Evaluating the Antibacterial Potential of Commercial Herbal Mixtures against Clinical Isolate of *Salmonella typhi*: An *In-vitro* Experimental Analysis

Isaac Baffour Awuah [a], Dennis Bardoe [a*],
Nelson Danyomah Wepia [b], Denis Dekugmen Yar [a],
Daniel Hayford [c], Robert Bagngmen Bio [a], Simms Ofosu [a],
Collins Saanle Eng [d], Osei-Sarfo Kantanka [d],
George Ahiaka [a], Francisca Serwaa Oppong [a],
James Azupero [c] and Paul Acheampong [d]

[a] *Department of Public Health Education, Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development, Mampong, Ghana.*
[b] *School of Security and Global Studies, American Military University, West Virginia, USA.*
[c] *Department of Integrated Science Education, Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development, Mampong, Ghana.*
[d] *Department of Biological Science Education, Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development, Mampong, Ghana.*

Authors' contributions

This work was carried out in collaboration among all authors. Authors IBA, DB, DDY, NDW, SO, CSE, FSO, PA, OSK, DH, GA, JA, and RB conceived, designed and performed the experiments. Authors DB, IBA, and NDW analysed and interpreted the data. Authors DB, IBA, DDY, DH, SO, GA, JA and NDW contributed to reagents, materials, and analysis tools. Authors DB and NDW drafted the first manuscript and did data visualizations. Authors DDY, DH, RBB, DB, NDW, SO, and FSO validated and edited the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

_____

*Corresponding author: E-mail: izyanjr@gmail.com;

Original Research Article

ABSTRACT

Background: Typhoid fever incidence has continuously increased, particularly in developing countries. As a result, there has been an influx of preference for herbal remedies to meet the growing demand. However, the increasing demand for these herbal preparations has created room for false claims.

Aim: This study investigated the in vitro activity of commercial herbal mixtures against a clinical isolate of *S. typhi*.

Study Design: The study employed an experimental design to evaluate the vitro activity of four commercial herbal mixtures against a clinical isolate of *S. typhi*.

Methods: Four herbal mixtures (IBA-ENTR5, DB-TYFO222, DY-PHB, and BIA-TABH) were screened for phytochemical and antimicrobial activity. Screening and identification of bioactive secondary metabolites were performed using standard procedures. The antimicrobial activity and minimum inhibitory concentration (MIC) were determined using the agar well diffusion and dilution method.

Results: Based on photochemical screening, apart from the absence of glycosides in IBA-ENTR5, all other phytochemicals were present in the herbal mixtures. The highest activity per agar well diffusion was observed for IBA-ENTR5, followed by DB-TYFO222, DY-PHB, and BIA-TABH. The minimum inhibitory concentration values ranged from 150 to 250 mg/L, with IBA-ENTR5 and DY-PHB possessing 150 mg/L, DB-TYFO222 possessing 200 mg/L, and BIA-TABH possessing 250 mg/L. Compared with the positive control IAG-CPRO, only IBA-ENTR exhibited a higher inhibition zone.

Conclusion: This study revealed that herbal mixtures contain rich phytochemical constituents. The results also confirmed that the antimicrobial activity and minimum inhibitory concentration of the herbal mixtures were concentration-dependent. That is, the herbal mixtures sampled provided good results when their concentrations were increased. In conclusion, herbal mixtures in open Ghanaian markets may provide good antimicrobial efficacy.

ABBREVIATIONS

MIC   : Minimum inhibitory concentration
MTT   : 3-(4,5-Dimethylthiazol-2-yl)−2,5-Diphenyltetrazolium Bromide 0.125%
ml   : Millilitres; mm, Millimetre
mg/L   : Milligrammes per litre
µg/mL : Microgram per millilitre
µL   : Microlitre
g   : Gramme
FDA   : Food and Drug Authority
SSA   : Salmonella–Sigma-Aldrich agar (SSA)

1. INTRODUCTION

Typhoid fever is caused by *Salmonella enterica serotype typhi* (Lovane et al., 2016). Globally, this infection is a public health concern and accounts for several annual mortality. It is estimated that approximately 9 million people are infected, and an annual mortality of 110000 has been associated with typhoid fever (WHO, 2023). Evidence suggests that major parts of South Asia, Southeast Asia, and sub-Saharan Africa are prone to this infection (Akram, 2020). In Ghana, it is one of the most common illnesses, accounting for 0.92% of hospital admissions (Asamoah and Mensah, 2016). Transmission of the infection usually occurs through the oral–faecal route and the sources of infection are typically contaminated water and food. The most common clinical symptom of this infection is fever, which slowly increases in severity. Other symptoms include loss of appetite (anorexia), dry cough, muscle pain, headache, general discomfort (malaise), (myalgia), abdominal discomfort, coated tongue, hepatomegaly (enlarged liver) and/or splenomegaly (enlarged spleen), and diarrhoea (WHO, 2023). Although typhoid fever can be treated with antibiotics, current studies have established that *S. typhi* is resistant to several prescribed medications, including chloramphenicol, ceftriaxone, trimethoprim-sulfamethoxazole, ampicillin, ciprofloxacin, and azithromycin (Shaikh et al., 2016). making the treatment more complicated. Researchers and clinicians are keen to identify effective and preventive treatments for this infection (Obaro et al., 2017). Hence, the search for new antimicrobials is directed towards plant-based products because of their medicinal properties.

Medicinal plants contain several therapeutic compounds (Adeleye et al., 2022). The use of herbal drugs for the treatment of several diseases has gained considerable attention, although it has been in use for a long time, especially in Africa and Asia (Okaiyeto et al., 2021). Approximately 80% of individuals in Africa and Asia rely on medicinal plants for the treatment of infections (Mensah et al., 2019). This can be attributed to the accessibility, availability, and affordability of medicinal plants or concoctions derived from medicinal plants (Ssenku et al., 2022). Traditionally, plant parts such as leaves, stems, bark, roots, flowers, and seeds have been used to prepare herbal mixtures for personal or commercial purposes (Monika et al., 2023). The chemical compounds contained in these parts function and possess several defence mechanisms that are required for the treatment of infections. These compounds are products of secondary metabolism and are known as phytochemicals (Ashraf et al., 2023). Several phytochemicals, such as alkaloids, glycosides, tannins, flavonoids, saponins, and terpenoids, have been identified in medicinal plants or products and reported to have therapeutic effects (Alqethami and Aldhebiani, 2021). They also possess antiviral, anti-helminthic, antibacterial, antioxidant, antidiabetic, and antifungal properties (Alqethami and Aldhebiani, 2021). A single plant can contain different phytochemicals; hence, it is difficult to determine whether a whole plant can provide the full effects required for the treatment of diseases (Sheeba et al.,2019). The effectiveness of commercial herbal mixtures in the Ghanaian open markets is widely acknowledged in folkloric claims; however, the extent of their activity needs to be examined and studied. Given the recent surge in microbial contaminants in food (Bardoe et al., 2023) there is the need to ensure the safety of these commercial herbal formulations. Hence guidance is required for their selection, preparation, and application. Therefore, this study evaluated the antibacterial potential of commercial herbal mixtures against a clinical isolate of *S.typhi*.

2. MATERIALS AND METHODS

2.1 Study Design

This experimental study was designed to evaluate the antibacterial potential of commercial herbal mixtures against clinical isolate *S. typhi* isolates.

2.2 Study Area

This study was conducted in Asante Mampong, a town located in Mampong Municipality, the municipality's administrative capital. Mampong is approximately 57 km from the regional capital, Kumasi. The municipality is bounded to the south, east, and north by the Sekyere South District, Sekyere Central District, and Ejura Sekyeredumasi District.

2.3 Drug Sourcing and Sampling Procedures

Four commercial herbal mixtures and one non-herbal drug (used as a control) were purposively sampled between January and April 2021. All herbal mixtures sampled for this study were

produced locally using a series of extraction methods including maceration, digestion, decoction, infusion, percolation, and Soxhlet extraction, with solvent systems (methanol, distilled water, and ethyl acetate) with varying polarities. After preparation, they are offered for sale in pharmaceutical and authorised chemical stores across the country. The original names and batch numbers of the sampled mixtures were withheld to ensure confidentiality. The mixtures were coded as IBA-ENTR5, DB-TYFO222, DY-PHB, BIA-TABH, and IAG-CPRO (control). All herbal mixtures sampled in this study had a shelf life of more than two years. The retail stores where the mixtures were purchased maintained them at room temperature, away from sunlight, and in well-ventilated rooms. After sampling, the mixtures were stored in various packages at room temperature until drug analysis was performed at the Microbiology Laboratory of Akenten Appiah-Menka University of Skill Training and Entrepreneurial Development, Mampong, Ghana.

2.4 Preliminary Phytochemical Screening of Herbal Mixture Samples

Qualitative screening and identification of bioactive secondary metabolites in herbal mixtures were performed using standard procedures described in previous studies.

2.4.1 Screening for alkaloids

A volume (1 ml) of the sampled herbal mixture was treated with Wagner's test reagent (1.27 g iodine and 2 g potassium iodide in 100 ml of water). An observed colour change of brown (reddish-brown) or yellow precipitates indicated the presence of alkaloids (Balali et al., 2023, Hussen and Endalew, 2023).

2.4.2 Screening for saponins

A volume (1 ml) of each sampled herbal mixture was added to 2 ml of distilled water in a test tube and shaken vigorously. Foam formation indicates saponins (Balali et al., 2023, Hussen and Endalew, 2023).

2.4.3 Screening for tannins

Distilled water (2 ml) was added to 1 ml of each sampled herbal mixture. Two drops of ferric chloride were then added to the solution. The production of blue-black or greenish precipitates indicates Tannins (Hussen and Endalew, 2023, Chiangnoon et al., 2022).

2.4.4 Screening for Steroid and triterpenoid

Five (5) ml of each sampled herbal mixture were added to 2 millilitres of chloroform, along with five drops of concentrated sulphuric acid. The mixture was shaken and kept aside for some time, and the colour change was observed. The formation of a reddish-brown ring indicates steroids and terpenoids (Hussen and Endalew, 2023, Chiangnoon et al.,2022).

2.4.5 Screening for glycosides

A volume (2 ml) of acetic acid and 2 ml of chloroform were added to 5 ml of each herbal mixture. The mixture was then cooled, 1 ml of concentrated H_2SO_4 was added, and a colour change was observed. The appearance of a brown ring indicates glycosides (Hussen and Endalew, 2023).

2.4.6 Screening for flavonoid

Distilled water (5 ml) was added to a volume (1 ml) of each sampled herbal mixture, boiled for 2 min, and filtered. NaOH (20%) was added to 1 ml of the filtrate. For a confirmatory test, 1 ml of lead acetate was added to the filtrate and a colour change was observed. A change in colour to yellow indicated the presence of flavonoids (Kifle et al., 2020).

2.5 Microbial Analyses

2.5.1 Preparation of Inocula (Including Collection, Identification, and Maintenance of Bacterial Isolates)

S. typhi (clinical strain) was obtained from the Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR) at Kwame Nkrumah University of Science and Technology. Stool samples were inoculated overnight in an enrichment medium (Selenite broth), which inhibited all coliforms and allowed the growth of *S. typhi* species only. *S. typhi* species were subcultured on *Salmonella–Sigma-Aldrich* agar (SSA). For identification, *S. typhi* colonies were pink due to lactose fermentation or greyish because of the low production of hydrogen sulphide on a red background. A catalase test was performed to differentiate *S. typhi* (catalase-positive) from *Shigella* (catalase-negative) according to the manufacturer's instructions. *S. typhi* cultures were stored on nutrient agar at 37°C for 24 hours to obtain pure colonies.

Mueller–Hinton agar powder (100g) was weighed and prepared in distilled water and sterilised in an autoclave for 1 hour at 120°C to prevent contamination. A volume of 100 ml of the prepared medium was then poured into a Petri dish and allowed to solidify. The plates were incubated at 37°C for 24 hours to eliminate contaminants. The subcultured microbes were picked from the nutrient agar, spread on the media in Petri dishes using a micropipette, sterilised cotton swabs, and allowed to dry. Wells were made on the medium using a sterilised cork borer (3 mm in diameter). Appropriate labels were then assigned to each well.

2.5.2 Determination of the antimicrobial activity of herbal mixture samples

The antimicrobial activity of each herbal mixture was determined using the agar well diffusion method employed by Linthoingambi and Singh,2023. Concentrations of 100 µL of 10, 20, 40, 80, 100, 150$^\alpha$, 150$^\beta$, 200$^\alpha$, 200$^\beta$, and 250 mg/L of the sampled commercial herbal mixtures were inoculated into each well. The mixtures were allowed to diffuse into the medium and were subsequently placed in an incubator at 37 °C for 24 hours. The effects of the herbal mixtures on organisms showed a clear zone of inhibition after incubation. The results were recorded in millimetres (ml) by measuring the length of the inhibition zone using a ruler. The positive control was IAG-CPRO at a concentration of 50 µg/ml.

2.5.3 Minimum Inhibitory Concentration (MIC) of the herbal mixtures

The dilution method described by Wiegand et al. (2008) was used to determine the minimum inhibitory concentration (MIC) of different herbal mixtures. Different concentrations of the sampled herbal mixtures (BIA-TABH, IBA-ENTR5, DY-PHB, and DB-TYFO222) were prepared at concentrations of 10, 20, 40, 80, 100, 150$^\alpha$, 150$^\beta$, 200$^\alpha$, 200$^\beta$, and 250 mg/L. The same procedure was used for the control (IAG-CPRO). Using a

sterile pipette, 100 µl of double-strength broth were pipetted into four different 96-well microtiter plates labelled 'A, B, D, E' (for herbal drugs) and 'C' (for control). A volume of 80 ml of each sampled herbal mixture and control was added to the broth in four different 96-well tetra plates. The test organisms (20 µl) were added to the resulting mixtures in four separate 96-well microtiter plates. Microtiter plates were incubated at 37°C for 24 h. The minimum inhibitory concentration (MIC) was determined by adding 20 µl of 3-(4,5-Dimethylthiazol-2-yl) −2,5-Diphenyltetrazolium Bromide 0.125% (MTT) to each well and incubating for 30 min at 37 ℃. The results were qualitatively obtained by observing the reaction of the MTT solution with the mixtures after incubation for 30 min. Growth inhibition is indicated in yellow and bacterial growth in dark purple. The concentration at which there was no growth, as indicated by clear broth, was taken as the minimum inhibitory concentration (MIC) (Wiegand et al.,2022).

3 RESULTS

3.1 Active Constituents of Herbal Mixtures

Four herbal mixtures coded as IBA-ENTR5, DB-TYFO222, DY-PHB, and BIA-TABH were purposely selected for the study. The active medicinal plant constituents of the sampled herbal mixtures, as inscribed on their packaging boxes were listed, as presented in Table 1. The herbal mixtures contained different numbers of plant constituents ranging from four to seven, as shown in Table 1. In all, 17 medicinal plant constituents belonging to 15 plant families were present in the sampled herbal mixtures, as highlighted in Table 2. On average, each preparation contained four medicinal plant constituents. *Carica papaya* and *Cymbopogon citratus* emerged as the most commonly used plants, followed by *Ocimum viride* and *Citrus limon, which* were also documented twice, as illustrated in Table 2.

Table 1. Sampled herbal mixtures and their active plant constituents

Herbal mixtures	Active constituents
IBA-ENTR5	*Carica papaya, Mangifera indica, Citrus limon, Cymbopogon citratus.*
DB-TYFO222	*Ocimum viride, Vernonia amygdalina, Morinda lucida, Alstonia boonei, Carica papaya, Stigma maydis.*
DY-PHB	*Bidens pilosa, Cymbopogon citratus, Ananas comosus, Lantana camara, Musa acuminata, Carica papaya, Citrus limon.*
BIA-TAB	*Ocimum viride, Azadirachta indica, Tetrapleura tetraptera, Theobroma cacao, Cymbopogon citratus, Moringa oleifera*

Table 2. Distribution of medicinal plant constituents in the sampled herbal drugs, their families, and frequency of occurrence

Family	Plant constituent	Frequency of occurrence
Anacardiaceae	*Mangifera indica*	1
Apocynaceae	*Alstonia boonei*	1
Asteraceae	*Vernonia amygdalina*	1
	Bidens pilosa	1
Bromeliaceae	*Ananas comosus*	1
Caricaceae	*Carica papaya*	3
Fabaceae	*Tetrapleura tetraptera*	1
Lamiaceae	*Ocimum viride*	2
Malvaceae	*Theobroma cacao*	1
Meliacea	*Azadirachta indica*	1
Moringaceae	*Moringa oleifera*	1
Musaceae	*Musa acuminata*	1
Poaceae	*Cymbopogon citratus*	3
	Stigma maydis	1
Rubiaceae	*Morinda lucida*	1
Rutaceae	*Citrus limon*	2
Verbanaceae	*Lantana camara*	1

Table 3. Phytochemical constituents of IBA-ENTR5, DB-TYFO222, DY-PHB, and BIA-TABH

Phytochemicals	IBA-ENTR5	DB-TYFO222	DY-PHB	BIA-TAB
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Steroids	+	+	+	+
Triterpenoids	+	+	+	+
Glycosides	-	+	+	+
Flavonoids	+	+	+	+
(+) indicates Presence and (-) indicates Absence				

3.2 Phytochemical Properties of the Herbal Mixtures

The results obtained from the qualitative analysis of the bioactive compounds in IBA-ENTR5, DB-TYFO222, DY-PHB, and BIA-TABH, as presented in Table 3, indicated the presence of secondary plant metabolites. (+) indicates presence, whereas (-) indicates the absence of the components. The metabolites analysed included flavonoids, tannins, saponins, alkaloids, steroids, glycosides, and triterpenoids.

3.3 Antimicrobial Activities of Herbal Mixtures

Table 4 presents the zone of inhibition of *S. typhi* (clinical strain) when tested against IBA-ENTR5, DB-TYFO222, DY-PHB, BIA-TABH, and IAG-CPRO (control) at varying concentrations (10, 20, 40, 80, 100, 150^α, 150^β, 200^α, 200^β, and 250 mg/L). The results indicated that *S. typhi* exhibited resistance to IBA-ENTR5, DB-TYFO222, DY-PHB, and BIA-TABH at concentrations of 10, 20, 40, 80, and 100 mg/L, excluding the control (IAG-CPRO), as presented in Table 4. However, at increased concentrations of 150^α and 150^β mg/L, DB-TYFO222 and BIA-TABH failed to inhibit the growth. In addition, at 200^α mg/l, only BIA-TABH showed an inhibitory effect on *S. typhi*. Remarkably, at a concentration of 200^β mg/L, all sampled herbal mixtures (ENTR5, DB-TYFO222, DY-PHB, and BIA-TABH) produced an inhibition zone. Furthermore, at 250 mg/L, an inhibition zone was observed for all sampled herbal mixtures (ENTR5, DB-TYFO222, DY-PHB, and BIA-TABH), as presented in Table 4. When comparing the inhibition zones for each herbal mixture (DB-TYFO222, DY-PHB, and BIA-TABH) across increasing concentrations (10, 20, 40, 80, 100, 150^α, 150^β, 200^α, 200^β, and 250 mg/L), no significant difference was observed ($p > 0.05$), except for IBA-ENTR5, which exhibited significance ($p < 0.05$), as indicated in Table 5.

Table 4. Antimicrobial activities of the sampled herbal mixture at different concentrations

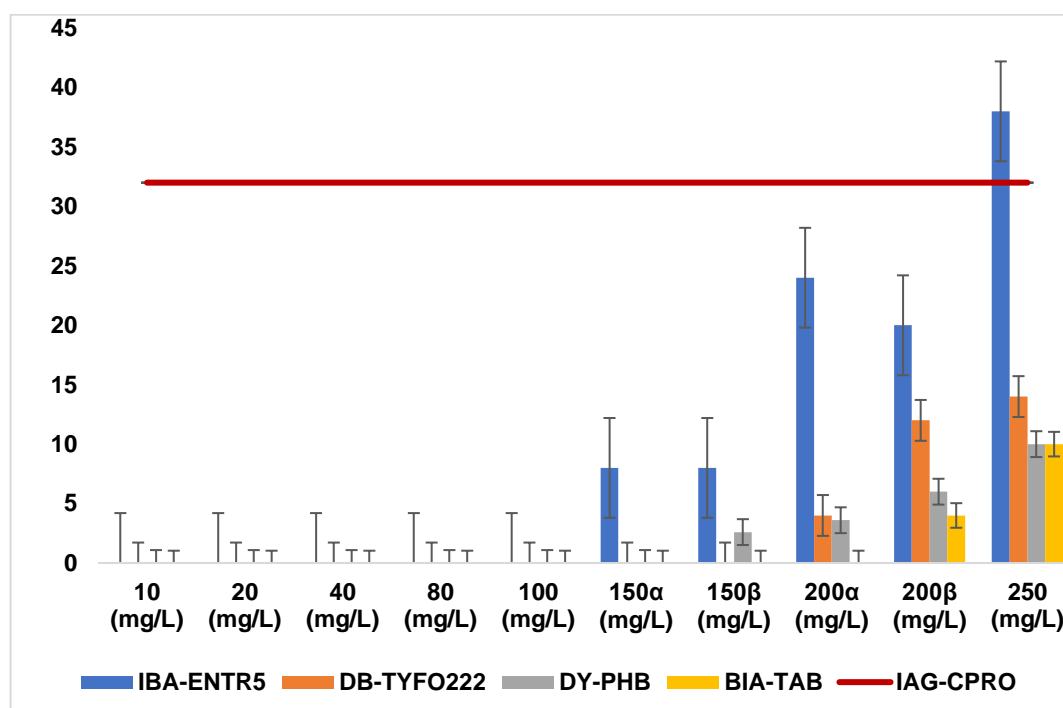| Concentration (mg/l)	IBA-ENTR5	DB-TYFO222	DY-PHB	BIA-TAB	IAG-CPRO
10 (mg/L)	0	0	0	0	32
20 (mg/L)	0	0	0	0	32
40 (mg/L)	0	0	0	0	32
80 (mg/L)	0	0	0	0	32
100 (mg/L)	0	0	0	0	32
150^{α} (mg/L)	8	0	0	0	32
150^{β} (mg/L)	8	0	2.6	0	32
200^{α} (mg/L)	20	4	3.6	0	32
200^{β} (mg/L)	24	12	6	4	32
250 (mg/L)	38	14	10	10	32
Total count (mm)	**98**	**30**	**22.2**	**14**	**320**
Mean (µmm)	**9.8**	**3**	**2.22**	**1.4**	**32**

Table 5. One-Sample T: IBA-ENTR5, DB-TYFO222, DY-PHB, and BIA-TABH

Sampled drugs	Mean	StDev	SE Mean	95% CI for µ	T-Value	P-Value
IBA-ENTR5	9.8	13.28	4.2	(3.30 – 13.30)	2.33	0.045*
DB-TYFO222	3	5.44	1.72	(0.89 – 5.89)	1.75	0.115
DY-PHB	2.22	3.44	1.09	(0.76 – 3.68)	2.04	0.072
BIA-TAB	1.4	3.27	1.03	(0.94 – 3.74)	1.35	0.209

3.4 Comparing Clear Zones among Different Concentrations

Differences in antimicrobial activity were determined using the agar well diffusion technique. The positive control (IAG-CPRO) was most inhibited, with a total inhibition of 320 mm across all 10 concentrations. Its activities were compared with those of IBA-ENTR5, DB-TYFO222, DY-PHB, and BIA-TABH, whose total inhibition was 98, 30, 22.2, and 14 mm, respectively (as shown in Figs. 1 and 2).

Fig. 1. Clear zones between different concentrations of IBA-ENTR5, DB-TYFO222, DY-PHB, and BIA-TABH against IAG-CPRO
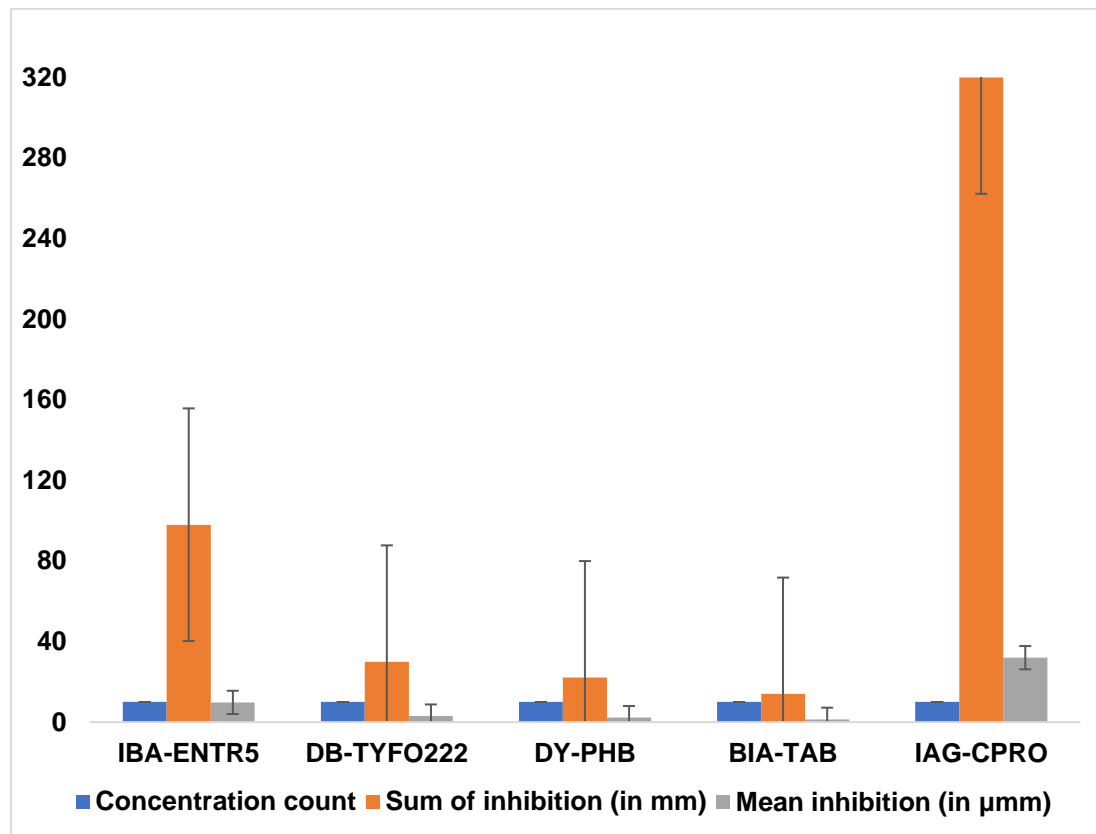
Fig. 2. The sum and mean inhibition of IBA-ENTR5, DB-TYFO222, DY-PHB, and BIA-TABH across different concentrations against IAG-CPRO

3.5 Inhibition Zones of IBA-ENTR5 at Different Concentrations

Fig. 3 presents the antimicrobial activity of IBA-ENTR5 against *S. typhi* at concentrations ranging from 10 to 250 mg/L. The findings revealed that IBA-ENTR5 showed the widest zone of inhibition against *S. typhi*, reaching a 38-mm inhibition zone at 250 mg/L. Conversely, as the concentration decreases, the clear zones decrease. Specifically, at concentrations of 200$^\beta$ and 150$^{\alpha\&\beta}$ mg/L, the clear zones were 20 and 8 mm, respectively. However, at concentrations of 100, 80, 40, 20, and 10 mg/L, no inhibition zones were observed. Comparing the observed mean inhibition zones of IBA-ENTR5 with that of IAG-CPRO (control) at different concentrations, there was a significant difference ($p < 0.05$), as shown in Table 6.

3.6 Inhibition Zone of DB-TYFO222 at Different Concentrations

Fig. 4 presents a summary of the results obtained from the agar well diffusion test with DB-TYFO222 at various concentrations

compared with the control (IAG-CPRO). The inhibition zones ranged from 0 to 14 mm, highlighting the varying efficacy. Notably, DB-TYFO222 demonstrated the second widest zone of inhibition against *S. typhi*. Specifically, at concentrations of 250 and 200$^\beta$ mg/L, the clear zones measured 14 and 12 mm, respectively. However, at concentrations of 150$^\beta$, 150$^\alpha$, 100, 80, 40, 20, and 10 mg/L, no zones of inhibition were observed. A comparison of the mean inhibition zones between DB-TYFO222 and the control (IAG-CPRO) at different concentrations revealed a significant difference ($p < 0.05$), as presented in Table 6.

3.7 Inhibition Zone of DY-PHB at Different Concentrations

The zones of inhibition observed for DY-PHB at different concentrations did not exhibit a significant difference as the concentrations increased ($p > 0.05$), as outlined in Table 5 and Fig. 5. The herbal mixture produced a notable zone of inhibition, reaching 10 mm at a concentration of 250 mg/L, followed by 6 and 3.6 mm at 200$^\beta$ and 200$^\alpha$ mg/L, respectively. At the

concentration 150β, a zone of inhibition of 2.6 mm was observed. However, at concentrations of 10 and 150α mg/L, no zones of inhibition were observed. Comparatively, when DY-PHB was evaluated against the positive control (IAG-CPRO), which consistently produced an inhibition zone of 32 mm across all concentrations, the mean inhibition zones showed a significant difference ($p < 0.05$), as detailed in Table 6.
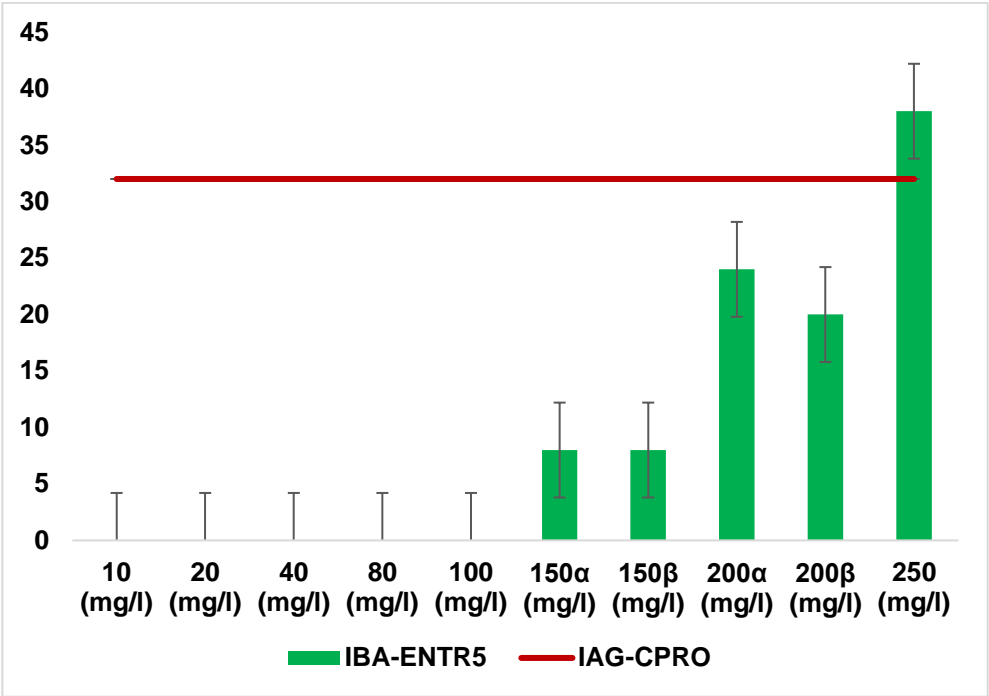

Fig. 3. Clear Zones for different IBA-ENTR5 concentrations
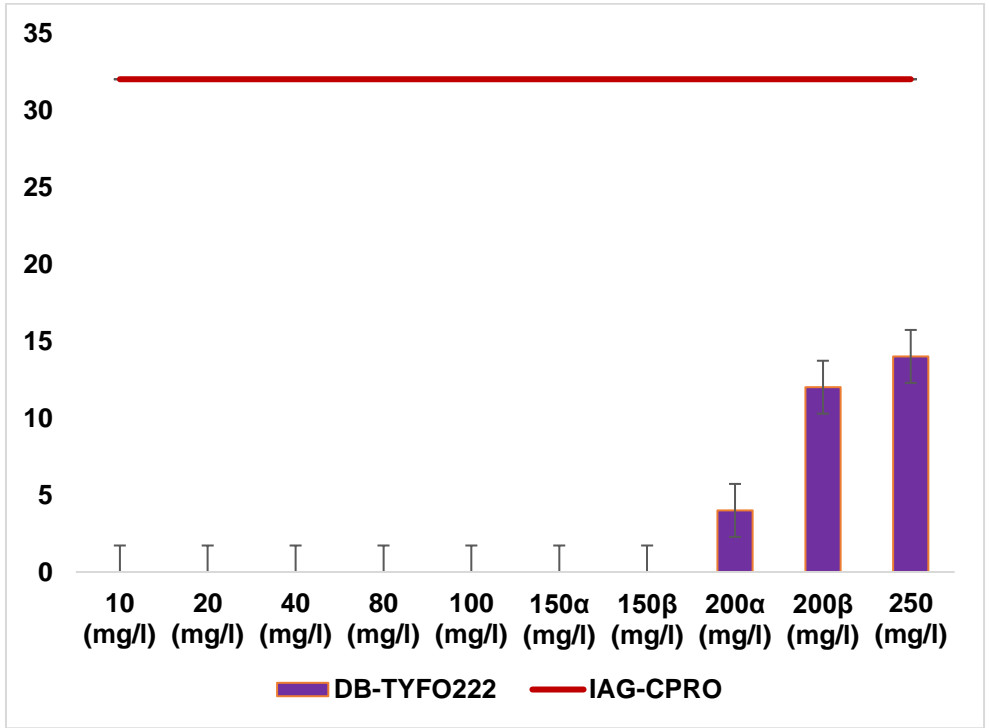
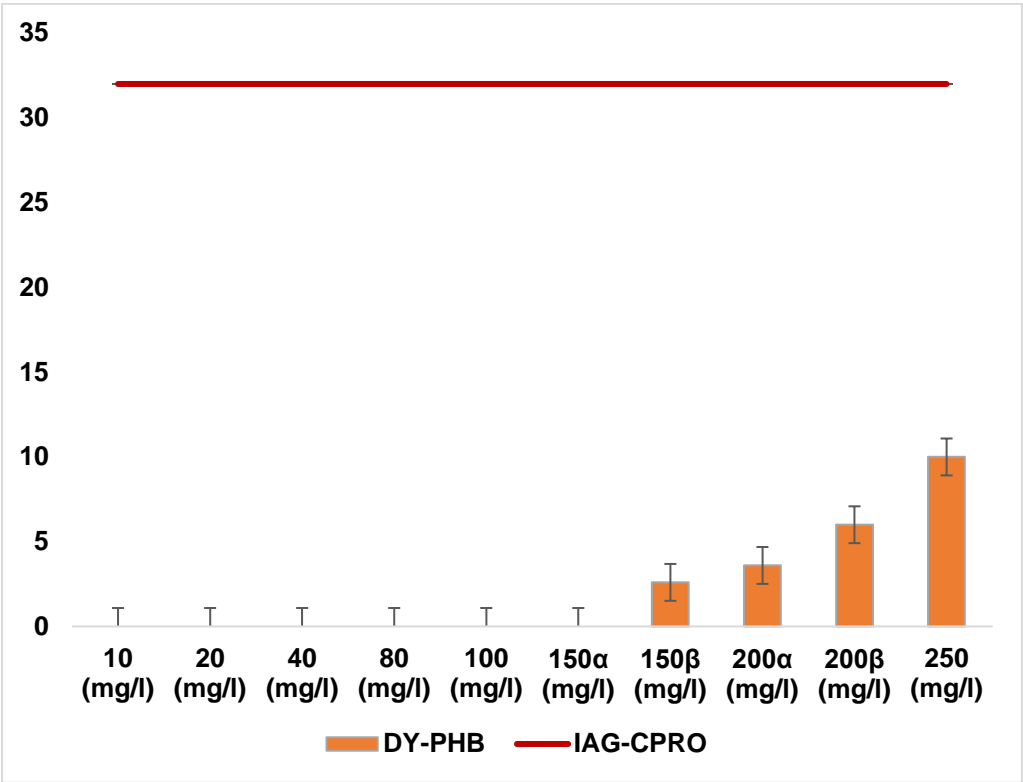**Fig. 4. Clear zones for different DB-TYFO222 concentrations**

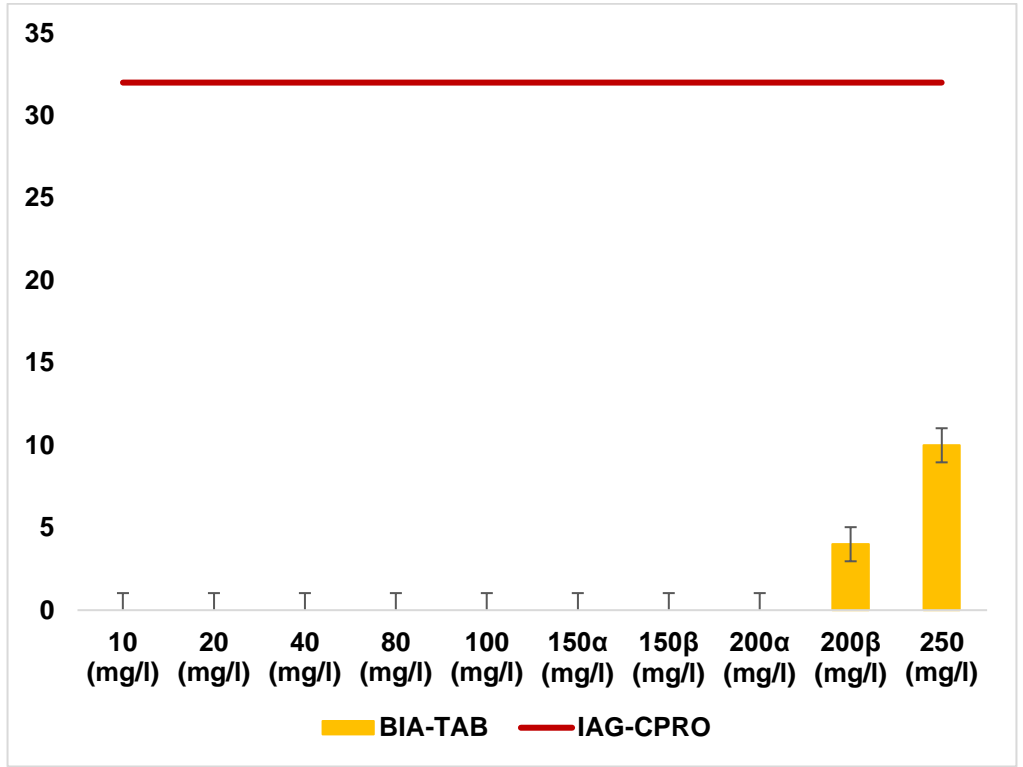**Fig. 5. Clear zones for different DY-PHB concentrations**

Fig. 6. Clear-Zones for the different concentrations of BIA-TABH

3.8 Inhibition Zone for BIA/TABH at Different Concentrations

Fig. 6 illustrates the inhibition zones of BIA/TABH at various concentrations. Notably, this herbal mixture produced clear zones of 10 and 4 mm at concentrations of 250 and 200^{β} mg/L, respectively. However, concentrations ranging from 10 to 150^{β} mg/L did not exhibit inhibition. A comparison of the mean inhibition zones of BIA-TABH with the control (IAG-CPRO) at different concentrations revealed a significant difference ($p < 0.05$), as detailed in Table 6.

3.9 Minimum Inhibitory Concentrations of the Herbal Mixtures

The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that can cause microbial death. For IBA-ENTR5 and DB-TYFO222, the MIC was determined to be 150 mg/L, indicating high inhibitory potency. DY-PHB had a minimum inhibitory concentration of 200^{α} mg/L, and BIA-TABH had the highest MIC of 250 mg/l. Fig. 7 visually illustrates the MICs of these drugs.

Table 6. Comparison of the mean inhibition zones of IBA-ENTR5, DB-TYFO222, DY-PHB, and BIA-TABH with those of IAG-CPRO (Control)

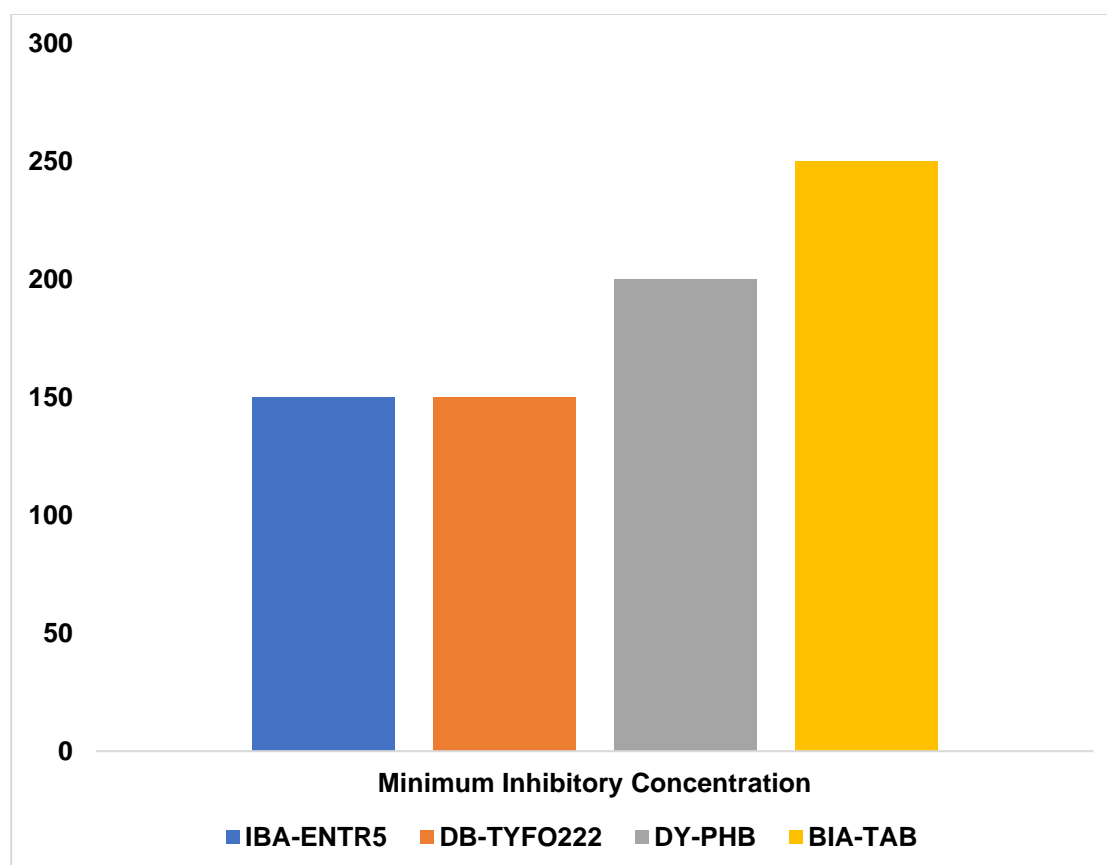| Sampled drug	Mean	StDev	SE Mean	95% CI for μ	T-Value	P-Value
IBA-ENTR5	9.8	13.28	4.2	(3.70 – 12.70)	5.29	0.001**
DB-TYFO222	3	5.44	1.72	(2.89 – 5.11)	16.87	0**
DY-PHB	2.22	3.44	1.09	(1.24 – 3.32)	27.38	0**
BIA-TAB	1.4	3.27	1.03	(1.11 – 2.26)	29.57	0**
IAG-CPRO	32	0	0	*	*	*
*p 0.05; **, p < 0.05						

Fig. 7. Minimum inhibitory concentrations of the herbal mixtures

4. DISCUSSION

4.1 Medicinal Plant Constituents of the Herbal Mixtures

The market for contemporary medicinal plants and raw herbal materials has rapidly expanded. This is due to the resurgence of interest in ethnopharmacology and traditional and alternative medicine. The medicinal value of herbal mixtures lies in chemical substances that have physiological functions in humans (Oladeji et al.,2019). Numerous plants have been used to treat typhoid fever in Ghana, but few have been documented. These include *Carica papaya, Morinda lucida, Citrus aurantifolia, Vernonia amygdalina Delile, Azadirachta indica, Cassia alata, Khaya senegalensis, Momordica charantia, Persea Americana, Cocos nucifera, Phyllanthus fratenus, Khaya ivorensis, Trema orientalis, Cryptolepis sanguinolenta, Psidium guajava, Cymbopogon citratus,* and *Pycnanthus angolensis* (Kisseih et al., 2017). This result affirms the numerous medicinal plant constituents identified during the study. A brief review of the medicinal plant constituents listed (presented in Tables 1 & 2) showed that they have been scientifically investigated for their therapeutic potency against typhoid and related symptoms (Al-Shalah et al.,2023). For example, Mangifera indica showed good anti-*Salmonella* activity against clinical isolates of *S. typhi* (Alaiya and Odeniyi, 2023). In addition, *Ocimum viride* has antibacterial potential against some pathogenic bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Shigella sp.* and *Salmonella sp* (Chukwuma et al., 2023). Finally, *Carica papaya* has been noted for its effectiveness against Gramme-negative and Gramme-positive bacteria, including *Escherichia coli*, *Salmonella typhi, Staphylococcus aureus, Klebsiella pneumoniae,* and *Proteus mirabilis,* with the highest activity against *S. typhi* (Dottoa and Abihudi, 2021). This could be the underlying reason why *Carica papaya* was one of the most used plant constituents in the sampled herbal mixtures. Many Ghanaians believe that a single herbal remedy could effectively address multiple health issues (Afriyie and Kumi-Kyereme, 2023) which could explain why a significant number of plant species (up to 16) were consistently found in all the sampled herbal mixtures. This suggests that these products were intentionally developed to address various ailments simultaneously, a practice commonly embraced by herbalists in Ghana (Nortey et al.,2023). Additionally, scientific evidence supports the idea that certain active ingredients can enhance their effects when combined with other herbs (Kahraman et al., 2020). However, it is essential to acknowledge the potential risks associated with this approach, including the possibility of negative outcomes and toxicities. Simply combining herbs does not guarantee optimal therapeutic activity, and the lack of clarity regarding plant parts used in these formulations further complicates matters (Nortey et al., 2020).

Herbalists often withhold information about the plant parts they use in their formulations to protect their brands from piracy (Asase, 2023). Nevertheless, disclosing this information is critical for the scientific validation of plant usage. Alternatively, the inclusion of multiple plants might stem from manufacturers' limited understanding of the active components of the extracts (Stéphane et al., 2021). To address these concerns, it is advisable to conduct bioactivity-guided isolation and characterisation studies on these formulations. This process helps identify active plant fractions and compounds, allowing for the exclusion of unnecessary or harmful elements from the formulations. Ultimately, this would enhance the safety and efficacy of the resulting products, potentially increasing their effectiveness in the treatment of typhoid through the use of higher concentrations of active ingredients.

4.2 Phytochemical Properties of the Herbal Mixtures

Phytochemical screening was qualitatively performed using several reagents to isolate various constituents of the plants used in the preparation of the sampled herbal mixtures to confirm their biological activity or medicinal uses. This analysis was important for identifying new sources of valuable therapeutic and industrial compounds in the herbal mixtures. Three of these four herbal mixtures (IBA-ENTR5, DB-TYFO222, DY-PHB, and BIA-TABH) contained the maximum number of plant constituents. The most commonly distributed compounds among the herbal mixtures were flavonoids, tannins, steroids, glycosides, alkaloids, saponins, and triterpenoids (Table 3).

The phytochemical constituents found in the herbal mixtures possess a wide range of medicinal properties that may protect against various diseases. For instance, previous studies have established that glycosides, for example, have sedative and digestive (Khan et al., 2020)

and anticancer properties (Du et al., 2021). In addition, tannins possess extremely stringent effects against diarrhoea and poisoning by heavy metals and alkaloids (Hussein et al., 2018) and stomach and duodenal tumours (Alqethami and Aldhebiani, 2021). They promote mucosal membrane inflammation and wound healing (Asase, 2024). In addition, alkaloids possess a variety of substituents, including amine, amide, phenol, and methoxy groups, which protect the body from chronic disorders (Tuzimski and Petruczynik, 2023). They have a significant impact on the nervous system and are also well known for their sedative, cardiac, respiratory, and relaxation effects, vasoconstriction, and muscle-relaxation effects (Hussein et al., 2018).

Saponins have analgesic, anti-inflammatory, antitumor, piscicidal, molluscicidal, spermicidal, sedative, and expectorant activities (Hussein et al., 2018). They also protect against hypercholesterolaemia (Sharmin et al., 2021). Furthermore, triterpenoids and steroids, which have analgesic effects, are generally used for pain relief and anaesthesia (Chen et al.,2018) and have antioxidant properties (Gadouche et al.,2023). In addition, flavonoids have antioxidant properties and can protect against heart disease and cancer (Li et al., 2023). They are also well known for their antiallergic, anti-inflammatory, and antithrombotic properties, as well as their ability to prevent tumour growth and preserve the stomach mucosa (Ullah et al., 2020). This observation was consistent with a series of earlier studies that found that plants used for the preparation of herbal mixtures contained the maximum number of secondary metabolites (phytochemicals) (Moomin et al., 2020).

4.3 Antimicrobial Activity of the Herbal Mixtures

Antimicrobial activity refers to the inhibition of unwanted microorganisms by antimicrobial agents (Romano et al., 2020). The herbal mixtures sampled showed good activity against the tested bacterial isolates. In this study, the highest antimicrobial activity per agar well diffusion was observed for IBA-ENTR5, followed by DB-TYFO222, DY-PHB, and BIA-TABH. Compared with the control (IAG-CPRO), only IBA-ENTR5 was highly susceptible at a concentration of 250 mg/L. This observation was supported by previous studies reporting higher antimicrobial activities of herbal mixtures compared with control samples (Sokoudjou et al.,2020). The various concentrations displayed

in Table 4 indicate that the higher the concentration, the greater the inhibition of the pathogen. No activity was recorded for BIA-TABH and DB-TYFO222 at a concentration of 150^α mg/L. This does not necessarily imply that the herbal mixtures sampled were ineffective; however, the extraction methods could account for the results (Zhang et al., 2020). This was supported by Iroha and co-authors, who observed that certain effective typhoid fever drugs in Nigeria did not exhibit any activity. The authors further attributed the drug concentration and extraction methods to these results (Iroha et al., 2023). In another study, the use of other solvents, such as ethyl acetate, water, ethanol, and ethanol, yielded very low quantities of plant extracts and showed poor activity against bacterial isolates (Mdee et al.,2009). However, other studies have reported higher yields of bacterial isolate extracts. This could be attributed to the processing methods of herbal mixtures, plant compositions, harvesting, and preservation procedures of plants in the herbal concoctions (Syamsuri et al., 2020). Therefore, the ability of an herbal mixture or plant extract to exhibit activity depends on the extraction method, the concentration of the herbal mixture or extract, harvesting, and preservation procedures used for medicinal plants (Vaou et al.,2021). Similarly, another study has posited that bacteria react differently to herbal mixtures. This phenomenon can be attributed to the cell wall structure of bacteria (Shenderov et al.,2013). Moreover, gram-negative bacteria possess an outer layer known as the phospholipid layer, which makes it harder for medicinal constituents of plants to penetrate (Shenderov et al., 2013).

4.4 Minimum Inhibitory Concentration (MIC) of the Herbal Mixtures

The minimum inhibitory concentration (MIC) test is a crucial step in drug discovery, providing valuable insights into the effectiveness of a substance in inhibiting the growth of pathogens (Balali et al.,2023). The lowest concentration of a drug or antimicrobial agent is required to prevent the visible growth of a microorganism. This test is particularly essential for understanding the potency and efficacy of the sampled mixtures because it helps determine the minimal dose required to exert an inhibitory effect on the target pathogen (Rodríguez-Gascón et al., 2021). Differences in MICs are usually caused by the organism, concentration, and nature of the drug (Kowalska-Krochmal and Dudek-Wicher, 2021). In this study, the MIC values ranged from 150^α to

250 mg/L. Because *S. typhi* is a gramme-negative organism, the recorded MIC values could be attributed to the fact that its cell walls contain a phospholipid layer that is difficult to penetrate, making it difficult for the plant extract to seep through to provide lower values (Vaou et al., 2021). Another factor that could explain the observed results was that the bacterial isolates of *S. typhi* could be resistant strains (Shaikh et al., 2023). Moreover, synergy among the phytochemicals of the sampled herbal mixtures could account for the MICs obtained (AlSheikh et al., 2020). The implication of this finding could be that increasing the concentrations of plant extracts used in the preparation of herbal mixtures could reduce the growth of microorganisms and slow down the development of resistant strains. In practice, various drugs have been proven effective in treating typhoid fever; hence, it was not surprising that the current study showed the effectiveness of the sampled herbal mixtures against bacterial isolates. This observation is consistent with previous studies reporting high MICs against *S. typhi* (Balali et al.,2023).

5. CONCLUSIONS

This study determined the antibiotic sensitivity of four selected herbal mixtures, IBA-ENTR5, DB-TYFO222, DY-PHB, and BIA-TABH, against the clinical strain of *S. typhi*. This study revealed that IBA-ENTR5 had a strong bactericidal effect, and the sensitivity of DB-TYFO222 and DY-PHB to the bacteria was intermediate. In contrast, *S. typhi* was somewhat resistant to BIA-TABH. The results obtained from this study also showed that the sampled herbal mixtures have high potential bactericidal effects against *S. typhi* at a concentration of 250 mg/L compared with the positive control (IAG-CPRO) and other concentrations used in our study. The results further revealed that the sampled herbal mixtures possess rich phytochemical constituents. The study also revealed that the herbal mixtures sampled provided good results when their concentrations were increased. Therefore, it can be concluded that commercial herbal mixtures in the open Ghanaian market may provide good antimicrobial results when there is an increase in the intake regarding the number of dosages.

6. STUDY'S LIMITATIONS AND FURTHER RESEARCH

Although this study provides valuable insights into the potential bactericidal effects of IBA-ENTR5, DB-TYFO222, DY-PHB, and BIA-TABH against *S. typhi*, it is important to acknowledge the limitations of this study that call for further studies. First, this study focused solely on the efficacy and antibiotic sensitivity of the selected herbal mixtures against only one clinical strain of Salmonella (*S. typhi*). The decision to focus exclusively on one strain was made due to logistic limitations. Therefore, to enhance the robustness and applicability of the proposed method to future research, a more diverse range of *Salmonella sp.* and other bacterial strains with varying levels of resistance should be considered. This will provide a more comprehensive overview of the efficacy of drugs across different resistance profiles and contribute to a more generalised interpretation of their potential applications. This study did not integrate High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) as part of compound identification and characterisation. Although HPLC-MS offers significant advantages for detailed phytochemical analysis, its absence in this study reflects the preliminary nature of the research, resource constraints, and specific focus on initial antimicrobial screening. Future studies could incorporate HPLC-MS to build on these findings and provide a more detailed understanding of the bioactive compounds present in herbal mixtures. Detailed compound identification and characterisation using HPLC-MS could be planned for follow-up studies after establishing efficacy. Despite these limitations, our study addresses the current gap in research on the resistance of microbial strains, such as *S. typhi*, to conventional drugs.

7. RECOMMENDATIONS

Most Ghanaians rely on herbal mixtures as their first-line treatment; hence, stakeholders such as the FDA, pharmaceutical companies, and herbal companies must focus particularly on ensuring that topical herbal preparations are up to the standard and can effectively eliminate typhoid fever. Second, the FDA, pharmaceutical companies, and herbal companies should regulate the dosage of these herbal mixtures to prevent microbial resistance. This approach could help prevent cost-related challenges in the incidence of drug resistance. In addition, consumers and the public should be made aware of the appropriate dosages to prevent increased resistance to *Salmonella sp.* This approach could help reduce typhoid treatment failures, especially in developing countries. In conclusion, to

increase the robustness of modern and herbal medicines, future studies are needed to validate the effectiveness of herbal preparations, such as those obtained in this study.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ACKNOWLEDGEMENT

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Adeleye, O. A., et al. (2022). Medicinal plants with potential inhibitory bioactive compounds against coronaviruses. *Advanced Pharmaceutical Bulletin, 12*(1), 7–16. https://doi.org/10.34172/apb.2022.003

Afriyie, J., & Kumi-Kyereme, A. (2023). Predictors of herbal medicine use in Ashanti Region of Ghana. *Advances in Integrative Medicine, 10*(2), 80–85. https://doi.org/10.1016/j.aimed.2023.05.005

Akram, J., et al. (2020). Extensively Drug-Resistant (XDR) Typhoid: Evolution, prevention, and its management. *Biomed Research International, 2020*, 6432580. https://doi.org/10.1155/2020/6432580

Alaiya, M. A., & Odeniyi, M. A. (2023). Utilisation of *Mangifera indica* plant extracts and parts in antimicrobial formulations and as a pharmaceutical excipient: A review. *Future Journal of Pharmaceutical Sciences, 9*(1), 29. https://doi.org/10.1186/s43094-023-00479-z

Alqethami, A., & Aldhebiani, A. Y. (2021). Medicinal plants used in Jeddah, Saudi Arabia: Phytochemical screening. *Saudi Journal of Biological Sciences, 28*(1), 805–812. https://doi.org/10.1016/j.sjbs.2020.11.013

Al-Shalah, L. A. M., et al. (2023). Antibacterial effect of aqueous extract of *Theobroma cacao* against Gram-negative and Gram-positive bacteria: An *in vitro* study. *Journal of Medicinal and Chemical Sciences, 6*(3), 464–471. https://doi.org/10.26655/JMCHEMSCI.2023.3.2

AlSheikh, H. M. A., et al. (2020). Plant-based phytochemicals as possible alternatives to antibiotics in combating bacterial drug resistance. *Antibiotics, 9*(8), Article 480. https://doi.org/10.3390/antibiotics9080480

Asamoah, C., & Mensah, E. (2018). Analysis of typhoid fever surveillance data, Cape Coast Metropolis, 2016. *Pan African Medical Journal Conference Proceedings, 1.* https://doi.org/10.11604/pamj.cp.2017.3.49.203

Asase, A. (2023). Ghana's herbal medicine industry: Prospects, challenges, and ways forward from a developing country perspective. *Frontiers in Pharmacology, 14.* Retrieved February 6, 2024, from https://www.frontiersin.org/articles/10.3389/fphar.2023.1267398

Ashraf, M. V., et al. (2023). Phytochemicals as antimicrobials: Prospecting Himalayan medicinal plants as sources of alternate medicine to combat antimicrobial resistance. *Pharmaceuticals, 16*(6). https://doi.org/10.3390/ph16060881

Balali, G. I., Yar, D. D., & Sylverken, A. A. (2023). Antimicrobial activities of *Hibiscus sabdariffa* and *Aspilia africana* against clinical isolates of *Salmonella typhi*. *Scientific African, 20*, e01667. https://doi.org/10.1016/j.sciaf.2023.e01667

Bardoe, D., et al. (2023). Evaluation of bacteria composition in smoked fish processed in Yeji-Pru East District, Ghana. *Journal of Advances in Microbiology, 23*(4), 50–65. https://doi.org/10.9734/jamb/2023/v23i4720

Chen, S., et al. (2018). Analgesic effects of triterpenoid saponins from *Stauntonia chinensis* via selective increase in

inhibitory synaptic response in mouse cortical neurons. *Frontiers in Pharmacology, 9,* Article 1302. https://doi.org/10.3389/fphar.2018.01302

Chiangnoon, R., et al. (2022). Phytochemical analysis, antioxidant, and wound healing activity of *Pluchea indica* L. branch extract nanoparticles. *Molecules, 27*(3), 635. https://doi.org/10.3390/molecules2703063 5

Chukwuma, I. F., et al. (2023). African and Holy Basil—a review of ethnobotany, phytochemistry, and toxicity of their essential oil: Current trends and prospects for antimicrobial/anti-parasitic pharmacology. *Arabian Journal of Chemistry, 16*(7), 104870. https://doi.org/10.1016/j.arabjc.2023.10487 0

Dotto, J. M., & Abihudi, S. A. (2021). Nutraceutical value of *Carica papaya*: A review. *Scientific African, 13*, e00933. https://doi.org/10.1016/j.sciaf.2021.e00933

Du, J., Jiang, L., Chen, F., Hu, H., & Zhou, M. (2021). Cardiac glycoside ouabain exerts anticancer activity via downregulation of STAT3. *Frontiers in Oncology, 11.* Retrieved March 31, 2023, from https://www.frontiersin.org/articles/10.3389 /fonc.2021.684316

Gadouche, L., et al. (2023). Triterpenoid and steroid content of lipophilic extracts of selected medicinal plants of the Mediterranean region. *Molecules, 28*(2), Article 2. https://doi.org/10.3390/molecules2802069 7

Hussein, R. A., & El-Anssary, A. A. (2018). Plants secondary metabolites: The key drivers of the pharmacological actions of medicinal plants. IntechOpen. https://doi.org/10.5772/intechopen.76139

Hussen, E. M., & Endalew, S. A. (2023). *In vitro* antioxidant and free-radical scavenging activities of polar leaf extracts of *Vernonia amygdalina*. *BMC Complementary Medicine and Therapies, 23*(1), 146. https://doi.org/10.1186/s12906-023-03923-y

Iroha, I., Ilang, D., Ayogu, T., Oji, A., & Ugbo, E. (2010). Screening for anti-typhoid activity of some medicinal plants used in traditional medicine in Ebonyi State, Nigeria. *African Journal of Pharmacy and Pharmacology.* Retrieved March 30, 2023, from https://www.semanticscholar.org/paper/23

7ab40e9cf28aa1bf0694823517d1c29c43b 054

Kahraman, C., Arituluk, Z. C., & Cankaya, I. I. T. (2020). The clinical importance of herb-drug interactions and toxicological risks of plants and herbal products. In *Medical Toxicology*. IntechOpen. https://doi.org/10.5772/intechopen.92040

Khan, H., et al. (2020). The analgesic potential of glycosides derived from medicinal plants. *Daru, 28*(1), 387–401. https://doi.org/10.1007/s40199-019-00319-7

Kifle, Z. D., Yesuf, J. S., & Atnafie, S. A. (2020). Evaluation of *in vitro* and *in vivo* anti-diabetic, anti-hyperlipidemic, and anti-oxidant activity of flower crude extract and solvent fractions of *Hagenia abyssinica* (Rosaceae). *Journal of Experimental Pharmacology, 12,* 151–167. https://doi.org/10.2147/JEP.S249964

Kisseih, E., et al. (2017). Herbal medicines used in the treatment of typhoid in the Ga East Municipality of Ghana. *International Journal of Tropical Disease & Health, 23*, 1–13. https://doi.org/10.9734/IJTDH/2017/31448

Kowalska-Krochmal, B., & Dudek-Wicher, R. (2021). The minimum inhibitory concentration of antibiotics: Methods, interpretation, clinical relevance. *Pathogens, 10*(2), Article 165. https://doi.org/10.3390/pathogens1002016 5

Li, M., Qian, M., Jiang, Q., Tan, B., Yin, Y., & Han, X. (2023). Evidence of flavonoids on disease prevention. *Antioxidants, 12*(2), Article 2. https://doi.org/10.3390/antiox12020527

Linthoingambi, W., & Singh, M. S. (2013). Antimicrobial activities of different solvent extracts of *Tithonia diversifolia* (*Hemsley*) A. Gray. Retrieved March 30, 2023, from https://www.semanticscholar.org/paper/e2 9abee992640aaf3c74aced8fa3caef56e3f2 0a

Lovane, L., et al. (2016). Carriage prevalence of *Salmonella enterica* serotype Typhi in gallbladders of adult autopsy cases from Mozambique. *Journal of Infection in Developing Countries, 10*(4), 410–412. https://doi.org/10.3855/jidc.7306

Madrigal-Santillán, E., et al. (2014). Review of natural products with hepatoprotective effects. *World Journal of Gastroenterology, 20*(40), 14787–14804. https://doi.org/10.3748/wjg.v20.i40.14787

Mdee, L. K., Masoko, P., & Eloff, J. N. (2009). The activity of extracts of seven common invasive plant species on fungal phytopathogens. *South African Journal of Botany, 75*(2), 375–379. https://doi.org/10.1016/j.sajb.2009.02.003

Mensah, M., et al. (2019). Toxicity and safety implications of herbal medicines used in Africa. https://doi.org/10.5772/intechopen.72437

Monika, Y. S., Dhruv, M., & Preeti, C. (2023). Medicinal uses of traditionally used plants in Bhatwari Block, District Uttarkashi, Uttarakhand, India. Retrieved March 29, 2023, from https://www.researchgate.net/publication/339171726

Moomin, A., et al. (2023). Season, storage, and extraction method impact on the phytochemical profile of *Terminalia ivorensis. BMC Plant Biology, 23*(1), Article 162. https://doi.org/10.1186/s12870-023-04144-8

Nortey, N. N. D., et al. (2023). Herbs used in antimalarial medicines: A study in the Greater Accra Region of Ghana. *Evidence-Based Complementary and Alternative Medicine, 2023*, Article 6697078. https://doi.org/10.1155/2023/6697078

Obaro, S. K., Iroh Tam, P.-Y., & Mintz, E. D. (2017). The unrecognized burden of typhoid fever. *Expert Review of Vaccines, 16*(3), 249–260. https://doi.org/10.1080/14760584.2017.1255553

Okaiyeto, K., & Oguntibeju, O. O. (2021). African herbal medicines: Adverse effects and cytotoxic potentials with different therapeutic applications. *International Journal of Environmental Research and Public Health, 18*(11). https://doi.org/10.3390/ijerph18115988

Oladeji, O., Abraham, O., & Oloke, J. (2019). Phytochemical screening and antimicrobial investigation of *Moringa oleifera* leaf extracts. *African Journal of Science, Technology, Innovation and Development, 12*, 1–6. https://doi.org/10.1080/20421338.2019.1589082

Rodríguez-Gascón, A., Solinís, M. Á., & Isla, A. (2021). The role of PK/PD analysis in the development and evaluation of antimicrobials. *Pharmaceutics, 13*(6), Article 833.

https://doi.org/10.3390/pharmaceutics13060833

Romano, I., et al. (2020). Inhibition of bacterial growth on marble stone of the 18th century by treatment of nanoencapsulated essential oils. *International Biodeterioration & Biodegradation, 148*, Article 104909. https://doi.org/10.1016/j.ibiod.2020.104909

Shaikh, O. A., et al. (2023). Antimicrobial resistant strains of *Salmonella typhi*: The role of illicit antibiotics sales, misuse, and self-medication practices in Pakistan. *Journal of Infection and Public Health, 16*(10), 1591–1597. https://doi.org/10.1016/j.jiph.2023.08.003

Sharmin, F., Koyama, T., Koyama, H., & Ishizaki, S. (2021). Cholesterol-binding ability of saponin from Japanese starfish. *Journal of Food Science and Technology, 58*(8), 3056–3064. https://doi.org/10.1007/s13197-020-04809-4

Sheeba, H., Ali, M., & Anuradha, V. (2019). Bioactive compounds and antimicrobial activity of fungal crude extract from medicinal plants.

Shenderov, B. A. (2013). Metabiotics: Novel idea or natural development of probiotic conception. *Microbial Ecology in Health and Disease, 24*. https://doi.org/10.3402/mehd.v24i0.20399

Sokoudjou, J. B., et al. (2020). Isolation, characterization, and in vitro anti-salmonellal activity of compounds from stem bark extract of *Canarium schweinfurthii*.

Ssenku, J. E., et al. (2022). Medicinal plant use, conservation, and the associated traditional knowledge in rural communities in Eastern Uganda. *Tropical Medicine and Health, 50*(1), 39. https://doi.org/10.1186/s41182-022-00428-1

Stéphane, F. F. Y., et al. (2021). Extraction of bioactive compounds from medicinal plants and herbs. In *Natural Medicinal Plants*. IntechOpen. https://doi.org/10.5772/intechopen.98602

Syamsuri, F., et al. (2018). A review: Worldwide medicinal plants for typhoid fever. *Indian Journal of Public Health Research & Development, 9*(8), 1461. https://doi.org/10.5958/0976-5506.2018.00938.5

Tuzimski, T., & Petruczynik, A. (2023). New trends in the practical use of isoquinoline

alkaloids as potential drugs applied in infectious and non-infectious diseases. *Biomedicine & Pharmacotherapy, 168*, Article 115704. https://doi.org/10.1016/j.biopha.2023.115704

Ullah, A., et al. (2020). Important flavonoids and their role as a therapeutic agent. *Molecules, 25*(22), Article 5243. https://doi.org/10.3390/molecules25225243

Vaou, N., et al. (2021). Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. *Microorganisms, 9*(10), Article 2021. https://doi.org/10.3390/microorganisms9102041

Wiegand, I., Hilpert, K., & Hancock, R. E. W. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols, 3*(2), 163–175. https://doi.org/10.1038/nprot.2007.521

World Health Organization (WHO). (2023). Typhoid. Retrieved December 14, 2023, from https://www.who.int/news-room/fact-sheets/detail/typhoid

Wu, K., et al. (2023). Antioxidant, anti-inflammatory, and antibacterial tannin-crosslinked citrate-based mussel-inspired bioadhesives facilitate scarless wound healing. *Bioactive Materials, 20*, 93–110. https://doi.org/10.1016/j.bioactmat.2022.05.017

Zhang, Q.-W., Lin, L.-G., & Ye, W.-C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese Medicine, 13*, Article 20. https://doi.org/10.1186/s13020-018-0177-x

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/128486