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In vitro Susceptibility Patterns of Typhoidal and Non-typhoidal *Salmonella* to Selected Anti-typhoid Herbal Medicinal Preparations Sold in the Ga East Municipality of Ghana

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Authors' contributions

This work was carried out in collaboration between all authors. Author EOB conceptualized the study and wrote the first draft of the manuscript. Author EA conducted the HPLC experiments. Author PD managed the literature search and approved the final manuscript. Author AAA performed the market survey and the biological experiments.

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ABSTRACT

In Ghana, majority of the populace use herbal medicines for the treatment of typhoid. Though these herbal medications are widely acclaimed to be effective, there is a lack of validation of their efficacy with little or no information on the susceptibility patterns of the prevalent *Salmonella enterica* serovars to the numerous herbal medicines used for treating typhoid fever on the Ghanaian market. This research therefore investigates 16 anti-typhoidal herbal medicinal preparations for sale in the Ghanaian market and determines their activities against *S. typhi,*

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S. enteriditis, S. paratyphi, S. havana and *S. arizona* in the agar well diffusion assay. This study also screened the preparations for the presence of chloramphenicol by HPLC-UV analysis. Results showed that 38% of the herbal preparations sampled were active against all the five strains of *Salmonella*, 13% against three strains, 19% against 2 strains, 4% against 1 strain and 25% showed no activity against any of the tested strains. We concluded that 70% of the anti-typhoidal preparations were active against 2 to 5 strains of Salmonella and hence might be effective in the treatment of most typhoid infection due to its broad spectrum of activity. No extraneous chloramphenicol was detected to be present in the preparations.

Keywords: Salmonella enterica; herbal medicinal preparations; typhoid fever.

ABBREVIATIONS

NTS: Non-typhoidal Salmonella
TF: Typhoid Fever

1. INTRODUCTION

Typhoid fever (TF) is a systemic infection caused by *Salmonella enterica* serotype typhi (*S. typhi*) while a very similar but often less severe paratyphoid fever is caused by *S. paratyphi* A, B and sometimes C. Typhoid (enteric) fever is a collective term for both typhoid and paratyphoid fevers [1]. There is an estimated one case of paratyphoid fever for every four typhoid [2]. There is a lack of definitive diagnosis of typhoid infection in many Ghanaian patients before treatment, hence the term typhoid fever is widely used for the various types of enteric fevers in Ghana. Although many *Salmonella* serotypes exist, they can be broadly categorized as typhoidal or non-typhoidal *Salmonella* (NTS) depending on the clinical syndrome with which they are predominantly associated. The typhoidal Salmonella include the sub species *enterica* serotypes typhi and paratyphi which typically cause systemic illness with little or no diarrhea. The much larger group of NTS primarily induce acute, self-limiting gastroenteritis [3]. *Salmonella enterica* is a highly diverse Gram negative bacterial species containing more than 2600 different serovars that can be differentiated by their antigenic presentation [3]. Various serovars are characterized by their host specificity or by the clinical syndrome which ranges from asymptomatic carriage to invasive systemic disease. Most *S. enterica* serovars associated with diseases in humans and other warm blooded animals belong to subspecies group I consisting of both typhoidal and non-typhoidal serovars [3]. Examples of NTS serovars are *S. typhimurium* and *S. enteriditis* and that of typhoidal Salmonella are *S. typhi* and *S. paratyphi* [3]. The effect and incidence of TF in many parts of sub-Saharan Africa are largely unknown, mainly because diagnostic laboratories are lacking and fatal typhoid is frequently attributed to malaria [4]. The prevalent Salmonella strains in Ghana include *S. typhi, S. paratyphi, S. enteriditis and S. typhimurium* [4,5]. A study which included 1,456 children <15 years of age showed that, 2.5% isolates were positive for *S. enterica* ser. typhi. The frequency of TF was low among children <2 years of age (0.7%), increased among those 2 to <11 years of age (27.0%), and decreased among children ≥11 years of age (4.6%). The *S. typhi* isolates were resistant to chloramphenicol (73%), trimethoprim/sulfamethoxazole (71%), ampicillin/amoxicillin (70%), tetracycline (64%), gentamicin (46%), and amoxicillin/clavulanic acid (24%) [4]. In another study also conducted at the Korle-Bu teaching Hospital, a tertiary referral and teaching facility in Ghana, *Salmonella* bacteraemia had a prevalence of 6.5%. NTS was observed to have predominated over typhoidal salmonella, being 63% and 36% respectively [5]. Other Salmonellae present were *S. typhimurium* (11.5%) and *S. paratyphi* (9.9%) [5]. Although not much data from Africa is available, it is clear that typhoid is also highly prevalent in Nigeria, Mali, Ethiopia and Kenya [6]. The management of typhoid infections has various challenges which includes a lack of effective diagnosis and development of resistance to well known drugs such as ciprofloxacin, amikacin, cefotaxime, chloramphenicol, trimethoprim/sulfamethoxazole, ampicillin/amoxicillin and tetracycline [5-8]. In Ghana, ciprofloxacin is the treatment of choice for Salmonellosis. Alternatives such as azithromycin and ceftriazone may also be used [9]. Ciprofloxacin is highly active against both aerobic Gram negative and Gram positive bacteria including various species of *Vibrio, Shigella, Salmonella* etc. [10], however majority of patients still patronize herbal medicines for the treatment of typhoid. It is estimated that over 80 % of people in developing countries used herbal medicines for their primary healthcare [11]. As many as 70% of the population of Ghana rely on traditional medicine for their primary healthcare [12] hence there are a wide range of herbal products used in the treatment of typhoid on the Ghanaian market. There is little discrimination however when it comes to its use in the treatment of the various typhoid fevers. There is little or no information on the susceptibility patterns of typhoidal and non-typhoidal Salmonella to these preparations. Given the alarming incidence of antibiotic resistance in bacteria of medical importance [13], which includes the Salmonella species; there is a constant need to identify new and effective therapeutic agents and to ensure that whatever is being used as a remedy is active and effective. There is also some suspicion amongst Ghanaian consumers of herbal products, that some of these products might be lased with orthodox antibiotics. We therefore also screened these preparations for the presence of chloramphenicol, an antibiotic previously recommended for the treatment of typhoid and relatively cheaper than the current recommended therapy but also widely available on the Ghanaian market.

2. MATERIALS AND METHODS

2.1 Drug Collection

Between the periods of January to March, 2016, fifteen Pharmacies and six Herbal Medicines Retail Shops within the Ga East Municipal Area were visited and all herbal medicines indicated for the treatment of typhoid were purchased. Only herbal medicines that had FDA (Food and Drugs Authority of Ghana) registration numbers were bought. Those without FDA registration numbers were however noted. Sampling was stopped when no new anti-typhoid preparations were being discovered.

2.2 Sampling Sites

All the herbal products were collected within Haatso, Dome and Ashongman communities located within the Ga East Municipality of Accra Ghana (5° 44′ 17″ N, 0° 11′ 42″ W5.738056, -0.195). According to the Ghana Statistical Service, 2010 Population and Housing Census on the Ga East Municipality, it is located at the northern part of the Greater Accra Region and covers a land area of about 85.7 square kilometers. The population is almost 148,000. Males constitute 49% and females represent 51%. It has 40.3% of the population below 20 years. The population density of the Ga Municipal area stands at 1,725 persons per square kilometer. Households in the Municipal area are more of extended family (56.2%) than nuclear family (43.8%). Almost 97.5% of the population in the Municipal area is Ghanaians. Nearly 60% are literate. Of the employed population, 35.1% are engaged as service and sales workers while 22.6% are craft workers and traders [14].

2.3 Extract Preparation

Volumes of 200 – 400 mL of the herbal medicinal preparations were measured and concentrated *in vacuo* at 40°C. The concentrated extracts were then lyophilized to obtain dried extracts. The extracts were then dissolved in sterile distilled water to a concentration 50 mg/mL and sterile filtered through a sterile syringe filter (Corning Incorporated, NY, USA). The sterile extract was then diluted with sterile distilled water to obtain concentrations of 25, 12.5 and 6.25 mg/mL.

2.4 Test Organisms

Test organisms used were environmental isolates of *S. typhi, S. enteriditis, S. paratyphi, S. Havana and S. arizona.* These organisms were obtained from the Department of Pharmaceutics and the Department of Environmental Science, Kwame Nkrumah Universtiy of Science and Technology, Kumasi, Ghana.

2.5 Antimicrobial Activity Determination (Agar Well Diffusion Assay)

The susceptibility of the various strains of *Salmonella enterica* to the extracts was screened using the agar-well diffusion method [15,16]. Twenty millilitres of 45°C stabilized molten nutrient agar (Oxoid, United Kingdom) was seeded with 100 µL of a bacterial suspension equivalent to 0.5 McFarland (1.5 x10^8 colony forming units (CFU)/mL) of test organisms. Four wells equidistant from each other with a bore diameter of 13 mm (cork borer number 6) were made in the seeded agar. Two hundred microlitres of the different concentrations of the extracts (section 2.3.), were introduced into the wells and allowed to pre-incubate for 1 h. The nutrient agar plates were then incubated at 37°C for 24 h. Zones of growth inhibition were then measured to the nearest millilitre. The above procedure was repeated for the solvent (sterile distilled water, negative control) and for ciprofloxacin 5 µg/mL (positive control). The experiments were performed in triplicates and the average values calculated to determine the anti-salmonella activity.

2.6 Determination of Chloramphenicol in Herbal Formulations Using HPLC-UV Analysis

Determination of the presence of chloramphenicol in the herbal preparations was done using the USP 2006 method [17].

2.6.1 Preparation of standard chloramphenicol solution

An accurate weight of 100 mg Chloramphenicol was transferred into a 100 ml volumetric flask and dissolved in 20 ml of the mobile phase. The solution was sonicated for 5 min and allowed to cool. The solution was made up to the 100 ml mark using the mobile phase (1 mg/ml). A volume of 1 ml of the standard solution was pipetted and transferred into a 10 ml volumetric flask. The solution was made up to the 10 ml mark using the mobile phase and analyzed using the USP method to obtain a chromatogram for chloramphenicol.

2.6.2 Sample preparation

The herbal preparations were diluted with distilled water (1 in 10) and analyzed using the USP method described above for chloramphenicol.

2.6.3 Instrumental and chromatographic conditions

The HPLC analysis of the various preparations was done under the following Chromatographic conditions: Mobile phase: Methanol: Glacial acetic acid (55:45:0.1 v/v), Flow rate: 1 mL/min, Column: Phenomenex; Luna 5 micron C18; 4.6 x 100 mm, temperature: 22°C, Detector: Perkin Elmer 785A UV-Visible absorbance detector, Pump: Spectra series P100 Isocratic pump, Integrator software: Powerchrom, injection volume: 10 µL, detection wavelength: λ280 nm, and run time: 10 min.

3. RESULTS

Table 1 displays a summary of information on the anti-typhoidal preparations as stated on the product labels. It shows the volume of each product, the daily dose, indications and plant components. All the products were in the form of liquid decoctions with volumes that ranged from 330 to 1000 mL. After drying the herbal preparations, extract concentrations ranged from 0.8 to 13% W/V and Fig. 1 displays the distribution of the concentrations of the products.

In the agar well diffusion assay, the various anti-typhoidal products showed varying anti-salmonella activities (zones of inhibition) against the various Salmonella strains. Figs. 2 to 5 display the antimicrobial activities of the various products against the test organisms.

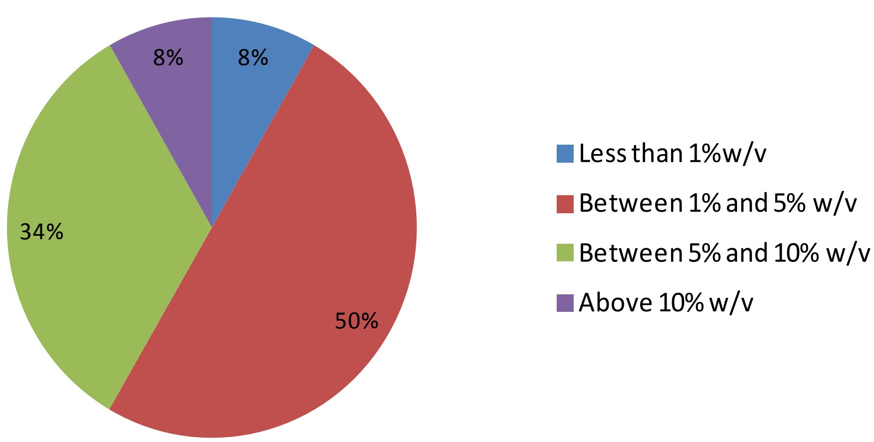

Fig. 1. Percentage extract yields (W/V) of the various herbal preparations

Table 1. Product information of anti-typhoid herbal preparations

Product code	**1**	**2**	**3**	**4**	**5**	**6**
Product volume	**1000 mL**	**430 mL**	**500 mL**	**330 mL**	**1000 mL**	**500 mL**
Indications	Typhoid fever Jaundice Malaria fever	Typhoid fever Malaria fever Jaundice	Typhoid fever Malaria fever Body pains Headache Tiredness Stress	Enteric fever	Typhoid fever Jaundice	Typhoid fever
Plant components	*Carica papaya leaves* *Cassia alata*	*Aloe schweinfurthii* *Khaya senegalensis* *Pileostigma thonningil* *Cassea siamea*	*Cryptolepis sanguinolenta* *Carapa procera*	*Spondiasis mombin* *Persea americana* *Psidium guajava* *Trema orientalis* *Cnest ferruginea* *Momordica charantia* *Vernonia amygdalina* *Lantana camara* *Paullina pinnata* *Citrus aurantifolia* *Morinda lucida* *Bidens spilosa*	*Nauclea latifolia* *Morinda lucida*	*Khaya senegalensis* *Ocimum gratissimum* *Zingiber officinale* *Cassia alata* *Vernonia amygdalina* *Anthocleista vogelii* *Cassia alata* *Cassia sieberiana*
Product code	**7**	**8**	**9**	**10**	**11**	**12**
Product volume	**280 mL**	**1000 mL**	**500 mL**	**300 mL**	**1000 mL**	**500 mL**
Indications	Enteric fever	Typhoid fever Malaria fever	Typhoid fever Malaria fever	Typhoid fever Malaria fever Headache Body pains	Typhoid	Malaria fever Typhoid fever Jaundice Body pains Menstrual pains
Plant components	*Psidium guajava* *Citrus aurantifolia*	*Ocimum viridi* *Vernonia amygdalina*	*Phylanthus fratenus* *Carica papaya*	*Mangifera indica* *Cocos nucifera*	*Morinda lucida* *Nauclea latifolia*	*Khaya ivorensis* *Mangifera indica*

	Vernonia amygdalina	*Morinda lucida* *Alstonia boonei* *Carica papaya* *Corn still*	*Cymbopogon citratus* *Vernonia amygdalina*	*Azadirachta indica* *Citrus aurantifolia*	*Paullina pinata* *Pycnanthus angolensis* *Rauwolfia vomitoria*
Product code	**13**	**14**	**15**	**16**	
Product volume	**1000 mL**	**500 mL**	**330 mL**	**1000 mL**	
Indications	Malaria Typhoid Jaundice	Malaria Typhoid	Typhoid fever	Typhoid fever	
Plant components	*Khaya senegalensis* *Azadirachta indica*	*Anthocleista nobilis* *Vitex grandifolia* *Phyllanthus fraternus*	*Azadirachta indica* *Momordica charantia*	*Azadirachta indica* *Momordica charantia*	

Table 2. Proportion of products active against *Salmonella* strains of public health importance in Ghana

Salmonella serovar	***S. typhi***	***S. paratyphi***	***S. enteriditis***
Number of products	9	8	6
Proportion of total number of products [%]	56	50	38

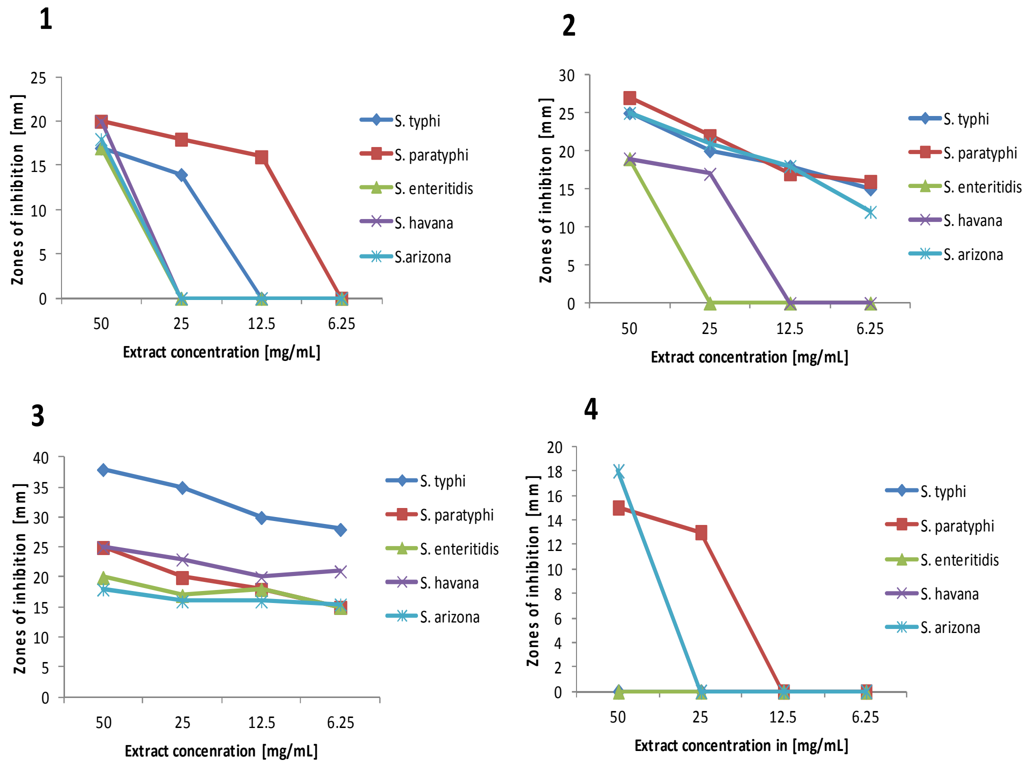

Fig. 2. Anti-Salmonella activities of anti-typhoid herbal preparations (Products 1 - 4). Positive control (5 µg/mL Ciprofloxacin) gave zones of *S. typhi* 42 mm, *S. paratyphi* 50 mm, *S. enteritidis* 39 mm, *S. havana* 50 mm and *S. arizona* 43 mm

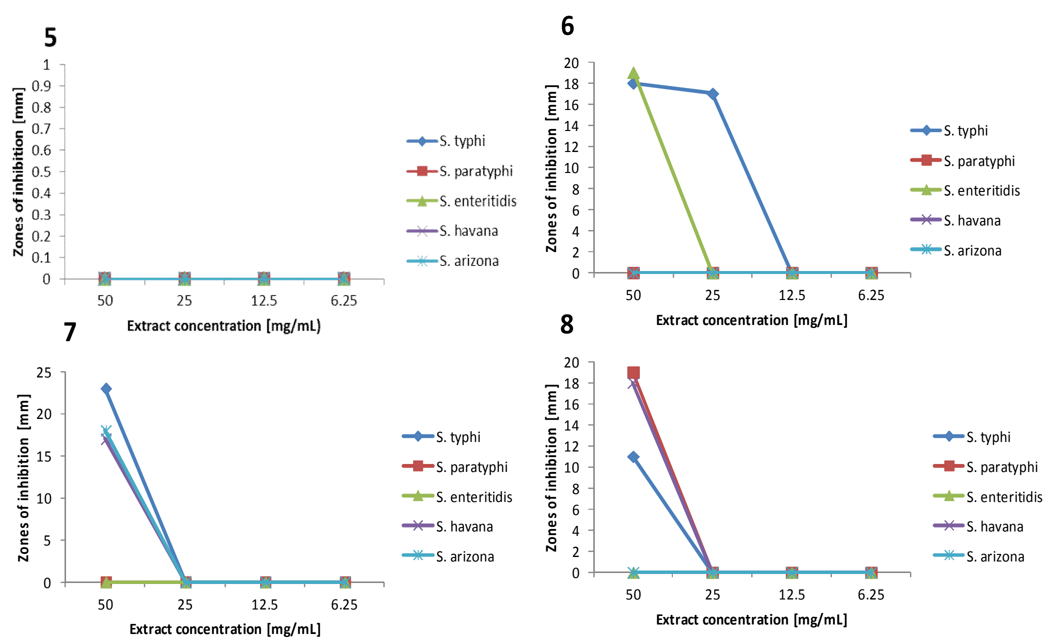

Fig. 3. Anti-Salmonella activities of anti-typhoid herbal preparations (Products 5 - 8). Positive control (5 µg/mL Ciprofloxacin) gave zones of *S. typhi* 42 mm, *S. paratyphi* 50 mm, *S. enteritidis* 39 mm, *S. havana* 50 mm and *S. arizona* 43 mm

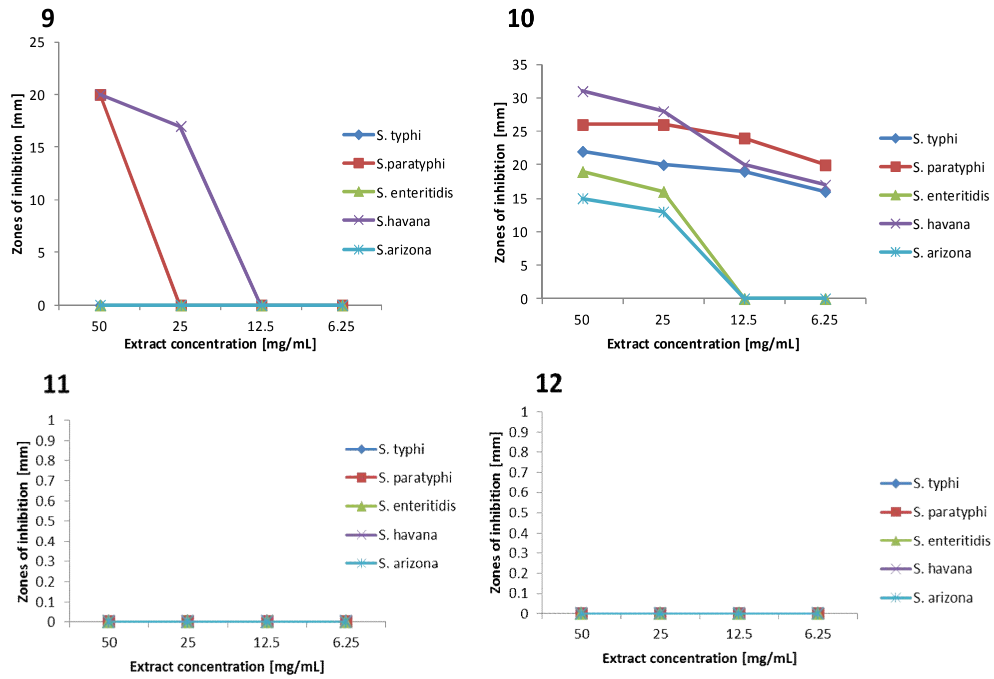

Fig. 4. Anti-Salmonella activities of anti-typhoid herbal preparations (Products 9 - 12). Positive control (5 µg/mL Ciprofloxacin) gave zones of *S. typhi* 42 mm, *S. paratyphi* 50 mm, *S. enteritidis* 39 mm, *S. havana* 50 mm and *S. arizona* 43 mm

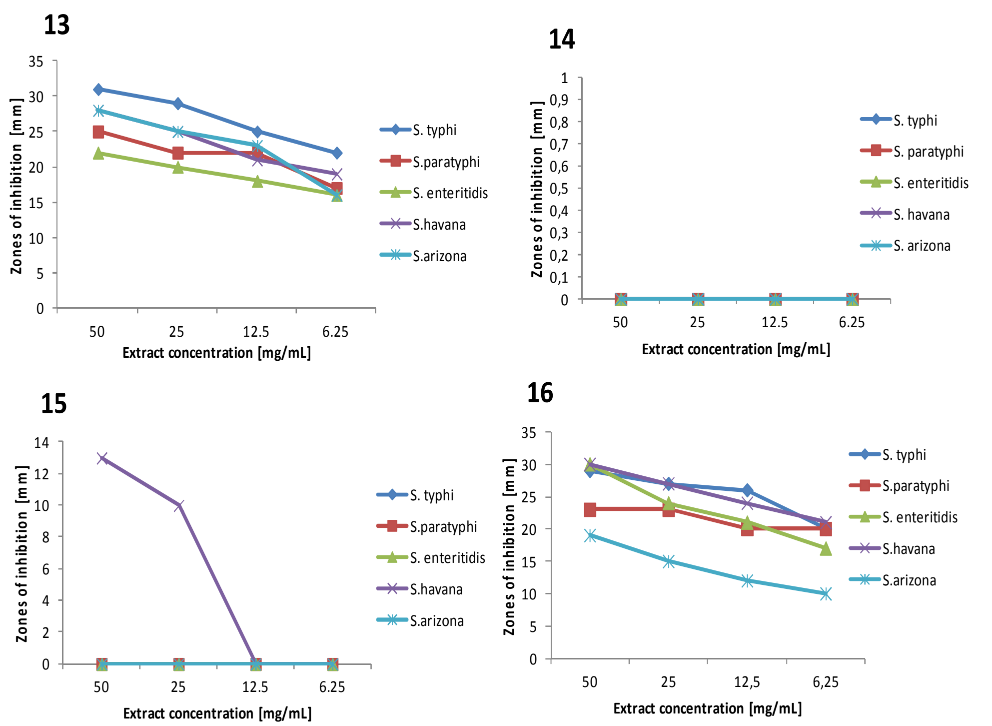

Fig. 5. Anti-Salmonella activities of anti-typhoid herbal preparations (Products 13 - 16). Positive control (5 µg/mL Ciprofloxacin) gave zones of *S. typhi* 42 mm, *S. paratyphi* 50 mm, *S. enteritidis* 39 mm, *S. havana* 50 mm and *S. arizona* 43 mm

Table 3. Chloramphenicol in herbal preparations

Sample	Chloramphenicol
1	-
2	-
3	-
4	-
5	-
6	-
7	-
8	-
9	-
10	-
11	-

As observed in Figs. 2 to 5, 56% (9 out of 16) were active against *S. typhi*, which is the most prevalent serovar of Salmonella that causes enteric fever in Ghana. Majority of the products were also simultaneously active against both *S. typhi* and *S. paratyphi* (8 out of 16, representing 50%), hence will most likely be effective in the treatment of most enteric fevers (both typhoid and paratyphoid fever). Table 2 provides a summary of the proportion of products active against the three strains of Salmonella (namely S. *enterica, S. typhi and S. paratyphi*) with the most public health significance in Ghana. As seen in Figs. 2 to 5, 38% (6 products) showed activity against all the three strains of public health importance in Ghana. When these products were screened for the presence of chloramphenicol, none was determined to be present in any of the preparations (Table 3).

4. DISCUSSION

It is estimated that 70% of Ghanaians rely on herbal medicines for their primary healthcare [12], hence a large majority of the populace probably patronize more of these herbals than orthodox medicines for the treatment of typhoid. There was therefore a need to evaluate the efficacy of these herbal medicines *in vitro*. Also, the increase in the incidence of resistant strains of *Salmonella* to existing antibiotics such as chloramphenicol, ampicillin, cotrimoxazole and ciprofloxacin is making current treatment protocols less effective [3,18]. Typhoidal and non-typhoidal bacteria of the genus *Salmonella* are amongst some of the most resistant bacteria pathogens, and in addition to being present in the food chain they also cause major human infections [7]. There is also an increasing development of resistance to the flouroquinolones, the drug of choice for treating typhoid infection in Ghana. The third generation flouroquinolones are now the second line treatment for salmonellosis. Similarly, the less severe NTS strains have also become resistant to ampicillin, chloramphenicol, kanamycin, streptomycin, trimethoprim and cotrimoxazole [3]. There is therefore a developing need to search for new treatment alternatives to Salmonella infections. Clinical confirmation of typhoid or paratyphoid fever requires isolation of *S. typhi* or *S. paratyphi*, respectively, from blood, bone marrow, stool, duodenal fluid or cultures from skin above rose spots of patients [2], however in Ghana diagnosis for typhoid fevers in the community is mostly based on clinical symptoms. However, typhoid and paratyphoid fevers cannot be readily distinguished from each other based on clinical symptoms only [3]. Both typhoid and paratyphoid patients often present with a sustained fever of 39 to 40°C, chills, abdominal pain, hepatosplenomegaly, rash, nausea, anorexia, diarrhea or constipation, headache and a dry cough. On another hand individuals with non-typhoidal salmonella also present with self-limiting, acute gastroenteritis and watery diarrhea, nausea, vomiting, abdominal pain and fever [3]. The clinical symptoms for both typhoidal and non-typhoidal salmonella infection therefore overlap and cannot be readily used to distinguish one disease from the other, it is important therefore that such herbal preparations preferably act with a broad spectrum of activity against the various serovars of *Salmonella enterica* serovars. Ciprofloxacin for example is known to have a broad spectrum of activity against various salmonella spp [18]. This study showed that 38% of the herbal preparations sampled were active against all the five strains of salmonella. These include samples 1, 2, 3, 10, 13 and 16 (Figs. 2-5). Samples 10, 13 and 16 showed a somewhat similar pattern of activity against *S. typhi, S. paratyphi* and *S. havana* and this may likely be due to the common plant *Azadirachta indica* present in all these products. *Azadirachta indica* is a plant known to be highly active against Salmonella [19]. Thirteen percent of the products were active against three bacterial strains, 19% against 2 strains, 4% against 1 strain and another 25 % showed no activity against any of the strains. The results showed that 11 out of the 16 (69%) products had broad spectrum anti-salmonella activity against both typhoidal and non-typhoidal salmonella. This could be the reason why these anti-typhoid herbal preparations are believed to be so effective and are therefore highly patronized. The numerous and effective treatment of typhoid fever in countries such as India and Nigeria with herbal medicines is not a new phenomenon [20, 21]. Numerous *in vivo* and *in vitro* studies have demonstrated the efficacy of herbal medicines in the treatment of enteric fever [20]. Also, evaluation of the products for the possibility of adulteration with the active pharmaceutical ingredient (API) chloramphenicol (Table 3) showed all the products to be free of the API. This indicates that the observed anti-salmonella activities observed with the products are very likely due to their herbal content.

5. CONCLUSION

We therefore concluded that the widespread patronage of these products seem to be well justified. However more efficacy studies will need to be conducted against the preparations that did not show any *in vitro* anti-typhoid activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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