



***In vitro* efficacy of five commercially available herbal preparations used in the treatment of typhoid fever in Bamenda Municipality, Cameroon**

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ABSTRACT

Typhoid fever is an infectious disease that has been a public health concern for millennia. The use of herbal preparations is getting popularity, with an estimated 80% of the world's population still depending on it for the management of various diseases including typhoid fever. However, data concerning their quality, safety and efficacy is not readily available. Our aim was to determine the *in vitro* efficacy of some commercially available herbal preparations used in the treatment of typhoid fever in Bamenda Municipality. Five herbal preparations indicated for the treatment of typhoid fever (coded P1 to P5) were bought from various outlets of the herbal producers and serial dilutions made and screened for their activities against clinical isolates of *Salmonella* Typhi and *Salmonella* Paratyphi using the agar well diffusion and dilution methods. The bacterial growth inhibition zone diameters of the herbal preparations were measured with a transparent ruler and compared with those of some standard antibiotics (ciprofloxacin and ceftriaxone). Two of the herbal preparations (P1 & P2) showed inhibition zone diameters against *S. Typhi* (20 and 14 mm respectively) while the rest (P3, P4 & P5) were inactive. P1 showed minimal activity on *S. Paratyphi* while the rest of the herbal preparations (P2, P3, P4 and P5) were inactive at all tested concentrations. The difference between the value of the inhibition zone diameters of the herbal preparations and that of the standard antibiotics on both *S. Typhi* and *S. Paratyphi* was statistically significant ($p < 0.05$). It was found out that most of the herbal preparations showed no activity against the tested bacterial isolates contrary to their label bogus claims.

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INTRODUCTION

Typhoid fever is an acute generalized infection of the mononuclear phagocyte system, intestinal lymphoid tissue, and gallbladder caused by *Salmonella enterica* serovar Typhi (*S. Typhi*) and associated with poor sanitation and untreated water supplies

(Plotkin, 2018). It is a common and sometimes fatal infection of both adults and children, causing bacteremia and inflammatory destruction of the intestine and other organs. It is endemic in countries, especially throughout Asia and Africa (Arndt et al., 2014). Chloramphenicol has been its

treatment of choice for 40 years, but the widespread emergence of multi-drug resistant *Salmonella* Typhi to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole has necessitated the search for other therapeutic options (Frenck et al., 2000). The disease continues to be a public health problem in sub-Saharan Africa and common in developing countries (Appiah-Korang et al., 2014). Currently fluoroquinolone family of antibiotics is the drug of choice in the treatment of enteric fever in Cameroon. Alternatives such as Macrolides and C3G families are also recommended (Davidson, 2010). The disease is concomitant with poor public health and low socio economic indices (Appiah-Korang et al., 2014). Residents of poor communities lacking good water and sanitation system are mostly affected. It is estimated that a total of 400,000 cases occur annually in Africa, with an incidence of 50 per 100,000 persons per year (Bhan et al., 2005; Kariuki, 2008). In 2010, the global estimate of typhoid fever was estimated to be 26.9 million cases with 217,000 deaths recorded (Buckle et al., 2012). To improve on their health people use both conventional and traditional medicines to treat and prevent this disease.

Traditional medicines have constituted a source of ingredients, with therapeutic values. These medicines, mainly from plant origin, make up a huge reservoir for drug development (Cristina et al., 2006; Sivakumar et al., 2013). This is welcoming since nowadays a good number of synthetic drugs against infectious agents are known to be less effective due to microbial resistance (Alfatah et al., 2013). Plants contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases (Okafor, 2001). These natural resources are widely relied upon by over 80% of people in developing countries and especially in rural communities because of inefficiencies and/or lack of hospitals and social services in these areas. Other reasons may include high costs of drug, cultural incompatibility, and self-reliance

(Shi-Lin et al., 2016; Kamatenesi-Mugisha et al., 2005). It is largely known that medicines derived from plants products are non-toxic and with little or no side effects when compared to synthetic medicines. These plant derived medicines constitute an alternative and effective therapeutic approach against most ailments (Fatemeh et al., 2018). Recent studies have shown that medicinal plant products could either be used for curative purposes or for the prevention of diseases (Sofowora et al., 2013). However some of these plant extracts/ secondary metabolites are known to be toxic and can cause health hazards due to indiscriminate use (Teke and Kuete, 2014). Assurances of safety, efficacy and quality of herbal medicines have been limited by many factors (lack of research methodology, inadequate evidence base for traditional medicine therapies and products, lack of international and national standards, lack of adequate regulation and registration of herbal medicines, lack of registration of traditional providers and inadequate support for such research efforts).

In Cameroon and in Bamenda municipality in particular, most of the herbal preparations are produced and marketed by traditional medicine practitioners. The widespread and hence availability of a wide range of herbal medicines used in the treatment of typhoid fever (concomitantly often used to treat other common ailments such as malaria, and jaundice just to name a few), indicate an increase need to evaluate objectively the biological efficacy of those herbal preparations that are claimed to be active against *Salmonella* Typhi and Paratyphi. This study was therefore sought to determine the *in vitro* efficacy of five commercially available herbal preparations used in the treatment of typhoid fever in Bamenda Municipality. This was done through the determination of microbial growth inhibition zone diameters and minimum inhibitory concentrations of the herbal preparations.

MATERIALS AND METHODS

Description of the study site

The study was a Microbiology Laboratory base analysis that lasted for a period 2 months, from May to July, 2018. It was carried out at the Bamenda Regional Hospital, located in the capital city of the North West Region of Cameroon. It acts as a national and referral hospital, serving the Bamenda town with a population of about 800,000 inhabitants and the entire North West Region of about 1,728,953 inhabitants.

Collection of herbal samples

Within the study period, six herbal medicines retail shops in the Bamenda Municipality were visited and Five (5) different liquid herbal preparations (Table 1) indicated for the treatment of typhoid fever were bought from the various outlets of the herbal producers (Figure 1) in the city of Bamenda. These products were coded (P1 to P5) so as to keep the producer's identity secret. A questionnaire was administered to vendors to get some information on how the products were prepared, the place and the time of plant collection, the mode of conservation before and after preparation.

Collection, identification and maintenance of bacterial isolates

The microbial organisms were clinical isolates of *Salmonella* Typhi and *Salmonella* Paratyphi obtained from the Medical Microbiology Laboratory of the Bamenda Regional Hospital. Briefly, stool samples were first inoculated overnight in selenite F broth which is an enrichment medium that inhibits all coliforms in stool and permits just the growth of *Salmonella* species. The *Salmonella* species were then subcultured on *Salmonella Shigella* agar. *S. Typhi* colonies were pink due to lactose fermentation or grayish due to a small production of hydrogen sulphide on a red background while *S. Paratyphi* colonies were dark on a red background due to the high

production of hydrogen sulphide. A catalase test was done to differentiate *Salmonella* (catalase positive) from *Shigella* (catalase negative) according to the manufacturer. *Salmonella* bacterial cultures in Mueller Hinton medium (Cheesbrough, 2000) were stored and maintained on nutrient agar at 4 °C to be used later.

Preparation of inoculums

Freshly activated bacterial cultures of *S. Typhi* and *S. Paratyphi* on nutrient agar plates after 24 hours of growth were used. The isolate suspensions were prepared using the direct suspension technique. Briefly, two to five uniform colonies were picked and suspended in sterile distilled saline water and the turbidity compared with that of a 0.5 Mac Farland standard as described by Cockerill (2012). The standard solution of Mac Farland 0.5 was prepared by adding 0.6 ml of barium chloride solution to 99.4 ml of sulphuric acid solution in a clean glass bottle and the solution was well mixed. A volume of 5 ml of the turbidity standard solution was transferred to a sterile labelled plain tube of the same type and size as that used to prepare the suspension standard (Cheesbrough, 2000).

Preparation of herbal test concentrations

Various concentrations of the herbal test solutions were prepared using the Standard Operating Procedure. The raw solution of 10,000 µg/ml obtained from the dealers was considered 100%. From this solution, further two fold serial dilutions in sterile distilled water were made to obtain other test concentrations of 50 (5000 µg/ml), 25 (2500 µg/ml), 12.5 (1250 µg/ml) and 6.25% (625 µg/ml).

Activity of herbal samples against test organisms

Agar well diffusion assay was used to test the various concentrations of herbal preparations using Mueller Hinton Agar

media against *S. Typhi* and *S. Paratyphi*. A quantity of 20 µl of inoculum of test organisms prepared as indicated above was put into 25 ml of molten sterile Mueller Hinton Agar and homogenized. This was then poured into 90 mm Petri dishes and swirled to distribute the medium homogeneously. After solidification, wells were then made using a 6 mm cork borer as stated by Patel et al. (2004) and Teke et al. (2011). A quantity of 50 µl of a given herbal preparation at various concentrations was introduced into each well accordingly and allowed to stand for 30 min at room temperature in order for it to diffuse before incubation at 37 °C for 24 hours. Ciprofloxacin and ceftriaxone were used as standards prepared at 2.5% (25 µg/ml). After incubation, the antityphoid activity was evaluated by measuring the bacterial growth inhibition zone diameters for each test concentration using a millimeter ruler. All tests were done in triplicates.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) here was defined as the lowest concentration of antimicrobial agent resulting in no microbial growth after 18 to 24 hours of

incubation (Prescott et al., 2005) and it was determined using agar diffusion method as described by Bonev et al. (2008). MIC of the herbal preparations which showed significant activity against the test bacterial isolates (diameter of inhibition zone assay) were determined by preparing two fold dilutions to concentrations of 100% (10,000 µg/ml), 50% (5000 µg/ml), 25% (2500 µg/ml), 12.5% (1250 µg/ml), 6.25% (625 µg/ml) and 3.12% (625 µg/ml) (Akinyemi et al., 2005). An amount of 19 ml of molten sterile MHA was introduced into sterile Petri dishes and 1 ml of each concentration of the herbal preparations was added and mixed for homogeneity. After the agar had solidified, inoculums of the test bacterial isolates were streaked on the surface of each plate using a sterile cotton swap and incubated aerobically at 37 °C for 24 hours. Two control plates were maintained for each test batch. These included antibiotic control (plate containing agents and the growth medium without the inoculums) and organism control (plate containing the growth medium and the inoculums). The lowest concentration of the agent that produced no visible growth when compared with the control plate was regarded as the MIC. All tests were done in triplicates.

Table 1: Codes, composition and brand names of herbal preparations.

Sample code	Brand name	Indicated composition and parts used
P1	Herbal mixture	<i>Lantana camara</i> (leaves), <i>Musa acuminata</i> (blossom), <i>Carica papaya</i> (leaves), <i>Bidens pilosa</i> (leaves), <i>Citrus lemon</i> (fruits), <i>Ananas comosus</i> (unripe fruits), <i>Cymbopogon citrates</i> (leaves).
P2	Body-Colla H4	<i>Carica papaya</i> (roots), <i>Mangifera indica</i> (leaves), <i>Citrus lemon</i> (fruits), <i>Cymbopogon citrates</i> (leaves).
P3	Desefection	<i>Emilia coccinea</i> (whole plant).
P4	Fever off	<i>Telfaria occidentalis</i> (leaves).
P5	Malsoline	<i>Gossypium arboretum</i> (whole plant), <i>Carica papaya</i> (leaves).



Figure 1: Liquid herbal preparations collected from different shops.

Statistical analysis

Statistical analysis was performed for inhibition zone diameters values with the help of Statistical Package for Social Sciences (SPSS) version 20. Level of statistical significance was considered at $p < 0.05$ with Student Newman Keuls. MIC values were reported as obtained.

Ethical consideration

Administrative authorization to conduct this study was obtained from the Regional Delegation of Public Health (Ref. n°424/NWR/RDPH), Northwest-Cameroon.

RESULTS

Antibacterial activity against *S. Typhi*

The herbal preparations were not all able to prevent the growth of *S. Typhi*. Preparations P1 and P2 at the tested concentrations inhibited bacterial growth with zone diameters ranging from 7-20 mm (Table 2). The susceptibility of *S. Typhi* to these two herbal preparations were rated as 59.7 and 41.8% respectively. Greater antibacterial activity was observed at 10,000 $\mu\text{g/ml}$ concentration of herbal preparation even

though at this concentration Desefection, Fever off and Malsoline were not active.

Antibacterial activity against *S. Paratyphi*

It was observed that only P1 (Herbal mixture) preparation inhibited the growth of *S. Paratyphi* (inhibition zone diameter of 11mm) and at a test concentration of 10,000 $\mu\text{g/ml}$. This bacterium was rated susceptible.

Minimum inhibitory concentrations

The minimum inhibitory concentrations of the herbal preparations P1 and P2 were obtained. *S. Typhi* had the least MIC value of 6.25% (625 $\mu\text{g/mL}$) for the herbal preparation P1 while *S. Paratyphi*, had the highest MIC values of 100% (10,000 $\mu\text{g/mL}$) for the same product. However, no MIC was determined for P2 against *S. Paratyphi* since it did not show any inhibition zone diameter (Table 3). The other herbal preparations (P3, P4 and P5) with no growth inhibitory activity could not afford MIC values.

Table 2: Diameter of growth inhibition zones of *S. Typhi* to herbal preparations at various concentrations.

Sample Code	Commercial names of herbal preparations	Sample test concentration						Susceptibility of bacteria* (%) **
		2.5	6.25	12.5	25	50	100 %	
		25	625	1250	2500	5000	10,000 µg/mL	
Diameters (mm) of growth inhibition zones								
P1	Herbal mixture	-	0	7 ^a	11 ^b	16 ^c	20 ^d	Susceptible (59.7)
P2	Body-Colla H4	-	0	0	7 ^a	10 ^b	14 ^c	Susceptible (41.8)
P3	Desefection	-	0	0	0	0	0	-
P4	Fever off	-	0	0	0	0	0	-
P5	Malsoline	-	0	0	0	0	0	-
Reference substances	Ciprofloxacin	21	-	-	-	-	-	Susceptible
	Ceftriaxone	24	-	-	-	-	-	Susceptible

^{a,b,c,d}Values with the same superscript are not significantly different for the same test; Student Newman Keuls at $p > 0.05$.

*diameter >7 mm was considered susceptible and <7 mm as resistant at 100% test concentration

**mean zone diameter produced by herbal preparation X100/mean zone diameter produced by the reference substances.

Table 3: MIC of the herbal preparations against tested bacterial isolates.

Bacterial species	MIC of herbal preparations in percentage (µg/mL)	
	P1	P2
	<i>S. Typhi</i>	6.25 (625)
<i>S. Paratyphi</i>	100 (10,000)	-

MIC: Minimum inhibitory concentration, CIP: Ciprofloxacin, CEF: Ceftriaxone, -: no MIC value, P1: Herbal mixture, P2: Body-Colla H4.

DISCUSSION

In this study, out of the five (5) herbal preparations indicated for the treatment of typhoid fever, only P1 and P2 showed inhibition zone diameters on *S. Typhi* while the rest of the herbal preparations (P3, P4 and P5) showed no inhibition zone diameters. For *S. Paratyphi*, only P1 showed minimal

inhibition zone diameter. This study tied with the one carried out by Syed et al. (2011) in India and by Ikegbunam et al. (2013) in Nigeria. These authors showed that majority of the herbal preparations indicated for the treatment of typhoid fever showed no inhibition zone diameters. Anibijuwon et al. (2010) indicated that failure of some of these

herbal preparations to exert antibacterial effect on test organisms may not be enough to conclude that they do not contain substances that can exert antibacterial activity. This is because the potency of these herbal preparations depends on the methods of production. Moreover, the age of plants when harvested and the season of harvest determine the amount of the active constituents (Dougnon et al., 2017). Since the active ingredients of plants can vary in quality and quantity from season to season, their efficacy can thus be affected.

The active plant components of the anti-typhoidal formulations used probably indicate that the resulting products could be highly active. The problem seems to be at the level of products formulation which reduces the daily dosage and product volumes.

Our results also showed that the herbal preparations had higher minimum inhibitory concentration (MIC) values on *S. Typhi* and *S. Paratyphi*. This result is in line with a study carried out by Syed et al. (2011) in India, though contrary to a study carried out by Ikegbunam et al. (2013) who found out that minimal concentrations of the herbal preparations were needed to inhibit the activity of *S. Typhi* and *S. Paratyphi*. The reason for the higher MIC values can be attributed to the methods of production where the herbal preparations are over diluted, thus reducing their activity against the test bacterial isolates. It can also be explained by the fact that different combinations of plants were used in preparing the herbal preparations as well as different strains of the test bacterial isolates might have been used in the various studies.

Conclusion

Two of the herbal preparations (P1 and P2) showed inhibition zone diameters against *S. Typhi* while only one (P1) showed inhibition zone diameters on *S. Paratyphi* providing scientific evidence for the use of

these two herbal preparations in the treatment of typhoid fever. However, most of the herbal preparations used in this study, had no activity against the test bacterial isolates contrary to their label bogus claims.

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COMPETING INTERESTS

The authors declare no competing interests regarding the publication of this paper.

AUTHORS' CONTRIBUTIONS

FNN and GNT designed the study, monitored laboratory work, and analyzed the data. AC did the laboratory work, collected and comment the results. NNF drafted and finalized the manuscript for publication. GNT and KFHL edited the manuscript. All authors contributed to the writing of the paper and approved the final version.

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