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Ethnobotanical Survey and Antimicrobial Evaluation of Medicinal Plants used by the Samburu Community (Kenya) for treatment of Diarrhorea

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ABSTRACT
The Samburu are a marginalized nomadic people who have no access to conventional medical services. The Samburu therefore depend on traditional medical practice and medicinal plants for most of their medicare. The medicinal plants used have not been tested for efficacy especially on diarrhoreal diseases which are endemic in the community. This study evaluated plants commonly used for the treatment of diarrhoea *in-vitro* for antimicrobial activity against standard Gram positive and Gram negative bacteria. Results obtained show that the zones of inhibition for the active plants ranged between 16mm to 36.33mm. The MICs of the most active plants ranged from 0.9375 mg/50 µl to7.5 mg/50µl. The MBCs ranged between 0.9375 mg/50µl to 7.5 mg/50µl. These results were significant at p< 0.01. The findings show that most of the medicinal plants used by the Samburu community have significant activity against *E.coli* (*Acacia nilotica*- 21.66 mm) *S. typhi* (*Acacia horrida*- 19mm) and *Pseudomonas aeruginosa* (*Cordia monoica*- 36.33 mm) which are human pathogens especially *Escherichia coli* and *Salmonella typh*i which cause diarrhoea.
Keywords: diarrhoea; medicinal plants; phytochemicals; antidiarrhoeal activity; ethno botany.

INTRODUCTION

The Samburu community is one of those communities that are marginalized in Kenya in terms of 'HEALTH CARE FOR ALL' as a basic human right and prerequisite to social-economic development. The frequent use of medicinal plants by the Samburu for health care is as a result of the unavailability of health care services from the government in this remote region of Kenya (1).

It is estimated that about 85% of the Samburu community medicare is from medicinal plants (1). The problem is compounded by high poverty rate, poor sanitary conditions and inadequacy of clean water. For instance, pastoralism is a normal practice of the inhabitants' that leads to sharing of water with both domestic and wild animals which makes inhabitants using water without proper treatment as it is scarce most of the year. This has led to an increase in diarrhoreal diseases.

The Samburu people like the rest of the world contribute to the estimated 4.6 million people, including 2.5 million children, who die from diarrhoeal diseases every year particularly in developing countries (2, 3). Diarrhoea is caused by various agents like the viruses (rotavirus, adenovirus e.t.c), bacterial (*E. coli, Vibrio cholerae* and *S. typhi)* and parasites (*Cryptosporidium* and *Giardia)* (4).

Nevertheless, acute diarrhoeal diseases among children younger than 5 years remain a major cause of morbidity and mortality worldwide (5). An Expert Committee of the WHO recently estimated that diarrhoea causes 18% of the 11 million deaths among children younger than the age of 5 yrs in the world, nearly the same mortality as pneumonia (19%) which is the leading cause of infant mortality (6,7). The adults are affected with an estimated incidence of 1.4 episodes/adult/year (8). The disease requires special attention in treatment and management in adults, because of their multiple comorbidities, immunosenescence, frailty, and poor nutritional status

(9). Diarrhoea is identified as a major opportunistic disease among HIV/AIDS patients (10, 11).

However, there have been numerous reports on the use of traditional plants for the treatment of diarrhoeal diseases (4). In recent years, secondary plant metabolites (phytochemicals), with unknown pharmacological activities, have been extensively investigated as a source of medicinal compounds (12). The results obtained have been phenomenal. For instance, in one study organic extracts of *Punica granatum*, *Ozoroa insignis*, and *Indigofera daleoides* were active against *Staphylococcus aureus* ATCC 25923, *Salmonella typhi* ATCC 0232, *Vibro cholera*, *Escherichia coli* ATCC 35218 and *Shigella* spp. (*Shigella dysentery*, *Shigella flexneri*, *Shigella sonnei*, *Shigella boydii*). Water extracts of *Punica granatum* were equally active as organic extracts against bacteria such as *Staphylococcus aureus*, *Shigella sonnei* and *Shigella flexneri* (13). Therefore, medicinal plants are increasingly being projected as a suitable alternative source because of their often multiple targets, minor side-effects, low potentials to cause resistance and low costs (14).

There are high rates of occurrence of stomach ailments among the Samburu. The diseases get magnified given the fact that they lack proper medication because of high poverty rates hence prefers the use of local treatment by use of medicinal plants. The community believes in medicinal plants first before the patient is hospitalized and in most cases hospitalization is as a result of intoxication due to overdoses. It becomes necessary therefore to carry out a survey of the most common plants the Samburu use for the treatment of stomach ailments and to validate the efficacy of the plants.

MATERIALS AND METHODS

Ethnobotanical survey

A survey was carried out in Wamba division, Samburu district, Kenya on the major medicinal plants the community uses for the treatment of diarrhoreal diseases (fig 1). Questionnaires were used to identify the plants used by the herbalists and the community in the treatment of diarrhoreal diseases.

Collection of plant material

Fresh plants/plant parts used by the community for treatment of diarrhoreal diseases were collected from Samburu-Wamba Conservancies as shown in Fig 1. The taxonomic identities of these plants were confirmed by a taxonomist at the Kenyatta University herbarium where the voucher specimens are deposited.

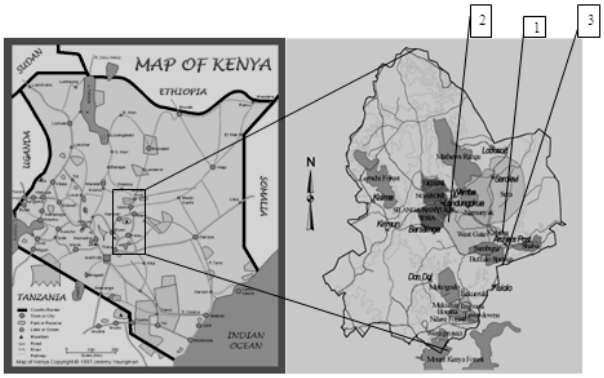

Fig1: Map of Kenya showing the location of Wamba Division and its conservancies

Key: Site 1. Namunyak conservancy, Site 2. Ngaroni conservancy, Site 3. West gate conservancy.

Extraction

Dried, ground plant materials (50g) were soaked in 300 ml of 80% methanol (MW-32.04) for 12-48h with intermittent shaking to allow the active phytochemicals to dislodge in the solvent. The methanol soaked plant extracts were filtered by use of Whatman No. 1 filter paper and the filtrates evaporated until dry weight of each extract was obtained by using the rotary evaporator (VV 2000 Heidolph, Germany) set at 40-50°C. The extract moisture was reduced to constant weight by additional drying over copper sulphate in a desicator under vacuum.

Antimicrobial screening/ bioassay.

a. Test cultures

Test cultures were obtained from Kenyatta National Hospital in Nairobi-Kenya, which included *Staphylococcus aureus* (Gram +ve cocci) - ATCC 20591, *Bacillus subtillis* (Gram +ve spore forming rod) - local isolate, *Salmonella typhi* (Gram –ve rod) - ATCC 2202, *Escherichia coli* (Gram-ve rod) - STD-25922 and *Pseudomonas aeruginosa* (Gram-ve rod) - ATCC 25852. All the microorganisms were maintained at 4 ºC on nutrient agar slants. Some of the micro organisms were selected on the basis of their natural differences and cell wall properties, but others such as *Escherichia coli* and *Salmonella typhi* were identified in Samburu as actual causes of diarrhoea (unpublished data).

b. Disc diffusion method.

The antimicrobial bioassay was performed by agar disc diffusion method using 18h test cultures for methanol extracts (15, 16). The Mueller Hinton agar (Biotec) was prepared following the manufacturers instructions and was inoculated with 100 µl of the inoculum that is equivalent to MacFarland turbidity standard of 0.5×10^6

CFU/ml which were spread plated. Then a disc (6 mm) was saturated with 100 µl of the plant extract, allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated overnight at 37 ºC. Microbial growth was determined by measuring the diameter from the end of growth to the disc at one end to the beginning of growth at the other end including the diameter of the disc. For each bacterial strain, Amoxicillin (250 µg) was used as a positive control and methanol as the negative control. The results were again obtained by measuring the zones of inhibition. The experiment was repeated three times and the mean values recorded.

c. Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

A micro titre -dilution technique using 96 well micro-plates, (17) was used to obtain MIC values of the crude extracts against all the test bacteria. Each plant extract was serially diluted to obtain 7.5mg/50µl, starting from the first well. Similar serial dilutions were performed for Cefrodoxima (250mg), as a positive control. The starting concentration for Cefrodoxima in the first well after the dilution was 7.5mg/50µl. An equal volume of 50µl log-phase bacterial cultures were added to each of the wells. Micro titre-plates were covered and incubated at 37ºC overnight. The MIC values were determined as the lowest concentrations of the extract showing no growth. All the wells where no growth (not turbid) was observed were sub cultured, and the lowest concentration of the plant extracts that did not yield any colony on the solid nutrient medium after sub-culturing and incubating for 12-24h was taken as the MBC. All tests were performed in triplicate (18).

Phytochemical screening

Qualitative phytochemical analysis of the crude powder of the plants collected was determined by established methods (19, 20). Tannins presence was determined by use of 200 mg plant material which were dissolved in 10 ml distilled water, and then filtered. 2 ml of the filtrate was taken and 2 ml iron (III) chloride solution added. A blue-black precipitate indicated the presence of tannins. For the presence of alkaloids 200 mg plant extract was dissolved in 10 ml methanol, and filtered. 1 ml of the filtrate was mixed with 6 drops of Wagner's reagent (made by mixing 1.27g iodine and 2g potassium iodide in 100ml of water). A creamish/brownish-red/orange precipitate indicated the presence of alkaloids.

Saponins presence was determined by the frothing test method (20) where 0.5 ml of the filtrate was mixed with 5 ml distilled water. Frothing persistence indicated presence of saponins. Cardiac glycosides presence was determined by Keller-Kiliani test (19) where 2 ml of the filtrate was mixed with 1 ml glacial acetic acid, followed by three drops of Iron (III) chloride and concentrated sulphuric acid. Green-blue colour indicated the presence of cardiac glycosides. Terpenoids presence was determined by taking 5mls of the plant extract that was mixed with 2mls of chloroform, and 3mls of concentrated sulphuric acid was then carefully added to form a layer. If a reddish brown coloration of the interface formed, it indicated the presence of terpenoids. Flavonoids presence was determined by taking 5 ml of dilute ammonia solution that was added to a portion of the aqueous filtrate of each plant extract followed by the addition of concentrated sulphuric acid. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing.

Statistical analysis

The mean zones of inhibition for each test culture were analyzed by one-way analysis of variance (ANOVA) to get the differences among group means. A P- value < 0.05 was considered as significant. The means were separated by Tukey's test. The computer software STATISTICA® was employed for the statistical analysis.

RESULTS

Ethnobotanical survey.

After carrying out the survey, 16 medicinal plants were found to be used by the community in the treatment of diarrhoreal diseases as presented in Table 1. The family of Mimosaceae had the highest number of the medicinal plants (six), Vetaceae family had two plants and the rest of the families had one medicinal plant each. Various parts were harvested depending on the parts the community preferred to use in the treatment of the diarrhoreal diseases. The bark, roots and the leaves were the ones that were harvested, but the part that is used most was found to be the bark of the stem, followed by the roots and then the leaves.

Disc diffusion assay

The antibacterial activities of 16 plant species were assayed *in-vitro* by agar disc diffusion method against 5 bacterial species (Table 2). Table 2 summarizes the average microbial growth inhibition of the methanol extracts of the screened plant species against the test cultures. From the findings it was observed that most

TABLE 1: Medicinal plants collected

	Botanical name	Family name	Samburu name	Part used	Diseases treated	AREA FOUND
1.	*Acacia ethaaica* Schweinf.	Mimosaceae	Lchakwai	Bark	Diarrhoea	Namunyak (1)
2.	*Acacia horrida* (L.) Willd.	Mimosaceae	Lerai	Bark	Diarrhoea	West gate (3)
3.	*Acacia nilotica* (L.) Del.	Mimosaceae	Lkiloriti	Bark/ roots	Diarrhoea	Namunyak (1)
4.	*Acacia nubica* Benth.	Mimosaceae	Ldepe	Bark	Diarrhoea	Ngaroni (2)
5.	*Acacia senegal* (L.) Willd.Var. *persica*	Mimosaceae	Lderekesi	Bark	Diarrhoea	Ngaroni (2)
6.	*Acacia tortilis* (Forssk.) Hayne.	Mimosaceae	Ndapes	Roots	Diarrhoea	Namunyak (1)
7.	*Acokanthera friesiorum* Markgr.	Apocynaceae	Nchipilikwa	Roots/leaves	Diarrhoea	Namunyak (1)
8.	*Albizia anthelmitica* Brongn.	Leguminosae	Lumurtana	Roots/bark	Deworming	Ngaroni (2)
9.	*Aloe secundiflora* Engl.	Aloaceae	Sukuroi	Whole	Diarrhoea	Namunyak (1)
10.	*Balanites aegyptiaca* (L.) Del.	Balanitaceae	Sirai	Roots	Diarrhoea	Ngaroni (2)
11.	*Boscia angustifolia* Guill. and Perr.	Capparaceae	Lororoi	Bark	Diarrhoea	Ngaroni (2)
12.	*Cissus rotundifolia* Forsk. Vahl.	Vitaceae	Raraiti	Root	Diarrhoea	Ngaroni (2)
13.	*Cissus quadrangularis* L.	Vitaceae	Sukurtut	Stem	Diarrhoea	Ngaroni (2)
14.	*Clerodendrum myriacoides* (Hochst.)Vatke subsp. *napperae* Verdc	Verbenaceae	Makutukuti	Roots	Diarrhoea, Malaria, cold, & polio	West gate (3)
15.	*Commiphora africana* (A. Rich) Engl. Var. *persica*	Burseraceae.	Lcheni-Ngiro	Bark	Diarrhoea	Ngaroni (2)
16.	*Cordia monoica* Roxb.	Boraginaceae	Seki	Roots	Diarrhoea	West gate (3)

Table 2: Average zones of inhibition (mm) of the plant extracts against the test cultures.

BOTANICAL NAME	*S. aureas*	*B. subtilis*	*S. typhi*	*E. coli*	*P. aeruginosa*
1) Acacia ethaaica Schweinf.	19.33	19	16.66	17.66	19.33
2) Acacia horrida (L.) Willd.	18	17.66	19	18.66	28.66
3) Acacia nilotica (L.) Del.	20.33	18	21	21.66	27.66
4) Acacia nubica Benth.	17.33	15.66	13.33	13.33	15.33
5) Acacia senegal (L.) Willd.Var. *persica*	11.33	15	11	12.66	18.33
6) Acacia tortilis (Forssk.) Hayne.	15	13	12.33	11.33	21.33
7) Acokanthera friesiorum Markgr.	14	11	11	10.66	19
8) Albizia anthelmitica Brongn.	16	13.33	13.33	10	28.66
9) Aloe secundiflora Engl.	13.66	13.33	12.33	13.66	27
10) Balanites aegyptiaca (L.) Del.	15.66	12.66	16	18	17
11) Boscia angustifolia Guill. and Perr.	7	8	10.33	11.33	20
12) Cissus rotundifolia Forsk. Vahl.	14.33	13.66	9.66	11.33	17.66
13) Cissus quadrangularis L.	10.33	12.66	12.66	13.66	23
14) Clerodendrum myriacoides (Hochst.) Vatke subsp. *napperae* Verdc	14.33	15	14	16	25.33
15) *Commiphora africana* (A. Rich) Engl. Var. *persica*	13	14.33	16	16.33	21.66
16) Cordia monoica Roxb.	14	18	15.66	16.33	36.33
17) Amoxicillin	21.33	17.17	24.17	23.58	17.58
18) -ve control	6	6	6	6	6

Table 3: The MICs (mg/50µl) and MBCs (mg/50µl) produced by the selected Samburu medicinal plants against the test cultures.

	S. aureas		***B. subtilis***		***S. typhi***		***E. coli***		***P. aeruginosa***	
BOTANICAL PLANT NAME	**MIC** (mg/ 50µl)	**MBC** (mg/ 50µl)	**MIC** (mg/ 50µl)	**MBC** (mg/ 50µl)	**MIC** (mg/ 50µl)	**MBC** (mg/ 50µl)	**MIC** (mg/ 50µl)	**MBC** (mg/ 50µl)	**MIC** (mg/ 50µl)	**MBC** (mg/ 50µl)
Acacia ethaaica Schweinf.	0.9375	0.9375	0.9375	1.875	1.875	1.875	0.9375	0.9375	0.9375	0.9375
Acacia horrida (L.) Willd.	3.75	3.75	3.75	7.5	3.75	3.75	3.75	3.75	1.875	1.875
Acacia nilotica (L.) Del.	1.875	1.875	1.875	3.75	1.875	1.875	1.875	1.875	0.9375	0.9375
Acacia nubica Benth.	3.75	7.5	3.75	3.75	3.75	7.5	3.75	3.75	1.875	3.75
Acacia senegal (L.) Willd.Var. *Persica*	3.75	7.5	3.75	7.5	3.75	3.75	1.875	1.875	3.75	3.75
Acacia tortilis (Forssk.) Hayne.	1.875	1.875	1.875	3.75	3.75	7.5	1.875	1.875	1.875	3.75
Acokanthera friesiorum Markgr.	3.75	3.75	3.75	7.5	1.875	3.75	1.875	1.875	1.875	3.75
Albizia anthelmitica Brongn.	1.875	3.75	3.75	3.75	3.75	3.75	3.75	3.75	1.875	1.875
Aloe secundiflora Engl.	3.75	3.75	3.75	7.5	3.75	3.75	3.75	3.75	3.75	7.5
Balanites aegyptiaca (L.)Del.	1.875	3.75	1.875	3.75	1.875	1.875	3.75	3.75	1.875	3.75
Boscia angustifolia Guill. and Perr.	3.75	3.75	3.75	7.5	3.75	7.5	3.75	3.75	3.75	7.5
Cissus rotundifolia Forsk. Vahl.	1.875	3.75	3.75	7.5	3.75	7.5	3.75	3.75	1.875	3.75
Cissus quadrangularis L.	3.75	7.5	3.75	7.5	3.75	3.75	3.75	3.75	3.75	3.75
Clerodendrum myriacoides (Hochst.) Vatke subsp. *napperae* Verdc	1.875	1.875	1.875	1.875	1.875	1.875	1.875	1.875	1.875	1.875
Commiphora africana (A. Rich) Engl. Var. *persica*	3.75	3.75	1.875	3.75	1.875	1.875	1.875	1.875	1.875	1.875
Cordia monoica Roxb.	3.75	3.75	3.75	7.5	3.75	7.5	3.75	7.5	1.875	3.75
Cefrodoxima +ve Control	1.875	1.875	1.875	1.875	1.875	1.875	1.875	1.875	0.9375	0.9375

Table 4: Phytochemical screening results.

Botanical plant name	Tannins	Saponins	Flavonoids	Terpenoids	Cardiac glycosides	Alkaloids (Wagner's test)
Acacia ethaaica Schweinf.	+	++	++	+++	++	+
Acacia horrida (L.) Willd.	++	++	-	++	++	+++
Acacia nilotica (L.) Del.	+++	++	+++	++	+	++
Acacia nubica Benth.	-	-	+	++	++	-
Acacia senegal (L.) Willd.Var.*persica*	+	++	+	++	+	-
Acacia tortilis (Forssk.) Hayne.	++	+	+	+	++	++
Acokanthera friesiorum Markgr.	++	-	++	-	+	-
Albizia anthelmitica Brongn.	+	++	+	-	-	++
Aloe secundiflora Engl.	+	-	++	++	-	-
Balanites aegyptiaca (L.) Del.	+++	+	++	+	-	+
Boscia angustifolia Guill. and Perr.	+	-	-	-	-	+
Cissus rotundifolia Forsk. Vahl.	++	+++	-	-	-	++
Cissus quadrangularis L.	++	+	+	+++	+	+
Clerodendrum myriacoides (Hochst.) Vatke subsp. *napperae* Verdc	+++	+	+++	++	+++	++
Commiphora africana (A. Rich) Engl. Var. *persica*	++	+	-	-	+++	+++
Cordia monoica Roxb.	++	+	-	+	-	-

Key: +++ (Most abundant), ++ (Abundant), + (Less abundant) and - (Not present)

plants had good activity against *P. aeruginosa* with some producing very wide mean zones of inhibition like *Acacia nilotica* (L.) Del. (27.66mm), *Clerodendrum myriacoides* (Hochst.)Vatke subsp. *napperae* Verdc. (25.33mm), *Acacia horrida* (L.) Willd. (28.66mm), and *Cordia monoica* Roxb. (36.33mm). *Acacia ethaaica* Schweinf., *Acacia nilotica* (L.) Del., *Acacia horrida* (L.) Willd., *Clerodendrum myriacoides* (Hochst.)Vatke subsp. *napperae* Verdc. and *Acacia tortilis* (Forssk.) Hayne. extracts showed great activity against the test cultures used. For instance *Acacia ethaaica* Schweinf. produced a zone of inhibition of 19mm against *S. aureas, B. subtilis* and *P. aeruginosa* respectively. *Acacia nilotica* (L.) Del., on the other hand produced average zones of inhibitions of 20.33mm, 21mm, 21.66mm and 27.66mm against *S. aureas, S. typhi, E. coli* and *P. aeruginosa* respectively. Some of these extracts like *Acacia horrida* (L.) Willd. (28.66mm), *Cordia monoica* Roxb. (36.33mm), *Clerodendrum myriacoides* (Hochst.)Vatke subsp. *napperae* Verdc. (25.33mm) e.t.c had wider zones of inhibition than the positive control (Amoxicillin) against *P. aeruginosa*. The means of the zones of inhibition of the test cultures were significantly different at P≤ 0.01 except in *E. coli* and *B. subtilis* that showed no significant difference even at P≤ 0.05. Nevertheless more activity of the extracts was observed in the Gram negatives than the Gram positives bacteria.

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

Most of the extracts produced good MICs and MBCs as shown in Table 3. *Acacia ethaaica* Schweinf., *Acacia nilotica* (L.) Del., *Acacia horrida* (L.) Willd., and *Acacia tortilis* (Forssk.) Hayne. showed great activity producing MICs and MBCs ranging from 0.9375 mg/50µl to 1.875 mg/50µl in the test cultures. Some had active concentrations like *Acacia ethaaica* Schweinf. with MICs of 0.9375 mg/50µl against *S. aureas, B. subtillis, E.coli* and *P. aeruginosa* which are lower than that of the positive control (Amoxicillin MIC 1.875 mg/50µl) in the same test cultures except for *P. aeruginosa* where amoxicillin produced an MIC of 0.9375 mg/50µl. Incidentally some of the extracts such as *Cissus quadrangularis* L., Acacia *nubica* Benth., *Acacia senegal* (L.) Willd.Var. *Persica* e.t.c showed lower MBCs against *S. aureas* (7.5 mg/50µl) than the MBCs produced by the positive control (1.875 mg/50 µl).

Phytochemical screening results

Various phytochemicals were found to be present in the medicinal plants used by the Samburu community as summarized in Table 4. The tested phytochemicals were tannins, saponins, flavonoids, terpenoids, cardiac glycosides and alkaloids. Tannins were found to be the most common phytochemical in the extracts screened especially in *A. nilotica* (L.) Del., *B. aegyptiaca* (L.)Del., and *C. myriacoides* (Hochst.) Vatke subsp. *napperae* Verdc. Saponins and flavonoids were also found in a number of the extracts such as *A. ethaaica* Schweinf., *A. horrida* (L.) Willd., and *C. rotundifolia* Forsk. Vahl. compared to cardiac glycosides and terpenoids. *A. nilotica* (L.) Del., *A. ethaaica* Schweinf., *C. quadrangularis* L., *A. tortilis* (Forssk.) Hayne.and *C. myriacoides* (Hochst.) Vatke subsp. *napperae* Verdc. were found to possess at least all the screened phytochemicals.

DISCUSSION

Sixteen plants used for the treatment of diarrhoeal diseases were identified after carrying out the survey. It was clear that the community uses mostly the bark of these medicinal plants. Nevertheless roots and leaves are also used. This explains why most of the medicinal plants were becoming scarce due to non-sustainable harvesting of the bark which has been reported to accelerate the death of a tree (21). This means that the community should be encouraged to adopt sustainable harvesting methods and traditional healers domesticating some of these plants, an idea that is becoming very popular in other regions of the world (22).

Disc diffusion was carried out to ascertain whether the plant extracts had activity against the test cultures. Some extracts like *Acacia nilotica, Acacia horrida, Acacia ethaaica, Cordia monoica, Acacia nubica* e.t.c showed wide zones of inhibition among the test cultures. But generally, most extracts had substantial inhibitions against the test cultures, although *Boscia angustifolia* showed low inhibition zones against the Gram positive test cultures- *S. aureas* (07mm) and *B. subtilis* (08mm). Our findings of less antibacterial activity by the methanol extract of *Boscia angustifolia* are contradicted by what is reported (38) where the extract produced zones of inhibition of 13mm for *S. aureas, P. aeruginosa* (11mm), *E.coli* (14mm) and *S. typhi* (21mm). Some extracts had higher zones of inhibition than that of the positive control.

For example *Cordia monoica* had a zone of inhibition of 36mm and Amoxicillin had a zone of inhibition of 17.58mm against *P. aeruginosa* which is one of the most difficult microorganism to be managed by many antibiotics due to the nature of its cell wall (23). It is possible to theorize that *Cordia monoica* extracts have

a higher diffusion rate or the degree of sensitivity of the tested microorganisms to the extract is higher as compared to that of the positive control. Perhaps *Cordia monoica* could have compounds that can be used to control diseases caused by *P. aeruginosa*. On the other hand plant extracts could be host specific in their antibacterial activity since zones of inhibition varied for each test culture. The different rates of inhibition could be due to the molecular size of the phytochemical compounds present in the extracts. For instance Catechins, the most reduced form of the C_3 unit in flavonoid compounds has been found to inhibit *V. cholerae* O1 *in vitro* (24), *Streptococcus mutans, Shigella*, and other microorganisms (25).

MIC and MBC results show that the extracts had substantial inhibitory concentrations against the test cultures (Table 3). *Acacia ethaaica* particularly produced the lowest inhibitory concentration in all the test cultures (0.9375 mg/50µl) a concentration that was lower than that of the positive control (Cefrodoxima). Other extracts like *Acacia nilotica, Acacia ethaaica, Acacia tortilis, Clerodendrum myriacoides,* and *Commiphora africana* produced low inhibitory concentrations that were between 0.9375 mg/50µl and 1.875 mg/50µl against most of the test cultures. The results also appear to confirm the antibacterial potential of the plants investigated and their usefulness in treatment of diarrhoea. But for an antibiotic to be effective, killing of the infective agent must be achieved at the site of the infection. The killing will be influenced by the route of administration, the dose, frequency of administration, rate of absorption and distribution of the antibiotic. The above named plants had a lot of tannins and alkaloids which are known to be cytotoxic to bacterial cells and that could explain the high killing rate observed (26).

Phytochemical screening revealed that the extracts had a good number of the most active phytochemicals such as tannins, saponins, flavonoids, and terpenoids. These can be a good source of bioactive principles with antimicrobial potency. Tannins were the most abundant. Previous work (27) shows that *A. tortilis* possess saponins, glycosides, tannins, alkaloids and flavonoids. Further work on *A. tortilis* revealed that the extract was active against *B. subtilis*, (NCTC8236) *E. coli*, (ATCC 9637), *S. aureus* (ATCC 13709) and *P. aeruginosa* (ATCC 27853); findings that are in contrast with this study. The phytochemicals found in *A. tortilis* are known to possess some antimicrobial activities (26, 28). For instance tannins can be toxic to filamentous fungi, yeasts, and bacteria. Condensed tannins bind cell walls of ruminal bacteria, preventing growth and protease activity (26). Alkaloids have also been known to have lead molecules of therapeutic importance. They possess heterocyclic indole compounds which have proved to be having pharmacological properties such as hypotensive activity, anticonvulsant activity, antiprotozoal, antidiarroheal and antimalarial activities (29).

Thus presence of different phytochemicals in different plants screened can be the reason why the community uses more than one plant to make a concoction for the treatment of a given disease. This is because different phytochemicals from the different plants are combined and tend to have some additive or synergistic activity against the pathogens that cause the diseases. It is not surprising that there are differences in the antimicrobial effects of plant species, due to the phytochemical properties and differences among the species. It is also possible that the active chemical constituents were not soluble in methanol or the drying process could have caused disintegration reactions that lead to production of other non-active chemicals (20).

In general, more activity was observed in the Gram negatives, with the highest activity observed in *P. aeruginosa*. This was a good finding as *P. aeruginosa* is known to be difficult to manage by commonly used antibiotics because of the cell wall properties (23). Among the Gram positive test cultures higher activity was observed in *S. aureas*. This may not be significant as *S. aureas* is affected by most compounds thus further screening of the extracts should be done on the Methicillin Resistant or Multi-drug Resistant (MDR) *Staphylococcus aureas*. However, since food intoxication is caused by *S. aureas* this finding is still valid. *Acacia nilotica, Acacia horrida, Acacia ethaaica,* and *Cordia monoica* are among the most active extracts that produced significant activity against all the test cultures. The activity of these extracts can be ascribed to the presence of such active phytochemicals as tannins, saponins, flavonoids, terpenoids, alkaloids, and cardiac glycosides that have been known to have antibacterial properties (28, 30, and 31). The ability of these extracts to be sensitive to both Gram positive and Gram negative bacteria makes them good candidates for the isolation of broad spectrum antimicrobials. The mode of action is not clearly understood, and the active principles not isolated. These issues will be reported when determined at a later date.

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REFERENCES

1. R. W. Bussmann. Ethnobotany of the Samburu of Mt. Nyiru, South Turkana, Kenya. *J. Ethnobiol. Ethnomed.* **2**: 35 (2006).
2. S.J. Allen, B. Okoko, E. Martinez, G. Gregorio and L.F. Dans. Probiotics for treating infectious diarrhoea. *Cochrane Database Syst. Rev. Issue* **4**: I-10 (2003).
3. N. Thapar and I.R. Sanderson. Diarrhoea in children: an interface between developing and developed countries. *Lancet.* **363**: 641– 653 (2004).
4. A. Palombo. Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: Modes of action and effects on intestinal function. *Phytother. Res.* **20:** 717-724 (2006).
5. B. Olesen, J. Neimann and B. Bottiger. Etiology of diarrhea in young children in Denmark: a case-control study. *J. Clin. Microbiol*; **43:**3136–3641(2005).
6. J. Bryce, C. Boschi-Pinto and K. Shibuya. The WHO Child Health Epidemiology Reference Group. WHO estimates of the causes of death in children. *Lancet*. **365:**1147–1152 (2005).
7. M. Kosek, C. Bern and R.L Guerrant. The global burden of Diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bull World Health Org*; **81**: 197-204 (2003).
8. C. Trinh and K. Prabhakar, K. Diarrhoeal diseases in the Elderly. *Clinics in Gastric medicine*.**23 (4)**:833-856 (2007).
9. S.F. Altekruse, D.A. Street, S.B. Fein and A.S. Levy. Consumer knowledge of food borne microbial hazards and food handling practices. *J. Food Prot.* **59**:287-294 (1995).
10. W.M. Kone, K.K. Atindehou, C.Terrwux, K. Hostettmann, D. Traore and M. Dosso. Traditional medicine in North Côte-d' Ivoire: screening of 50 medicinal plants for antibacterial activity. *J. Ethnopharmacol*. **93**: 13–49(2004).
11. A.Gassama, P.S. Sow, F. Fall, P. Camara, A. Gueye-N'diaye, R. Seng, B. Samb and S. M'Boup, A. Aidara-Kane. Ordinary and opportunistic enteropathogens associated with diarrhoea in Senegalese adults in relation to Human Immunodeficiency Virus serostatus. *International Journal of Infectious Diseases* **5**: 192–198 (2001).
12. A.V. Krishnaraju, T.V.N. Rao and D. Sundararaju. Assessment of bioactivity of Indian medicinal plants using Brine shrimp (*Artemia salina*) lethality assay. *Int J. Appl. Sci. Eng.* **2**: 125-134 (2005).
13. S. Voravuthikunchai, A. Lortheeranuwat, W. Jeeju, T. Sririrak, S. Phongpaichit and T. Supawita. Effective medicinal plants against Enterohaemorrhagic *Escherichia coli* O157: H7. *Journal of Ethnopharmacology*.**94**:49–54(2004).
14. S.A. Jassim and M.A. Naji. Novel antiviral agents: a medicinal plant perspective. *J. Appl. Microbiol.* **95**: 412–427 (2003).
15. National Committee for Clinical Laboratory Standards (NCCLS).Performance standards for antimicrobial susceptibility testing. Twelfth Informational Supplement M100–S12 NCCLS. Wayne (2002).
16. S. Paulo. Antimicrobial activity of *Syzygium cumini* (Myrtaceae) leaves extract. *Braz. J. Microbiol.* **38**: 2 (2007).
17. J.M. Andrews. Determination of minimum inhibitory concentrations. *J. Antimicrob. Chemother*. **48**: 5–16 (2001).
18. C. E. Evans, O. Banso and S. Adeyemo. Efficacy of some nupe medicinal plants against *Salmonella typhi*: an *in vitro* study. *J. Ethnopharm.* .**80:** 21–24 (2002).
19. H.O. Edeoga, D. E. Owu and B.O. Mbaebie. Phytochemical constituents of some Nigerian medicinal plants. *Afri. J. Biotechn*. **4 (7):** 685-688 (2005).
20. P. Jigna and V. C. Sumitra. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turk. J. Biol*. **31**:53-58 (2007).
21. O.M. Grace, H.D.V. Prendergast, J. Van Staden, and A.K. Jager. The status of bark in African traditional health care. South African Journal of Botany. **68:** 21–30 (2002).
22. C.P. Kala. Status and conservation of rare and endangered medicinal plants in the Indian trans-Himalya. Biological Conservation **93:** 371–379 (2000).
23. P.G. Higgins, A.C. Fluit, D. Milatovic, J. Verhoef and F.J. Schmitz. Antimicrobial susceptibility of imipenem-resistant *Pseudomonas aeruginosa. J. Antimicrob. Chemother.* **50:** 299-301 (2002).
24. R.P**.** Borris. Natural products research: perspectives from a major pharmaceutical company. *J. Ethnopharmacol.* **51:**29-38 (1996).
25. S. Sakanaka, N. Shimura, M. Aizawa, M. Kim, and T. Yamamoto. Preventive effect of green tea polyphenols against dental caries in conventional rats. *Biosci Biotechnol. Biochem.* **56**:592–594 (1992).
26. G. A. Jones, T. A. McAllister, A. D. Muir, and K. J. Cheng. Effects of sainfoin (*Onobrychis viciifolia* Scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. *Appl. Environ. Microbiol*. **60:**1374-1378(1994).
27. P. Rani and N. Khullar. Antimicrobial Evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant S*almonella typhi. Phytother. Res.* **18:** 670–673 (2004).
28. M.M. Nakano and P. Zuber. "Anaerobic growth of a "strict aerobe" (*Bacillus subtilis*)". *Annu. Rev. Microbiol.* **52**: 165-90 (1998).
29. P.B. Mallikharjuna, L.N. Rajanna, Y.N. Seetharam and G.K. Sharanabasappa. Phytochemical Studies of *Strychnos potatorum* L.f.- A Medicinal Plant. *Journal of Chemistry*. **4 (4):** 510-518, (2007).

30. C.J. Pretorius and E. Watt. Purification and identification of active components of *Carpobrotus edulis* L. *J. Ethnopharmacol*. **76**: 87-91 (2001).

31. K.N. C. Murthy, Vanitha, M.M. Swamy and G.A. Ravishankar. Antioxidant and antimicrobial activity of *Cissus quadrangularis* L. *Journal of Medicinal Food*; **6 (2):** 1-7 (2003).

