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COMMERCIALLY AVAILABLE HERBAL PREPARATIONS USED IN THE TREATMENT OF TYPHOID FEVER IN THE NORTH WEST REGION OF CAMEROON: DETERMINATION OF THE IN VITRO EFFICACY

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ABTRACT

The use of herbal preparations is gaining popularity, with an estimated 80% of the world's population still depending on herbal medicine for the treatment of various diseases. However, data concerning their quality, safety and efficacy is not readily available.

Aim

The main aim of the study was to determine the in vitro efficacy of some commercially available herbal preparations used in the treatment of typhoid fever in Bamenda.

Materials and methods

Five herbal preparations indicated for the treatment of typhoid fever (coded P1 – 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 agar dilution methods. The inhibition zone diameters of the herbal preparations were measured with a transparent ruler and compared with that of some standard antibiotics (ciprofloxacin and ceftriaxone).

Results

Two of the herbal preparations (P1 & P2) showed inhibition zone diameters against S.typhi while the rest (P3, P4 & P5) were in active. On S.paratyphi, P1 showed minimal activity while the rest of the herbal preparations (P2, P3, P4 and P5) were inactive. The difference between the value of the inhibition zone diameters of the herbal preparations and that of the standard antibiotics on both S. typhiand S. paratyphiwas statistically significant (p< 0.05). Conclusion

It was found out that most of the herbal preparations showed no activity against the test bacterial isolates contrary to their label bogus claims.

Keywords: Herbal preparations, Salmonella typhi, Salmonella paratyphi, typhoid fever ceftriaxone).

1.0 INTRODUCTION

Typhoid fever is a gastrointestinal infection caused by Salmonella typhi and to a lesser extent by Salmonella paratyphi [1]. This disease is common worldwide and is transmitted by the ingestion of food or water contaminated with faeces from an infected person [2]. This disease is estimated to affect at least 16 million persons worldwide, most of whom reside in the developing countries of South East Asia and Africa [3]. Advances in public health strategies, technology and hygiene have led to the eradication of typhoid fever in the developed world but since 1800, typhoid fever has remained an endemic disease in many developing countries [4]. Reports from the Ministry of Public Health in Cameroon showed that 1800 and 5300 patients were affected in 1987 and 1989 respectively [5], and recent reports suggest that the diagnosis of typhoid fever is becoming more and more frequent in health facilities in Cameroon resulting in a public scare [6]. Herbal medicine is the art or practice of using herbs and herbal preparations to maintain health and to prevent, alleviate, or cure disease [7]. Herbal medicinal products are becoming increasingly popular all over the world [8]. An estimated 80% percent of the world's population still depends on traditional herbal medicine for their health security [9]. In most African countries including Cameroon, herbal medicine is recognized as an important component of health care system, especially among rural dwellers that constitute about 70% of the population [10]. Also the ever increasing cost of orthodox health care services coupled with the site effects of certain synthetic drug therapies has further caused a large proportion of patients in developing countries to resort to alternative herbal health care which they feel is natural, safer, more accessible, more economical and takes into the consideration the peoples social values[11]. Authentication of herbal products is the foundation of the safe and correct use of plant-based natural health products. Without proper authentication as a starting point, the safe use of quality products cannot be guaranteed. There is recognition within industry and government that there is a need to protect access and choice by consumers when it comes to natural health products and at the same time, consumers have a right to expect that these products can be used with confidence regarding their safety and quality [12]. Assurances of safety, efficacy and quality of herbal medicines have been limited by 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 [13]. Herbal medicines have been used extensively to treat a wide range of medical conditions and an estimated 80% percent of the world's population and Cameroon not exempted still depends on traditional herbal medicine for their health security [9]. Recent years have witnessed an increase in the use of herbal medicines around the world including Cameroon, but questions remain concerning their quality, safety and efficacy [14]. The widespread availability of herbal medicines in today's world and the claims of most traditional practitioners in Bamenda on the efficacy of their herbal preparations used in the treatment of typhoid fever indicated an increase need to evaluate objectively the biological activity of those herbal preparations that were claimed to be active against *Salmonella typhi* and *paratyphi*[15].

2.0 Methodology

2.1 Study design

The study was an experimental procedure, following a laboratory study design.

2.2. Study setting

This study was carried out in the North West Regional city of Bamenda and laboratory base analysis was done at Ringland Medicals. Ringland medical is a hospital located at Foncha street opposite St Jonh catholic church Bamenda, Cameroon. It is situated in the Bamenda III subdivisional municipality of the Mezam Division.

2.3. Study period

This study lasted for a month. Laboratory work and analysis began from 1st to 30th October 2019

2.4. Data collection

2.4.1. Sample collection and sampling techniques

2.4.1.1. Herbal preparations

A convenient sampling of Five (5) different liquid herbal preparations (table 1) indicated for the treatment of typhoid fever was bought and collected from the various outlets of the herbal producers in the North West Region of Cameroon and were assigned with codes so as to keep the producers identity secret.

Table 1: Products codes and brand names

Sample code Brand name Plants present in products

- P1 Herbal mixture Cymbogogoncitratus, Carica papaya leaves and Zea mays silk
- P2 Body-Colla H4 Carica papaya roots, Magiferaindica leaves, Citrus limon fruit
- P3 Desefection Carica papaya leaves
- P4 Fever off Telfariaoccidentalis leaves
- P5 Malsoline Gossypium arboretum whole plant

P: product code

2.4.1.2. 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. 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 sub cultured on Salmonella Shigella agar. S. typhi colonies were pink due to lactose fermentation or greyish due to a small production of hydrogen sulfite on a red background while S. paratyphi colonies were dark on a red background due to the high production of hydrogen sulfite. A biochemical test (catalase test) was done to differentiate salmonella (catalase positive) from shigella(catalase negative).

2.5. Laboratory procedure

2.5.1. Importance and procedure for Gram stain technique

The gram stain is the most important staining procedure in microbiology. It is used to differentiate between gram positive and gram negative organisms, hence a differential stain. The procedure was done through the following steps [73]

Sterile glass slides were labelled. A drop of sterile normal saline was placed on each sterile glass slide and smears were made on the glass slides.

 \succ The bacteria smear on the slide was heat fixed by passing it over the Bunsen burner flame.

> Each slide was covered with the crystal violet stain for one minute.

> The slide was rapidly and carefully washed using clean water.

➤ Grams iodine was added to each slide for 30 seconds. This step fixes the crystal violet stain on to the bacteria cell wall.

> Each slide was tilted to remove excess iodine.

 \succ Each slide was rapidly decolourized with acetone for 20 seconds and then washed with clean water.

> The slides were then counter stained with Carbolfuschin for 20 seconds and washed with clean water thereafter.

➤ The slides were dried and a drop of immersion oil added to each. They were then examined microscopically using the oil immersion objective (X100 objective)

2.5.2. Preparation of turbidity standard

A 0.5 McFarland standard was prepared by adding 0.6ml of barium chloride solution to 99.4ml of sulphuric acid solution in a clean glass bottle and the solution was mixed. A volume of 5ml 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 [73].

2.5.3. Preparation of inoculums

The following isolates were used, S. typhi and S. paratyphi. The isolate suspensions were prepared using the direct suspension technique, were two to five uniform colonies were picked and dissolved in sterile distill water and turbidity compared with that of a 0.5 Mac Farland standard as described by Jan (2009) [74].

2.5.4. Preparation Mueller Hinton culture medium

Mueller Hinton agar (product of TULIP DIAGNOSTICS, India) was prepared following manufacturer's instructions [73]:

> 38g of Mueller Hinton agar was dissolved in 1000mlof sterile distilled water according.

> 30 plates were to be prepared each having a volume of 25mlof the agar.

> The total volume required for 30 plates was therefore $25 \text{ ml} \times 30 = 750 \text{ ml}$.

> The mass of the agar to be weighed was $Xg = 38g \times 750 \text{ m}/1000 \text{ m} = 28.5g$.

 \succ 750ml of distilled water was measured in a clean beaker and transferred in to a clean labelled glass bottle.

 \succ 30g of the agar was weighed on an electronic balance and dissolved in 750ml of distilled water in the glass bottle.

> The solution was stirred while heating to completely dissolve the agar.

> The bottle with its content was capped with aluminium foil and sterilized in an autoclaved at 121oC for 15minutes.

 \succ The bottle was allowed to cool until it could be touched with the hand for 5minutes; approximately 25ml of the agar was then poured into each labelled sterile Petri dishes 90mm in diameter in a flat horizontal surface under aseptic conditions such that each plate should have an agar depth of approximately 4mm.

> The plates were partially closed with their lids and allowed to stand for the agar to form.

 \succ The surface of the agar was dried by flaming after it has been allowed to cool down for some time.

> A small quantity of the formed agar was applied on a pH paper to determine its pH, which was 7.3.

> One plate was incubated in an incubator at 35-37oC for 24 hours for sterility testing and the others were covered and put in a plastic bag, sealed and placed in the refrigerator.

2.5.5. Preparation of various concentrations for sensitivity testing

Various concentrations of the herbal preparations were prepared using the Standard Operating Procedure (SOP) below obtained from the biochemistry unit of Ringland Medicals, Bamenda, Cameroon:

> To prepare a volume per volume percent (v/v) solution, the following formula was used:

 $(v/v) = [volume of solute (\mu l) / volume of solution (\mu l)] x 100.$

> To prepare 1000 μ l of a 5% by volume solution of the herbal preparation in water, the calculation was done as follows:

• 5% was first expressed as a decimal which is equal to 0.05. This decimal was multiplied by the total volume: $0.05 \times 1000 \mu$ l = 50 μ l (of herbal preparation needed)

• The volume of solute (herbal preparation) was then subtracted from the total solution volume that is 1000μ l - 50μ l = 950μ l (water needed).

> 50μ l of the herbal preparation was then dissolved in 950 μ l of water to make up 1000 μ l, so 50 μ l of herbal preparation / 1000 μ l x 100 = 5% (v/v) herbal preparation solution.

The above procedure was also followed in preparing the other concentrations.

2.5.6. Antibiotic sensitivity testing

About 0.1ml of the overnight broth culture of S. typhi and S. paratyphiwas taken and aseptically transferred into labelled sterile Petri dishes. Then 25ml of molten sterile Muller Hinton agar was poured into the seeded Petri dishes and swirled to distribute the medium homogenously. After solidification, different concentrations (5, 10, 25, 50 and 100%) of the standard antibiotics were aseptically poured into the wells made on the solidified Muller Hinton agar and then incubated aerobically for 24 hours at 37oc.A seeded Muller Hinton agar plate without an antibiotic disc was used as control [75].

The agar well diffusion assay was used according to Perez et al [75] to test various concentrations of herbal preparations using Mueller Hinton Agar (MHA) media [76] against S.typhi and S.paratyphi. 25ml of molten sterile Muller Hinton agar was poured into the seeded Petri dishes and swirled to distribute the medium homogenously. The MHA plates were then seeded with a suspension of the test organisms using a sterile cotton swap. The tested bacteria suspension was adjusted previously using a freshly prepared 0.5 McFarland turbidity standard. Wells were then prepared using an 8 mm cork borer as used by Patel et al.[77]. 100µl of the herbal preparations at various concentrations were introduced into each well and allowed to stand for 30mins at room temperature inorder for it to diffuse before incubation at 37oc for 24 hours. Ciprofloxacin and ceftriaxone were used as standards. After incubation, the antityphoid activity was evaluated by measuring the inhibition zone diameters for each concentration by using a meter rule.

2.5. Concentration (MIC 8. Determination of minimum inhibitory)

Minimum inhibitory concentration (MIC) is the lowest concentration of the antimicrobial agent resulting in no growth after 18 to 24 hours of incubation [78]and it was determined using agar diffusion method as described by Mendoza [79]. MIC of the herbal preparations which showed significant activity against the test bacterial isolates were determined by preparing two fold dilutions to concentrations of 100%, 50%, 25%, 12.5%, 6.25% and 3.12% [79]. 19ml of sterile MHA was introduced into sterile petri dishes and 1ml 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 37oC for 24 hours. The standard antibiotics ciprofloxacin and ceftriaxone were also screened under similar conditions for comparison. Two control plates were maintained for each test bacte. 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 (higher dilution) of the agent that produced no visible growth when compared with the control plate was regarded as the MIC.

2.6. Data management and analysis

The values of the inhibition zone diameters were measured using a meter rule and results recorded in a logbook and entered into Microsoft excel. Statistical analysis was done using Statistical Package for Social Sciences version 20 and results presented in tables and figures.

2.7. Ethical consideration

Research authorization was obtained from the authorities of the School of Health and Medical Sciences of kesmonds international University and from the Regional delegation of Public Health Bamenda.

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3.0 RESEARCH FINDINGS AND RESULTS

3.1. Results

3.1.1. Inhibition zone diameters of the herbal preparations on *Salmonella typhi* and *Salmonella para typhi* isolate. There were growth inhibitions of both species of *Salmonella* as indicated on culture plates by inhibition zone diameters. P1 (Fig. 1a) and P2 (Fig. 1b) showed best inhibition zone diameters on *S. typhi* that is 20mm and 14mm respectively at their initial concentrations, but there were reductions in the inhibition zone diameters as the samples were diluted. The rest of the herbal preparations (P3, P4, and P5) showed no activity on *S. typhi* (Fig. 1c). On *S. paratyphi*, only P1 showed minimal activity (Fig. 1d) while the rest of the herbal preparations showed no activity. The results are illustrated in tables 2 and 3.



: Inhibition zone diameters 🛛 🗲

Figure 1: Zones of inhibition by P1 on *S. typhi* (a), P2 on *S. typhi* (b), No zone of inhibition (c), minimal activity P1 on *S. paratyphi*(d)

Sample	Brand	Inhibition zone diameters (mm) against				
Code	Names	various concentrations				
		5%	10%	25%	50%	100
						%
P1	Herbal mixture	0	5	11	16	20
P2	Body-Colla H4	0	0	4	10	14
P3	Desefection	0	0	0	0	0
P4	Fever off	0	0	0	0	0
P5	Malsoline	0	0	0	0	0

 Table 2: Sensitivity of S. typhi against various concentrations of herbal preparations

Table 3: Sensitivity of S. paratyphi against various concentrations of herbal preparation.	Table 3: Sensitivity of S.	paratyphi against various	s concentrations of herb	al preparations
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Sample	Brand	Inhibitio	n zone	diamete	ers (mm)	against
code	Name	various concentrations				
		5%	10%	25%	50%	100%
P1	Herbal mixture	0	0	0	0	1
P2	Body-Colla H4	0	0	0	0	0
Р3	Desefection	0	0	0	0	0
P4	Fever off	0	0	0	0	0
P5	Malsoline	0	0	0	0	0

3.1.2. Inhibition zone diameters of standard antibiotics on *S.typhi* and *S. para typhi*

After perform antibiotic sensitivity testing, it was found out that are all test organisms were susceptible to the standard antibiotics. Inhibition zone diameters on *S. typhi* ranged from23mm at 5% (ciprofloxacin Fig. 2a) to 48 mm at 100% (ceftriaxone Fig. 2b). The inhibition zone diameters on *S. para typhi* ranged from 15mm at 5% concentration (ciprofloxacin Fig 2c) to

1000

40mm at 100% concentration (ceftriaxone Fig. 2d). The results are illustrated in Tables 4 and 5 below.



: Inhibition zone diameters 🔶

Figure 2: Inhibition zone by, Ciprofloxacin on *S. typhi* (a), Ceftriaxone on *S. typhi* (b), Ciprofloxacin in *S. para typhi* (c), Ceftriaxone on *S. para typhi* (d)

Table 4: Sensitivity of S. typhi against various concentrations of standard antibiotics

Standards	Diameters of inhibition zones (mm) against				
antibiotics	various concentrations				
	5%	10%	25%	50%	100%
Ciprofloxacin	10	13	21	27	32
Ceftriaxone	14	19	24	29	35

	Standards	Diameter	of inhi	bition zo	ones(mm)	against
antibiotics various concentrations						
		5%	10%	25%	50%	100%
	Ciprofloxacin	5	8	15	21	26
	Ceftriaxone	10	14	20	25	30

 Table 5: Sensitivity of S. para typhi against various concentrations of standard antibiotics

3.1.3. Minimum inhibitory concentration (MIC) of herbal preparations and standard antibiotics on *S. typhi* and *S. para typhi*

Table 5 shows that *S.typhi* had he least MIC value of 6.25 % for the herbal preparation P1and 0.39 % for the standard antibiotic ceftriaxone. On *S. paratyphi*, the herbal preparation (P1) and the standard antibiotics(ciprofloxacin and ceftriaxone) had MIC values of 100%, 1.56% and 0.78% respectively. However, no MIC was determined for P2 against *S. paratyphi*since it did not show any inhibition zone diameter.



Table 6: MIC of the herbal preparations and standard antibiotics against test bacterial isolates

Bacterial	MIC of herbal preparations (%)		MIC of standard antibiotics (%)	
species	P1	P2	CIP	CEF
S.typhi	6.25	25	0.5	0.39
S.paratyphi	100	-	1.56	0.78

CIP: Ciprofloxacin, CEF: Ceftriaxone, -: no MIC value

3.1.4 Comparison of the activity of the herbal preparations with the activity of ciprofloxacin and ceftriaxone on *S. typhi* isolates

Comparing the activity of the herbal preparations with that of the standard antibiotics on *S.typhi*, it was found that all herbal preparations showed no activity at 5% contrary to the standard antibiotics. P1 was the only herbal preparation that showed activity at 10% and had an inhibition zone diameter of 5mm while ciprofloxacin and ceftriaxone had inhibitory zone diameters of 13

mm and 19 mm respectively at the same concentration (Fig. 3). At 100%, P1 and P2 showed inhibitory zone diameters of 20mm and 14mm respectively while no inhibition zone diameters were noticed for P3, P4 and P5 at the same concentration. Ciprofloxacin and ceftriaxone meanwhile showed inhibitory zone diameters of 32 mm and 35 mm respectively at 100%. Ciprofloxacin and ceftriaxone had MIC values of 0.5% and 0.39% respectively contrary to P1 and P2, which had MIC values of 6.25% and 25%. The difference between the value of the inhibition zone diameters of the herbal preparations and that of the standard antibiotics on *S. typhi* was statistically significant (p= 0.006).



Figure 3: Comparison of the activity of the herbal preparations with the activity of ciprofloxacin and

ceftriaxone on *S. typhi* isolates

3.1.5. Comparison of the activity of the herbal preparations with the activity of ciprofloxacin and ceftriaxone on *S. Paratyphi*solates.

Out of the five herbal preparations, only P1 showed a minimal activity against *S. paratyphi* at 100%. No inhibition zones were observed for P2, P3, P4 and P5 at the same concentration while the standard antibiotics showed inhibition zones at all concentrations (Fig. 4). P1 had an inhibition zone diameter of 1 mm at 100%, which was far lesser to the inhibition zone diameters

showed by ciprofloxacin (26 mm) and ceftriaxone (30 mm) at the same concentration. Ciprofloxacin and ceftriaxone had MIC values of 1.56% and 0.78% respectively contrary to P1, which had a MIC value of 100% on *S. paratyphi*. The difference between the value of the inhibition zone diameters of the herbal preparations and that of the standard antibiotics on *S. paratyphi* was statistically significant (p=0.001).





4.0 Discussions, Conclusions and Limitations

4.2. Discussions

Out of the five (5) herbal preparations used in this study that is 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.On*S.paratyphi*, only P1 showed minimal inhibition zone diameter while P2, P3, P4 and P5 showed no inhibition zones. This is in accordance with studies carried out by *Syed et al.* in India[38] andbylkegbunam*et al.*[39] in Nigeria. These authors showed that majority of the herbal preparations indicated for the treatment of typhoid fever showed no inhibition zone diameters.Anibijuwon*etal.* [80] 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 against the test organisms because the potency of these herbal preparations depends on their method of production. In addition to that, Sofowora*et al.* [81] also explained that the age of plants when harvested and the season of harvest determine the amount of the active constituents and since the active ingredients of plants can vary in quality and quantity from season to season, their efficacy can thus be affected.

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*,[38] in India and is contrary to a study carried out by Ikegbunam*et al*. [39] 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 strains of the test bacterial isolates might have been used in the various studies. The standard antibiotics showed higher inhibition zone diameters contrary to the herbal preparations. In addition, the standard antibiotics had higher inhibitory activities at minimal concentrations when compared with that of the herbal preparations.

The comparison of the activity of the herbal preparations with that of the standard antibiotics on both *Salmonella* species showed that the standard antibiotics are more active than herbal preparations as confirmed by Anibijuwon*et al.* [80] and Ikegbunam*et al.* [39] in their respective studies. The reason for standard antibiotics being more active can be explained by the fact that traditional practitioners lack knowledge on the exact concentration of their products which often requires higher doses of the products being administered to their patients contrary to standard antibiotics whose concentrations are known require lower doses. In addition, it can also be attributed to the fact that most herbal preparations are prepared using a combination of whole plants which contains varieties of different substances that may interact with each other antagonistically, thus reducing its potency. In our study, it was found out that the activity of the standard antibiotics was not uniform. The test bacterial isolates were more sensitive to ceftriaxone and showed higher inhibition zone diameters compared to ciprofloxacin against both *Salmonella* species. This result is in line with a study carried out by Amna *et al.* [82] in Pakistan where the authors found out that ceftriaxone showed better results in the treatment of typhoid fever than ciprofloxacin.

4.2 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 giving scientific evidence for the use of these herbal preparations in the treatment of typhoid fever. The comparison of

the inhibition zone diameters of the herbal preparations with that of the standard antibiotics on both S. typhi and S. paratyphi was statistically significant (p = 0.006 and p = 0.001 respectively) indicating that the standard antibiotics are more active than the herbal preparations. Most of the herbal preparations used in this study, had no activity against the test bacterial isolates contrary to their label bogus claims.

4.3 Limitations

 \succ Most of the traditional practitioners did not give their herbal preparations after they knew it was for research.

 \succ There is very limited information on the in vitro efficacy of herbal preparations in Africa and Cameroon in particular.

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COMPETING INTEREST

The authors declare no competing interest regarding the publication of this paper

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