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Antibacterial activity of five medicinal plants against *Salmonella typhi*

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Abstract
Typhoid remains one of the challenging waterborne diseases in Africa, especially in Kenya due to lack of clean drinking water in rural and slum areas. This study involved evaluation of herbal medicine for typhoid treatment using plants used by herbalists in Embu and Mbeere regions of Kenya The evaluation was done by carrying out bioassay studies to determine the activity of botanical microbial against disease causing agents using the Disk Diffusion method. The aqueous extracts were tested for their inhibitory activity against one selected strain of *Salmonella typhi* bacteria. All the selected plant extracts investigated exhibited activity against *Salmonella typhi* with inhibition zone diameters ranging from 4.4 – 18.5 mm. *Aloe secundiflora* gave the highest inhibition zones against *S. typhi* followed by *Vernonia brachycalyx* and *Terminalia brownii*. It was confirmed that *Carrisa edulis, Aloe secundiflora and Tithonia diversifolia* were also active against *Salmonella typhi*. These findings provided a scientific basis for the use of the tested herbal plant extracts in the treatment of typhoid and other bacterial diseases by herbalists in Eastern Kenya.

Keywords: *Salmonella typhi*, Typhoid, *Vernonia brachycalyx*, *Aloe secundiflora*, *Tithonia diversifolia*

1. Introduction
Typhoid fever is a common worldwide bacterial disease transmitted by the ingestion of food or water contaminated with the feces of an infected person, which contain the bacterium *Salmonella enterica subsp. enterica*, *Serovar typhi* [3].

The causative agent, *Salmonella enterica typhi* referred to as *Salmonella typhi*, is an obligate parasite that has no known natural reservoir outside of humans. This disease is characterized by the sudden onset of a sustained and systemic fever, severe headache, nausea, and loss of appetite. Other symptoms include constipation or diarrhea, enlargement of the spleen, possible development of meningitis, and/or general malaise. Untreated typhoid fever cases result in mortality rates ranging from 12-30% while treated cases allow for 99% survival. Worldwide, typhoid fever affects roughly 17 million people annually, causing nearly 600,000 deaths [4].

Salmonella typhi is a multi-organ pathogen that inhabits the lympathic tissues of the small intestine, liver, spleen, and bloodstream of infected humans. It is not known to infect animals and is most common in developing countries with poor sanitary systems and lack of antibiotics.

Salmonella is found worldwide in both cold-blooded and warm-blooded animals, and in the environment. They cause illnesses such as typhoid fever, paratyphoid fever, and food poisoning [5].

Transmission of *S. typhi* has only been shown to occur by fecal-oral route, often from asymptomatic individuals. 2-5% of previously infected individuals become chronic carriers who show no signs of disease, but actively shed viable organisms capable of infecting others. These highly infectious carriers pose a great risk to public health due to their lack of disease-related symptoms.

The temperature rises slowly, fever fluctuations, headache, and cough. A bloody nose is seen in a quarter of cases, and abdominal pain is also possible. There is a decrease in the number of circulating white blood; blood cultures are positive for *Salmonella typhi* or *paratyphi*. Also high fever in plateau around 40 °C (104 °F) develops. Diarrhea and constipation can also occur **[6]**.

Sanitation and hygiene are the critical measures that can be taken to prevent typhoid. Transmission is from human to human. Typhoid can only spread in environments where human feces or urine are able to come into contact with food or drinking water. Careful food preparation and washing of hands are crucial to prevent typhoid. Also vaccines are available especially for travelers to areas where typhoid is endemic **[7]**.

Treatment of the disease with antibiotics reduces the case-fatality rate to approximately 1%. When untreated, typhoid fever persists for three weeks to a month. Death occurs in between 10% and 30% of untreated cases. In some communities, however, case-fatality rates may reach as high as 47% **[8]**.

The damage caused by typhoid fever is reversible and limited if treatment is started early in the infection. Some herbal plants used to treat typhoid in Eastern Kenya were studied and presented according to Kareru **[9]**.

2. Materials and method
2.1. Collection of plant material
From the ethanobotanical information obtained, five plants parts were collected, namely: *Terminalia brownii, Vernonia brachycalyx, Carrisa edulis, Aloe secundiflora* and *Tithonia diversifolia*. The sample parts were collected in sterile polyethylene bags and later dried under shade. The dried plant

materials (Table 1) were powdered and extracted using solvents of increasing polarity, that is, Hexane, Ethyl acetate, Ethanol and aqua, using soxhlet apparatus method. All the extracts were concentrated and spray dried to obtain a powders. These extracts were used for the in-vitro antimicrobial assays.

Table 1: Information of Medicinal plants used by herbalists

S. No.	Name of plant	Plant species	Part used	Disease treated
1	Mururuku	*Terminalia brownii*	Bark	Pneumonia
2	Mugirimura	*Vernonia brachycalyx*	Stem and leaves	Candidiasis
3	Mukawa	*Carrisa edulis*	Stem and leaves	Typhoid, Pneumonia
4	Githunju	*Aloe secundiflora*	Leaves	Pneumonia, Typhoid and Candidiasis
5	Kirurite	*Tithonia diversifolia.*	Leaves	Typhoid

2.2. Test organisms

The bacterial isolate *Salmonella typhi*, a clinical isolate, was obtained from KEMRI Nairobi. The anti-biotic agents (powder); gentamicin and erythromycin were purchased from Kobian Kenya Limited.

2.3. sensitivity testing using disk diffusion method

The antimicrobial screening was carried out using the disk diffusion method. The bacterium was grown in Nutrient Agar using *Salmonella typhi* as bacterial isolate. 28 g/L of Nutrient agar was dissolved and sterilized at 121 °C in an autoclave. The test microorganisms were inoculated into petri dishes of nutrient broth separately and incubated at 37 °C for 24 hours. The media was left to cool to around 40 °C and then adjusted to 0.5 McFarland turbidity. It was allowed to dry for 10 minutes. Paper disks were diffused into each of the sample extracts and dried at 40 °C. The disks were pressed gently onto the seeded agar plates with the tips of sterile forceps. Antimicrobials gentamicin and erythromycin were served as negative controls. The plates were incubated at 37 °C for 24 hours after which antimicrobial activity was determined by measurement of diameter zones of inhibition (Mm) (against the test organisms) around each of the extracts and the standard antibiotics [10].

3. Results and Discussion

The inhibition zone diameters for extracts were reported in Figure 1, and commercial antibiotics were used as controls, and their sensitivities towards the bacterial strain versus plant extracts reported in Figure 2.

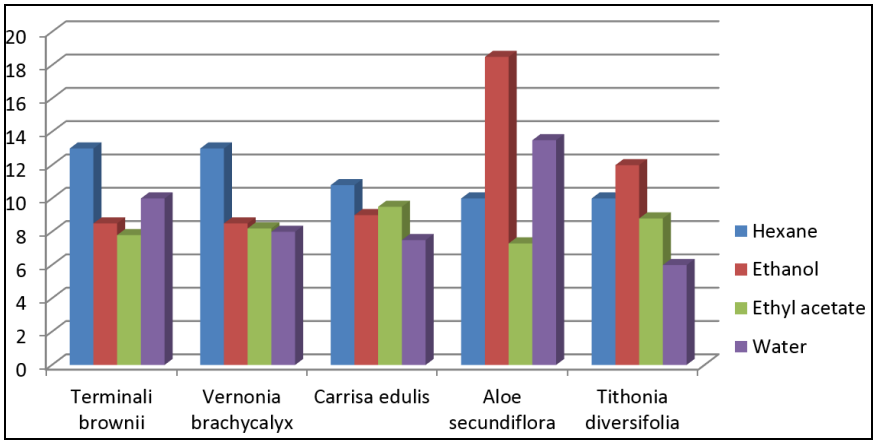

Fig 1: Inhibition zone diameters of plant extracts (mm)* against *Salmonella typhi*

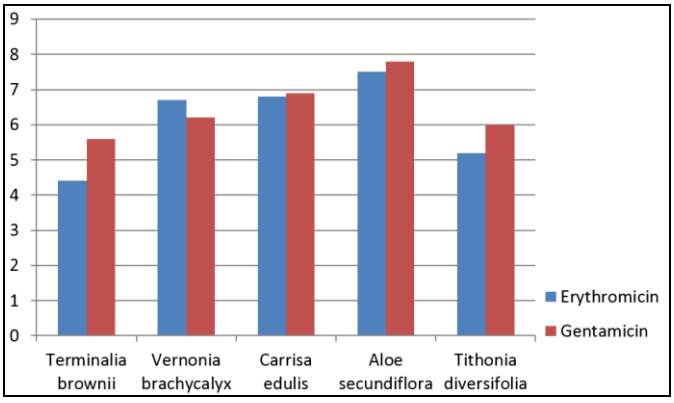

Fig 2: Inhibition zone diameters of plant extracts versus positive controls (mm)* against *Salmonella typhi*

The positive controls (erythromycin and gentamicin) showed higher inhibition values compared to the negative control (yeast). According to Bonferroni mean comparisons for activity, the different extracting solvents showed significant variations shown in Figure 3 to Figure 6 below. The deviation could be due to different extraction conditions and the type of herb. Environmental factors such as climate and soil condition could also have played a role in variation of activity. [11]

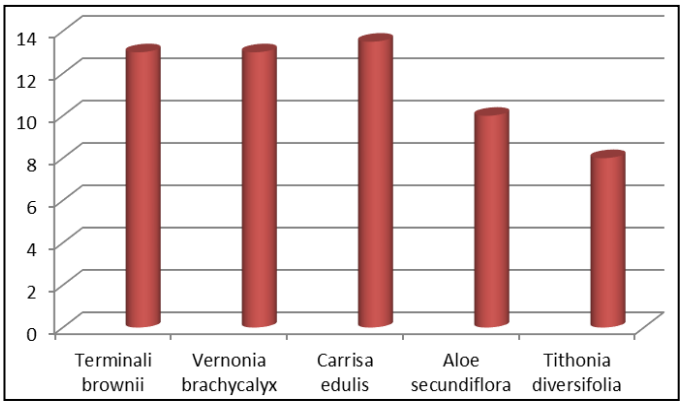

Fig 3: Activity of Hexane extracts against *Salmonella typhi*

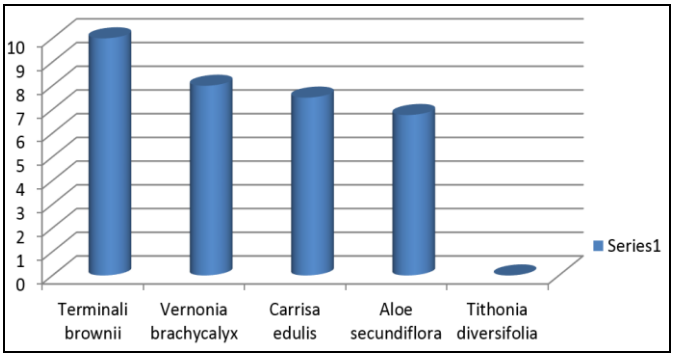

Fig 4: Activity of Water extracts against *Salmonella typhi*

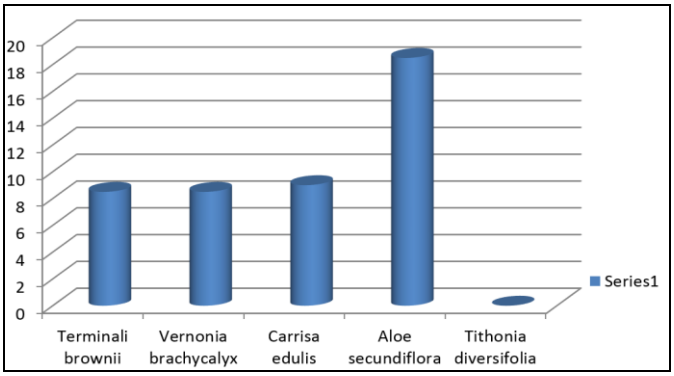

Fig 5: Activity of Ethanol extracts against *Salmonella typhi*

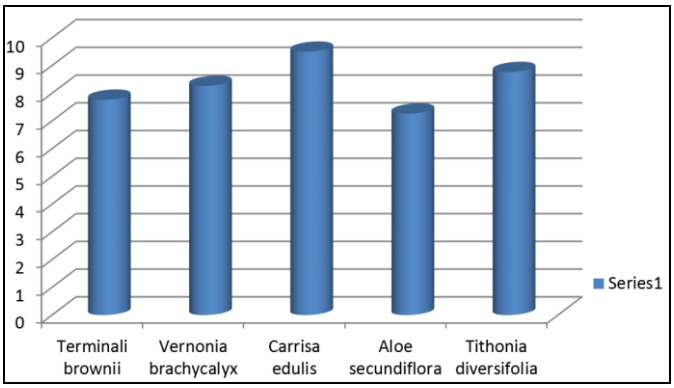

Fig 6: Activity of Ethyl acetate extracts against *Salmonella typhi*

The most active solvent extract for *Aloe secundiflora* was ethanol and the second was water extract of *Carrisa edulis* against *Salmonella typhi.* For ethanol and water extracts of *Tithonia diversifolia* very little activity was shown. Extraction has to be done with specific solvent to obtain maximum efficiency of the herbs used for treatment of a specific disease because significant difference was shown among the solvents used.

4. Conclusion

It was concluded that the plants investigated were effective against the microbial strains tested. The best solvent for extraction was ethanol while the most effective plant extract was *Aloe secundiflora*. Therefore from these findings, these plant extracts can be recommended for use by the herbalists for the treatment of bacterial infections against *Salmonella typhi*.

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5.2. Author's Contribution and Competing Interests

There were no competing interests.

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