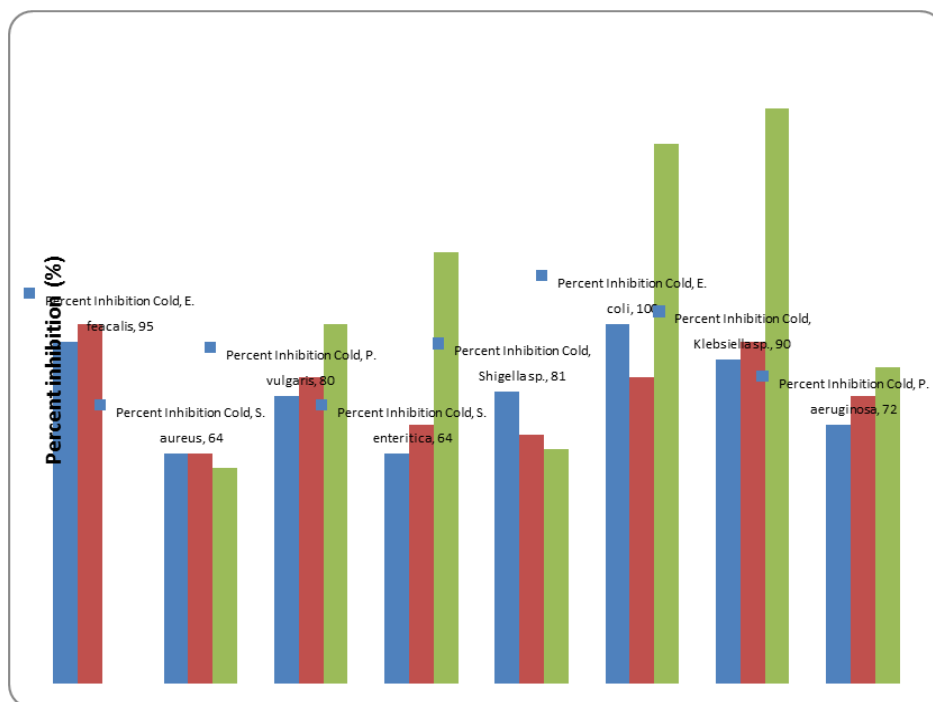


Figure 1: Percent inhibition of fecal isolates by herbal extracts compared to streptomycin

Legend: The highest inhibition zone produced by each of the three herbal extracts against the isolates was compared with that produced by the reference drug, streptomycin as follows:

$$\text{Percent inhibition} = \frac{\text{Zone of inhibition by herbal extract}}{\text{Zone of inhibition by streptomycin}} \times 100$$

Table 5: Minimum inhibitory concentration of the herbal extracts on fecal isolates

Isolates	Minimum inhibitory concentration		
	Adult men extract	Adult women extract	Children extract
<i>Enterococcus faecalis</i>	100%	50%, 48h	No inhibition
<i>Staphylococcus aureus</i>	50%	25%	100%
<i>Proteus vulgaris</i>	100%	25%	25%
<i>Salmonella enteritica</i>	100%	50%	50%
<i>Shigella</i> sp.	50%	50%	25%
<i>Escherichia coli</i>	100%	50%	25%
<i>Klebsiella</i> sp.	100%	25%	25%
<i>Pseudomonas aeruginosa</i>	100%	50%	50%

Legend: The MIC of the adult herbs against most of the isolates was obtained from the 24 hour extract except for *S. aureus* and *P. aeruginosa* which was obtained from the 48 hour extract; while in the case of the children's herbs, MIC was obtained from older extracts.

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