



## Anti-Bacterial Analysis of Some Herbal Medicines in Mbale, Eastern Uganda

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### Authors' contributions

This work was carried out in collaboration among all authors. Author HAA designed the study, performed the statistical analysis, wrote the protocol, carried out the laboratory analysis and wrote the first draft of the manuscript. Authors AMD and KA managed the analyses of the study, literature searches and introduction. All authors read and approved the final manuscript.

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### ABSTRACT

This study was aimed at investigating the anti-bacterial activity of some on shelf herbal medicines for treatment of Cholera and Typhoid in markets. The sample was collected from shops that sell herbal medicines using random sampling to investigate the effectiveness of these herbal medicines on *Escherichia coli* and *Salmonella enterica* serotype *typhi*. It was noticed that all the four herbal medicines that were subjected to antibacterial activities using *E. coli* and *S. typhi*, showed effect on the bacteria. Diameters of zones of inhibition was measured, which showed that zone of inhibition varies among the on shelf herbal medicines. Serial dilution was also carried out to check for the effect of concentration. Herbal Medicine for Typhoid 1 showed the highest zone of inhibition 14.36 mm and the lowest zone of inhibition was Herbal Medicine for Cholera 1 with 10.17mm on *S. Typhi*. While for *E. coli*, the highest zone of inhibition was 12.83mm shown by Herbal Medicine for Cholera 2 and the lowest was 10.25mm shown by Herbal Medicine for Typhoid 1.

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**Keywords:** Herbal medicine; antibacterial screening; *Salmonella enterica* Serotype Typhi; *Escherichia coli* HBT (herbal Medicine for typhoid); HBC (Herbal Medicine for Cholera); Mbale.

## 1. INTRODUCTION

Herbal medicines are defined as plant-derived material or preparations perceived to have therapeutic benefits, often contain raw or semi processed ingredients from one or more plants [1].

The populations of developing countries worldwide continue to rely heavily on the use of traditional medicines as their primary source of healthcare [2]. Ethnobotanical studies carried out throughout Africa confirm that native plants are the main constituent of traditional African medicines [3]. Furthermore, there is increasing reliance on the use of medicinal plants for traditionally used rural herbal remedies [4]. It is documented in a report by the WHO [5] that in some Asian and African countries, 80% of the population use plant-derived medicines for their primary healthcare. In countries for which more detailed data are available, the percentage of the population that uses traditional medicine ranges from 90% in Burundi and Ethiopia, to 80% in Burkina Faso, the Democratic Republic of Congo and South Africa; 70% in Benin, Cote d'Ivoire, Ghana, Mali, Rwanda and Sudan; and 60% in Tanzania and Uganda WHO [5]. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries, particularly Africa [6].

In Uganda, most people still rely on herbal medicines because they are cheaper compared to conventional drugs, the people using these herbal medicines take them without having any scientific proof that they cure those diseases.

Many locals in Mbale district are using medicinal plants to treat coughs and diarrhea [7]. However some of the therapeutic properties attributed to plants have proven to be wrong, such plants should be investigated scientifically to better understand their properties, and efficacy [8,9]. The research is aimed at using anti-bacterial screening to test the effectiveness of on shelf herbal medicine for treatment of typhoid and cholera in Mbale Municipality.

## 2 MATERIALS AND METHODS

### 2.1 Preparation of Medium

The antibacterial activity was carried out in the microbiology Laboratory at the Department of

Biological Sciences, Makerere University. the instructions from the manufacturer, 38 g Mueller-Hinton agar (Sigma-Aldrich Inc.) powder was mixed to one liter of distilled water and then boiled while continuously agitating the mixture purposely to enable agar powder component to melt and homogenize into the medium. The solution was autoclaved at 121°C at 15 psi for 15 minutes and cooled to 50°C in a water bath. It was then transferred into sterile Petri dishes of 9 cm diameter. It was allowed to cool and solidify for 24 hrs to ensure proper texture for the well establishment of the wells. It was incubated for 24 hours at 37°C to ensure that there is no microbial contamination.

### 2.2 Preparation of Plant Extract for the Test

A set of six sterile test tubes were prepared for each sample by washing thoroughly and autoclaved at temperature of 120°C for 20 min at pressure of 15bars to ensure sterility. Three mls of aqueous sample were pipetted and serially diluted using distilled water into 6 set of sample tubes [10]. The dilutions were subsequently inoculated and four wells were dug respectively on each of the agar plates using a 10 mm diameter corkborer.

### 2.3 Preparation of Standard Drug

1 gram of ampiclox was weighed and dissolved into 100 ml of sterile distilled water to make stock solution. 1 ml was pipetted from stock solution using sterile micro pipette and diluted to 5 ml using sterile distilled water and it was inoculated on to the agar-wells.

### 2.4 Inoculation of the Plates for Sensitivity Determination

The adopted experimental procedure was that proposed by Eloff [8,9] but with slight modification. Ten well isolated colonies of pure cultures of both *S. Typhi* (Fig. 1) and *E. coli* (Fig. 2) were obtained from the freezer at the Department of Medical Microbiology, College of Health Sciences, Makerere University and incubated for 18 hours. The isolates were sub cultured to obtain pure isolated colonies, which were used for the assays. The bacteria were harvested from culture agar-plates using

platinum wire-loop which had been sterilized by burning it red-hot on spirit lamp. Turbid suspension of 5 ml broth of the respective pathogens were harvested and incubated for 4hrs in the incubator at 37°C. Two ml of the bacterial broth were pipetted on the surface of the set Mueller Hilton agar that had been divided into four quadrants and bacterial culture evenly distributed on the surface of the medium by gently swirling the agar-plates and excess of the suspension were pipetted off the plates.

## 2.5 Application of the Test Drugs

After about 15 min of inoculation, using 10mm diameter corkborer, four uniformly cut wells of 10 mm Diameter wells were drilled in each of the quadrant in the agar-plates. The quadrants were labeled as plant extract (E), standard drug (A), solvent used in preparing solution (O) and blank or control (B) respectively after which they were filled with 0.5ml of the respective test dilutions as indicated below (Fig. 3). This was done in



Fig. 1. Colonies of *Salmonella Typhi*

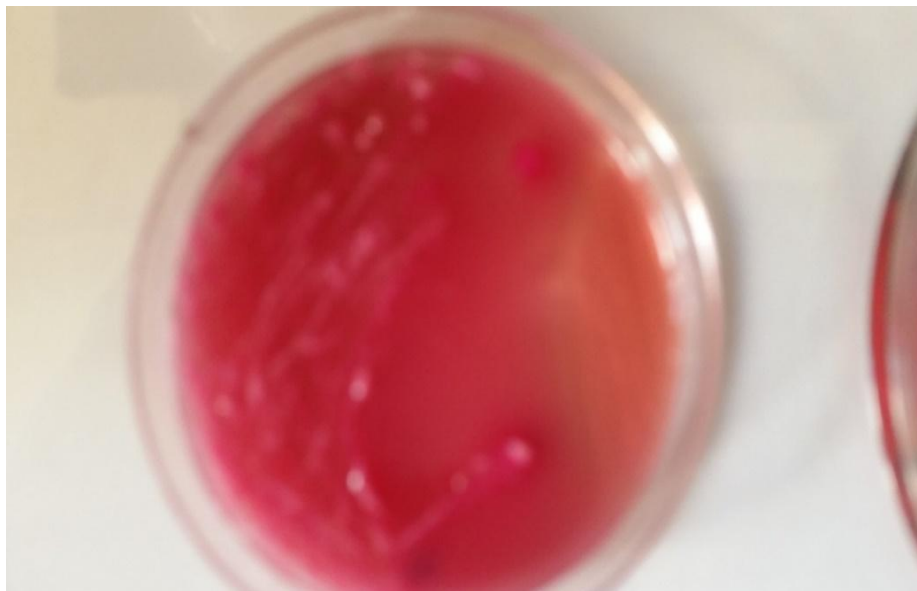
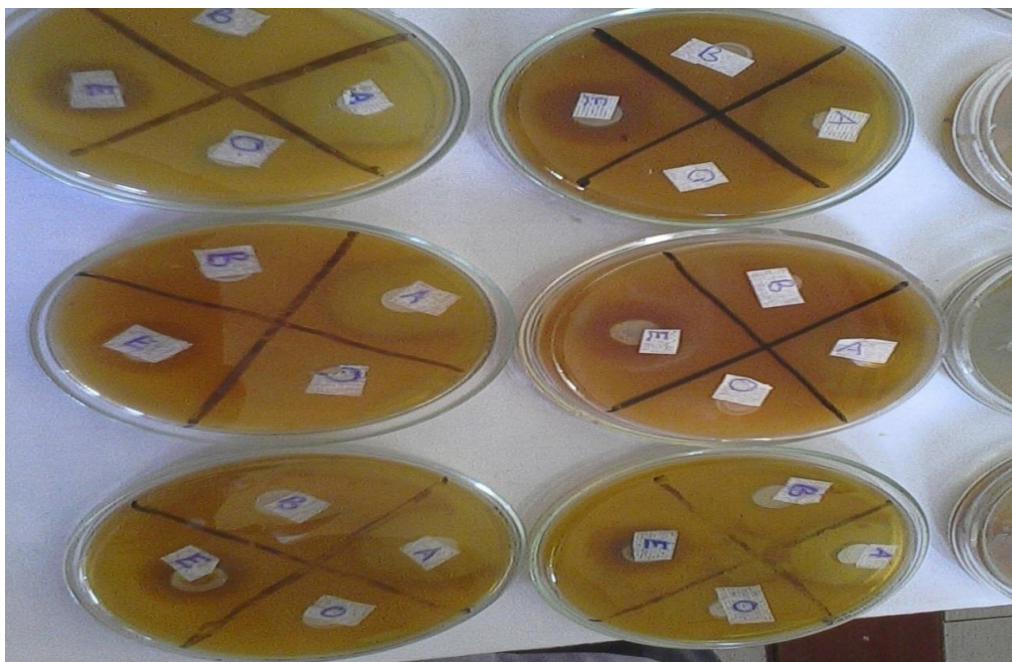


Fig. 2. Colonies of *Escherichia Coli*



**Fig. 3. Zones of inhibition**

triplicates for each dilution of the sample. The plates were then incubated in an upright position for 12 hours to allow diffusion of the test solutions into the wells. The plates were then turned upside down to allow aerobic incubation for 24 hours. The sensitivity of the test organisms to the extracts was determined by measuring the diameters of the zone of inhibition surrounding the wells with a metric ruler. A zone devoid of growth around the well indicated the capacity of the plant extract to inhibit growth. Average of each of the three plates was recorded [10].

### 3. RESULTS AND DISCUSSION

#### 3.1 Antibacterial Activity

Results of antibacterial activity of the selected products presented in Table 1. Shows the zone of inhibition of the antibiotic and herbal medicine on both *S. Typhi* and *E. coli* respectively. The readings were written following six serial dilutions to check the effect of concentration on the bacteria. The minimum inhibition concentration is the lowest concentration of the compound or extract that completely inhibits visible growth of the microorganism [10].

These samples were subjected to antibacterial activity using *S. Typhi* and *E. coli* to test their

effectiveness. It was noticed that all the four herbal medicine that were subjected to antimicrobial activities on the test organisms (*E. coli* and *Salmonella Typhi*), showed activity on the test organism which was similar to the findings of Periannan and Prabakaran, [11], Ali, [12], Biswas et al., [13], Even though the zone of inhibition varies among the samples. This proved that the herbal medicines are effective for treatment of cholera and typhoid. HBT1 showed the highest zone of inhibition 14.36mm and the lowest zone of inhibition was HBC1 with 10.17mm on *Salmonella Typhi*. While for *E. coli*, the highest zone of inhibition was 12.83mm shown by HBC2 and the lowest was 10.25mm shown by HBT1. The variation in zone of inhibition shows that the herbal medicines sold in some of the shops are more effective than others. It also proved that herbalists are using different plants when making this herbal medicines.

It was observed that the antibiotic has more zone of inhibition than the corresponding herbal medicine. This shows that the antibiotic (Amphiclox) used is more effective in treating cholera and typhoid than the on shelf herbal medicines that are used for treatment of cholera and typhoid in Mbale.

**Table 1. Test for zone of inhibition on *E. coli* and *Salmonella Typhi***

Sample	Dilution (mm)	<i>E. coli</i>		<i>S. Typhi</i>	
		Antibiotic (mm)	Herb (mm)	Antibiotic (mm)	Herb (mm)
HBT1	1	20.38	10.25	33.51	14.36
	2	20.00	10.10	31.98	13.37
	3	19.88	10.05	34.57	12.04
	4	18.50	10.00	38.65	10.01
	5	19.67	10.00	40.02	10.00
	6	19.70	10.00	40.05	10.00
HBC1	1	19.75	12.75	40.43	13.76
	2	20.17	12.50	40.15	10.17
	3	20.05	10.00	38.87	10.00
	4	19.83	10.00	37.15	10.00
	5	21.50	10.00	40.60	10.00
	6	18.67	10.00	42.05	10.00
HBT2	1	20.67	12.67	40.77	10.47
	2	16.17	10.73	32.15	10.20
	3	19.50	10.00	31.07	10.00
	4	18.00	10.00	39.25	10.00
	5	21.50	10.00	41.83	10.00
	6	20.50	10.00	38.90	10.00
HBC2	1	21.17	12.83	30.58	10.48
	2	18.33	11.17	36.82	10.33
	3	19.00	10.50	37.75	10.00
	4	21.33	10.00	45.32	10.00
	5	21.00	10.00	40.38	10.00
	6	20.83	10.00	42.17	10.00

Serial dilution was carried out using the samples to find out at which concentration the samples stop being active on the bacteria using the procedure described by Starke, [14] and Yamauchi et al., [15]. On *S. Typhi*, HBT1 showed no activity after the fourth dilution. HBC1, HBT2 and HBC2 showed no activity after the second dilution respectively. On *E. coli*, HBT1 showed no activity after the third dilution. HBC1 and HBT2 showed no activity after the second dilution, While HBC2 showed no activity after the third dilution which are shown on Table 1. This shows that beyond certain concentration, since most on shelf herbal medicines are administered in liquid form, even if they are to be taken for eternity, they will do no good in curing typhoid or cholera.

#### 4. CONCLUSION

Results of antibacterial activity of on shelf herbal medicine for treatment of typhoid and cholera from various shops in Mbale Municipality showed zone of inhibition on both *S. Typhi* and *E. coli* respectively.

The findings proved that herbal medicines used in treating typhoid and cholera in Mbale are

effective. It also proved that the antibiotic (Amphiclox) is more effective than the herbal medicines used in treating cholera and typhoid in Mbale.

The findings also proved that the herbal medicines are only effective at certain concentrations, beyond which they stop having effect on the bacteria. Which means beyond certain concentrations, the on shelf herbal medicines will not cure typhoid and or cholera. This factor is important to understand because most of the herbal medicines are taken in liquid form.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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