SUSCEPTIBILITY OF *Salmonella typhi* TO MEDICINAL PLANT EXTRACTS COMPARED TO CIPROFLOXACIN

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Abstract-
Background: **The development of bacteria resistance to present available antibiotics has necessitated the needs to search for new effective and cheaper antibacterial agents. Resistance of *Salmonella* to antibiotics is a serious health problem in the world more especially in developing countries and Cameroon in particular. This study aimed at comparing the antibacterial activity of medicinal plant extracts (*Cymbopogon citatus* leaves, *Carica papaya* leaves, *Bidens pilosa*, and *Mangifera indica* leaves) used by traditional herbalist against *Salmonella typhi*, compared to Ciprofloxacin.**
Methods: **This study was carried out at the Standard Medical Diagnostic and Research Center – Bamenda. Plant samples were collected, dried away from sunlight and ground into powder form. 25g of each plant powder was weighed to form a pool mixture of medicinal plant powder and dispensed in 95% methanol and distilled water and kept still for 7days. Isolation of *Salmonella typhi* from human feces was done using Salmonella Shigella agar (SSA) and sub-cultured on Muella Hinton Agar. Two fold serial dilutions were made on the methanol and water while two methods of sensitivity testing (well diffusion and disc diffusion) were carried out on different Mueller Hinton agar plates and incubated at 37°C for 18-24hrs. The zones of inhibition were measured in mm, compared and analyzed in SPSS version 21.**
Results: **The sensitivity studies conducted on the medicinal plant extracts and ciprofloxacin indicated that the buffered alcohol extract inhibited *S. typhi* to a zone of 13.34mm and 7.45mm for distilled water extract ($p<0.05$). Alcohol extract also recorded an inhibitory zone of 23.05mm by well diffusion and 19.55mm by disc diffusion ($p<0.05$). Again, sensitivity studies using ciprofloxacin 5µg gave a zone of inhibition of 5.0. Significant differences were observed among the extracts, on well diffusion versus disc diffusion and ciprofloxacin ($p<0.05$).**
Conclusion: **Despite the fact that methanol and aqueous extracts of the plants proved inhibitory actions to *S. typhi* more than the drug of choice in hospitals (Ciprofloxacin), Methanol extract of medicinal plants proved to inhibit the growth of *S typhi* strains more.**

Key words: **Susceptibility, *Salmonella typhi*, medicinal plant extracts, ciprofloxacin.**

BACKGROUND

Typhoid fever, also known as enteric fever, is a potentially fatal multisystemic illness caused primarily by *Salmonella typhoidal*, subspecies *typhoidal* serovar *typhi* and, to a lesser extent, related serovars *paratyphi* A, B, and C which are all Gram negative rods. Typhoid fever is a major cause of morbidity and mortality worldwide [1-3].
It is estimated that the global prevalence of morbidity due to typhoid fever stands at 21,650,974, and paratyphoid fever at 5,412,744 [4]. In Africa, typhoid fever is an important cause of morbidity and mortality with an estimated 12 - 33 million cases causing 216,000 - 600,000 deaths annually.[5-6] The highest incidence of this disease occurs in areas of high water contamination with human faeces, limited water supply, urbanization and weak health systems [5-7]. The unsatisfactory management of typhoid fever throughout the continents as well as in Cameroon which allows partially treated and relapsed patients to become sequentially resistant, may play a significant role in the development of resistance for *S. typhi* [8,9].
A major challenge in global health care is the need for novel, effective and affordable medicine to treat infectious diseases such as typhoid fever especially in developing countries of the world such as Cameroon [10].
Medicinal plants have been used by billions of people around the world for thousands of years to treat various diseases [11]. In fact, more than 80% of the population within developing countries relies on the use of medicinal

plants for their primary healthcare due to their lower cost and time tested nature [12] which probably why 25% of the conventional medicine is derived from plants.[11]

In Africa, attention has been directed towards medicinal plant research to substantiate the claims of the cure made by traditional healers, thus providing scientific basis for their efficacy. These medicinal plants; *Cymbopogon citatus* leaves, *Carica papaya* leaves, *Bidens pilosa*, and *Mangifera indica* leaves have been claimed by traditional medical practitioners to be effective when used for treatment of infectious diseases. These plants are rich in wide variety of secondary metabolites such as; tannins, terpenoids, phenols, alkaloids, and flavonoids [12-15]. An important characteristic of these compounds is their hydrophobicity which enables them to partition in the lipids of bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable [16].

Lack of access, less available, and elevated expenses on orthodox medications such as ciprofloxacin and the development of bacteria resistance to available antibiotics against *S. typhi* has necessitate the search for new [10] alternative therapeutic drugs including extracts from medicinal plants which are readily available, accessible and relatively cheap. This study therefore aimed at evaluating the inhibitory actions of alcohol and distilled water extracts of medicinal plant on *Salmonella typhi*, by comparing the effectiveness of well diffusion and disc diffusion methods for minimum inhibitory ratio of medicinal plant extracts on *Salmonella typhi* versus ciprofloxacin.

METHODS

The study was conducted at the Standard Medical Diagnostic and Research Center – Bamenda, utilizing an experimental study design. All plants were collected from Mile 7 Nkwen Bamenda. Plant materials collected were washed under running tap water, allowed to drain and shade dried in aluminum foil paper at room temperature, away from sunlight for two weeks. Separately, plant samples of *B. pilosa, C. citarus, C. papaya, and M. indica* were ground mechanically using a mortar and pestle. 25g of the various powdered plant samples were weighed using an electronic balance and dispensed in 500ml of distill water and 500ml of methanol. The mixtures were allowed for 7 days during which they were routinely mixed after every 12 hours by vigorously sharking to aid in the proper extraction of the active ingredients.

Two fold serial dilution techniques according to the method of Devkota and Dutta [17] was used in diluting the extracts to determine the minimum inhibitory ratio (MIR). Two fold serial dilutions (with distilled water) were made on both the methanol and aqueous extracts from crude, 1:2, 1:4, 1:8, and 1:16. An original 5µg of ciprofloxacin antimicrobial suspension solution was also used.

Motile black-centered H_2S *Salmonella typhi* producing colonies on SSA were sub-cultured into newly prepared SSA plates and allowed overnight at 37^0C for pure *Salmonella typhi* colonies. A disc diffusion technique using the Kirby-Bauer method, and a well diffusion method was applied. Circular discs from Whatmann paper were sterilized in a hot air oven for 1hour. Each disc was impregnated by soaking with the plant extract for different concentrations (Crude, 1:2, 1:4, 1:8 and 1:16) in duplicates for both the methanol and aqueous extracts. Two disks for ciprofloxacin were also impregnated with 5µg of the drug. All impregnated discs were air-dried for a few minutes aseptically beside a Bunsen flame. Each suspension of bacterial inoculum in 1ml of distilled water was poured onto 4 different labeled solidified Mueller Hilton agar plates using a sterile micropipette and spread evenly using a glass spreader.

Sensitivity disc of each plant extract and ciprofloxacin were aseptically transferred onto the two differently labeled MH agar plates using a sterilized forceps. On the remaining 2 dry bacteria seeded plates, wells of 5mm in diameter was made using sterilized micropipette tips. The labeled wells were filled to the brim with different concentration of the plant extract and ciprofloxacin suspension. These agar plates were incubated at 37°C for 24hrs. After this period, the minimum inhibitory ratio of the plant extracts was obtained by measuring the growth inhibitory zones using a ruler graduated in mm.

All statistical analyses were done using SPSS version 21 (IBM SPSS Statistics, IBM Corporation, Chicago, IL). The data was analyzed using the independent sample t-test (to compare significant differences between the means of two independent groups) and the analysis of variance (ANOVA) which was used to check if the means of two or more groups are significantly different from each other. Differences in means were done using the least significant difference (LSD) at significant values considered at $P < 0.05$. Authorization was gotten from the Regional Delegation of Public Health Bamenda.

RESULTS

Inhibitory action of methanol and distilled water extracts of medicinal plants (MP) on *Salmonella typhi*

After 24 hours of incubation at 37^0C, the extracts of MP evaluated against *S. typhi* found that the methanol extract showed good level of antimicrobial property against *S. typhi* than distilled water extract. The mean zone of inhibition on *S. typhi* from methanol was significantly different from the mean zone of inhibition on *S. typhi* from distilled water extract ($P = 0.0001$, $t = 4.64$) (Table 1). Increase in the dilution of the extracts with distilled water reduced the zones of inhibitions (Table 2, Figure 1)

Table 1: Inhibitory action of methanol and distilled water extracts of MP on *Salmonella typhi*

Type of extract	Zone of inhibition (*mm*)
Methanol extract	13.34±5.1
Distilled water extract	7.45±1.21
t – value	4.64
p – value	0.0001*

*- P < 0.05 (Significant at 0.05 significance level)

Table 2: Inhibitory action of methanol and distilled water (DW) extracts of MP on *Salmonella typhi* by dilution

Type of extract	Zone of inhibition (*mm*) per dilution				
	Crude	**1:2**	**1:4**	**1:8**	**1:16**
Methanol extract	21.3±6.20	18.4±4.30	14.6±2.11	10.1±1.10	2.3±0.94
DW extract	8.2±2.46	6.7±0.95	Nil	Nil	Nil
t – value	6.28	5.52	4.84	4.55	3.28
p – value	0.0001*	0.0001*	0.0001*	0.001*	0.0012*

*- P < 0.05 (Significant at 0.05 significance level)

Effectiveness of well diffusion versus disc diffusion methods for MIR (Minimum Inhibitory Ratio) of medicinal plant extracts MPEs on *S. typhi*

There was a significant difference ($P < 0.05$) between the mean inhibitory levels of well diffusion and disc diffusion methods. The well diffusion method for both the alcohol and distilled water extracts was more potent than the disc diffusion method in this study. However, the disc diffusion method showed no inhibition of *S. typhi* for the distilled water extract while there was a minimal zone of inhibition for the same extract using the well diffusion method. (Table 3, Figure 2)

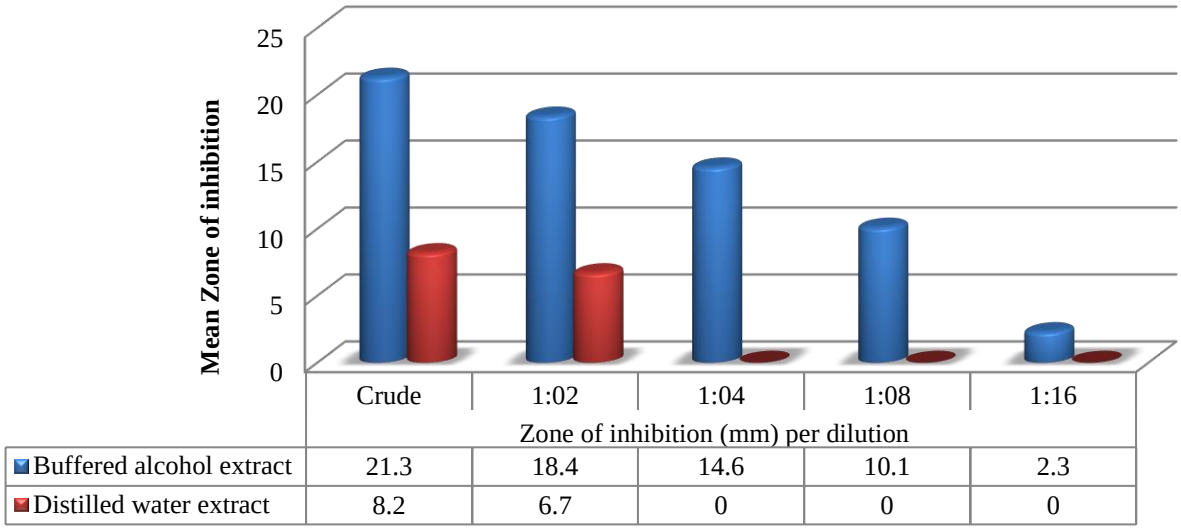

Figure 1: Inhibitory action of buffered alcohol and distilled water extracts of MP on Salmonella typhi by dilution

Table 3: Effectiveness of well diffusion versus disc diffusion methods for MIR (Minimum Inhibitory Ratio) of MPE on *S. typhi*

Type of extract		Zone of inhibition (*mm*) per dilution				
		Crude	**1:2**	**1:4**	**1:8**	**1:16**
Well difu	Methanol extract	23.05±6.01	20.01±3.04	16.26±3.02	12.32±0.97	1.10±0.54
Disc difu	Methanol extract	19.55±5.20	16.79±2.45	12.94±2.10	7.88±0.87	1.10±0.24
	p. value	**0.01***	**0.01***	**0.01***	**0.01***	**0.01***
Well difu	DW extract	8.2±2.46	6.7±0.95	Nil	Nil	Nil
Disc difu	DW extract	Nil (0±0)	Nil (0±0)	Nil	Nil	Nil
	p. value	**0.001***	**0.001***	**-**	**-**	**-**

*- P < 0.05 (Significant at 0.05 significance level). Difu: diffusion

Effectiveness of MPEs in the treatment of typhoid fever versus ciprofloxacin

MPEs showed greater antimicrobial effects against *S. typhi* than ciprofloxacin (5µg). Comparing the zones of inhibition between produced by ciprofloxacin and MPEs, there was a significant difference between them as

ciprofloxacin (5µg) only produced a zone of inhibition of 5mm. Compared to both extracts, *S. typhi* was not sensitive to ciprofloxacin as it was sensitive to both extracts. The zones of inhibition from ciprofloxacin (5µg) and the plant extracts were significantly different (P = 0.001) (Table 4)

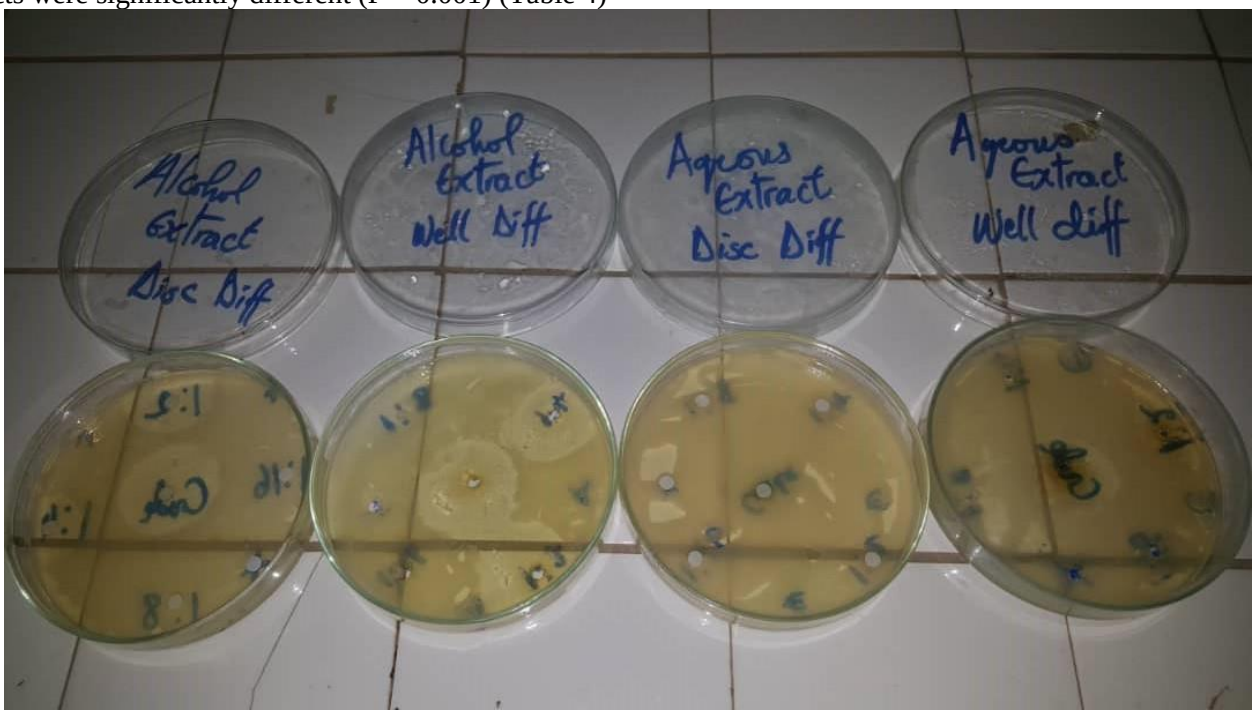

Figure 2: Effectiveness of well diffusion versus disc diffusion methods for MIR (Minimum Inhibitory Ratio) of MPE on S. typhi

Table 4: Effectiveness of MPEs in the treatment of typhoid fever vs ciprofloxacin

	Zone of inhibition (*mm*)
Methanol extract (ME)	13.34±5.1a
DW extract (DWE)	7.45±1.21b
Ciprofloxacin (5µg) (C)	5.0±0.01
F – value	12.43
p – value	0.001*

a = *P* < 0.05 when ME is compared with C, **b = *P* < 0.05** when DWE is compared with C

DISCUSSION

Efunwole *et al* had earlier reported that plants were the sole source of active principles capable of curing man's ailments before the development of chemistry and synthesis of organic compounds in the 19th century [18]. Our results indicate that the extracts of MP evaluated against *S. typhi* found that the methanol extract showed good level of antimicrobial property against *S. typhi* than distilled water extract. This may be because methanol is amphiphilic in nature [19] and may have extracted more of the anti-microbial components of the plants. Mostly, methanol is used for extracting various polar compounds even though certain groups of non-polar compounds are fairly soluble in methanol if not readily soluble. Methanol is commonly used for extraction of bioactive compounds [20]. These results did not however tie with that of Olukunle and Adenola who reported that their aqueous extract had the maximum inhibition against the isolates of *Salmonella typhi* followed by methanol and ethanol extracts [21]. The differences in these results could be due to the fact that their extracts were reconstituted using 30% v/v Dimethyl sulfoxide while the extracts in this study were only sterilized and used as crude.

In this present study, the well diffusion method for both the alcohol and distilled water extracts was more potent than the disc diffusion method. Agar well diffusion method has been reported to be used to evaluate the antimicrobial activity of plants or microbial extracts [22,23]. Valgas *et al.*, [24] had even reported that well diffusion methods are more potent in evaluating antimicrobial activity of plants extracts. The reason could be due to the fact that even though expensive than the disk diffusion methods, the antimicrobial agent has been shown to diffuse more into the agar than in the disc diffusion method. However, these results were not in line with the idea of Thavasi who explained that the choice of using any of the above methods depends on the diffusion of the drug molecules in agar or in discs. He added that some molecules won't diffuse in agar; in that case you have to use disc or well liquid methods. Solubility of the molecules play very important role [25].

It is universally admitted that the emergence of antimicrobial resistance, in particular, multidrug resistance (MDR) of *Salmonella* strains to ampicillin, chloramphenicol and cotrimoxazole, has complicated the treatment and management of salmonellosis [26]. MPEs showed greater antimicrobial effects against *S. typhi* than ciprofloxacin (5µg). *C. papaya* had been reported to have antimicrobial activity against *S. typhi*. This is because they contain flavonoids, sesquiterpene lactones, saponins, tannins, alkaloids, and sterols in varying degrees [27-29]. Despite the fact that *Cymbopogon citatus* leaves have been confirmed to have antimicrobial effects against *S. typhi* [30] Methanol, ethanol and water extracts of lemon grass from the study by [30] showed no antimicrobial activity. The disparities in these results may be due to the fact that the present study combined all medcicinal plants known to have been used as a pool in the treatment of typhoid while the later focused only on *Cymbopogon citatus*. However, the findings of Ewansiha *et al.,* [31] showed that chloroform extract of lemon grass demonstrated antimicrobial activity against isolate of *Salmonella typhi*. The inhibitory activities of the chloroform lemon grass extract on the test organisms indicate that the plant possess high active ingredients which may be chloroform soluble [31] Pfarelo *et al.,* [32] demonstrated the potential activity of using a combination of medicinal plants in investigation the action against microbes. The combination of two or more medicinal plants increases their potential and effectiveness in antimicrobial activities. Their conclusions are in line with the results obtained in this study. Farooqui *et al.,* [33] had shown that combining plant products together is effective in treating multidrug resistant infections. However, geographical and seasonal variations have been reported to affect the phytochemical constituents of plant and this in turn affects the result of antimicrobial activity [34]. No study however had been conducted to assess the effect of seasonal change on Cameroon medicinal plants.

Although medicinal plants are considered generally safe, they are known to contain potentially toxic, mutagenic, and/or carcinogenic substances. Since the MIC was not calculated due to the methods used in the antibiogram, it is unclear to state effectively if the plant extracts actually had bacteriocidal effects or were bacteriostatic.

CONCLUSION

Based on the findings, *Salmonella typhi* was highly susceptible to the alcohol extract with a zone of inhibition of 13.34mm than that of distilled water extract with 7.45mm. In addition, well diffusion was the best method for susceptibility testing with the highest zone of inhibition of 23.05mm. No zones of inhibition were observed for the disc diffusion, with the highest zone of inhibition of 8.2mm observed on well diffusion for DWE. Furthermore, the plant extracts proved inhibitory actions than ciprofloxacin (5µg) with a minimum zone of inhibition of 5.0mm.

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AUTHORS' CONTRIBUTIONS

The first and third authors performed the procedure, while the second author advised through the procedure. All authors discussed and contributed to the final manuscript.

CONFLICTS OF INTEREST STATEMENT

There are no conflicts of interest in this project.

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