



***In-vitro* Assessment of Antibacterial Activity of Local ‘Typhoid-Cure’ Cocktail of Leaves of Five Medicinal Plants Against Multidrug-Resistant *Salmonella typhi* from Clinical Sources**

Folasade Muibat Adeyemi^{a*}, Mahboob Adekilekun Jimoh^b, Yejide Posi Ajayi^a,
Mulikat Abiola Jimoh^b, Abideen Akinkunmi Wahab^a, and
Omotayo Opemipo Oyedara^c

^aDepartment of Microbiology, Faculty of Basic and Applied Sciences, Osun State University, P.M.B. 4494, 230212, Osogbo, Nigeria

^bDepartment of Plant Biology, Faculty of Basic and Applied Sciences, Osun State University, P.M.B. 4494, 230212, Osogbo, Nigeria

^cDepartment of Biotechnology, Faculty of Basic and Applied Sciences, Osun State University, P.M.B. 4494, 230212, Osogbo, Nigeria.

ABSTRACT

For centuries, medicinal plants have been used for curative purposes in traditional healthcare systems worldwide. In Nigeria, many indigenes opt for local herbs made from medicinal plants. However, the efficacy of local herbs needs to be consistently validated. This study evaluated the effectiveness of the extracts of five types of plant leaves (*Azadirachta indica*, *Carica papaya*, *Gossypium hirsutum*, *Jatropha curcas* and *Ricinus communis*) against clinical isolates of *Salmonella typhi*. The antibiotic susceptibility of the isolates was evaluated using the disk-diffusion method. Additionally, the crude aqueous extracts, along with a combined mixture of all five plant extracts, were assessed for in vitro antimicrobial activity through the agar well diffusion assay. Phytochemical analysis of the plant samples was conducted to identify and evaluate the presence of secondary metabolites. Phytochemical analyses revealed the presence of steroids, flavonoids, glycosides, and tannins in all plants, but no terpenoids. Altogether, 57 *S. typhi* isolates were isolated from 280 rectal swabs collected from hospital patients (20.4%). All were multidrug-resistant, with 100% resistance to sulfonamide, followed by ticarcillin and fosfomycin at 93.0% each, while resistance to nitrofurantoin was lowest at 26.3%. Neither crude extracts nor the reference sample displayed any antibacterial activity. However, a particular preparation of *A. indica* (10g extract in 50mL hot water) revealed zones of inhibition ($\geq 7\text{mm}$ and $\leq 12\text{mm}$) against 10.5% of isolates (6/57). None of the plant aqueous extracts exhibited noteworthy antibacterial activity against any of the 57 *S. typhi* strains. Therefore, there is an urgent need to curtail the regular dispensing of these local herbs until further studies are done to validate their efficacy and establish appropriate administration regimes for optimum effect.

Keywords: *Azadirachta indica*; Medicinal plants; Multidrug resistance; Phytochemicals; *Salmonella typhi*; Typhoid fever.

INTRODUCTION

Salmonella enterica subsp. *enterica* serovar Typhi (*S. Typhi*) is a causative agent that causes Typhoid fever in human beings (Kim et al., 2023). Typhoid fever is a globally significant public health concern, particularly in low- and middle-income

countries (LMICs) where access to clean water and appropriate hygiene systems is inadequate (GBD, 2019; Mogasale et al., 2014). It is an insidious disease of high burden, predominantly in South Asia, Southeast Asia, and Sub-Saharan Africa where roughly 11 to 21 million people get infected each year, with annual mortality ranging between

*Corresponding author: folasade.adeyemi@uniosun.edu.ng

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128,000 to 161,000 people (GBD, 2020a; GBD, 2020b; Kim et al., 2017; Kirk et al., 2015). Typically associated with abdominal pain, headache, high fever, fatigue, vomiting, and diarrhoea, trailed by constipation and rashes (Ünüvar, 2018), typhoid fever is transmitted via contaminated food and water through the ingestion of contaminated food or water, entering via the intestinal lining, and spreading to intracellular spaces and host systems (Meiring et al., 2023). It can also be spread through person-to-person contact via unsanitary habits and faecal matter from infected individuals as humans are the sole reservoirs of the pathogen, serving as cases and/or carriers (Kanungo et al., 2008).

Globally, use of botanicals for therapeutic purposes in traditional healthcare delivery systems has existed for ages. Various human diseases all over the world have been treated with desirable plants variously formulated into powders, concoctions, decoctions, infusions, or poultices (Salim et al., 2019; Tabuti et al., 2003; Umair et al., 2019) and administered in diverse ways and dosages. Within communities in sub-Saharan Africa, particularly in Nigeria, many indigenes resort to preparations from medicinal plant parts—leaves, stems, barks, flowers, fruits, and roots—to treat typhoid fever (Aliyu et al., 2020). This stems from the belief that plants are natural and as such are non-toxic, organic, easily available and accessible, affordable, and have a lower propensity for adverse reactions or negative side effects (Umair et al., 2019). It is also believed that local cures are much more effective than conventional antibiotics and evade challenges posed by the therapeutic failure of antibiotics (possibly from antibiotic resistance) and the progressively rising cost of available drugs developed to treat typhoid fever. Just some of the plants believed to have medicinal properties available in Nigeria include *Allium sativum*, *Alchornea cordifolia*, *Ananas comosus*, *Citrus paradisi*, *Carica papaya*, *Mangifera indica*, *Moringa oleifera*, *Azadirachta indica*, *Gossypium barbandense* L., and *Vernonia amygdalina* (Aliyu et al., 2020; Okosodo and Mohapatra, 2021; Olusola et al., 2023; Zakariya et al., 2021).

Within the last few years, there has been a steady world-wide rise of interest in traditional cures for infectious and non-infectious diseases, and a significantly intensified search for bioactive compounds in plants (dos Santos et al., 2022, Adetunji et al., 2024, Zaky et al., 2024). In Nigeria, especially southwestern Nigeria, herbal medicines have been an integral part of the healthcare culture of the people for centuries, and to date, still constitute an intrinsic part of the treatment system

in a large percentage of indigenous communities (Oreagba et al., 2011, Li et al. 2020, Eruaga et al., 2024). Many local herb sellers have profound albeit undocumented traditional knowledge about the use of herbs and indigenous plant species in the treatment of a myriad of diseases. However, the efficacies of these locally procured herbs need to be validated. This study, therefore, was aimed at evaluating the efficacy of a cocktail of the extracts from the leaves of five different plants used in the traditional treatment of typhoid fever (*Azadirachta indica* [neem], *Carica papaya* [pawpaw], *Gossypium hirsutum* [cotton], *Jatropha curcas* [physic nut] and *Ricinus communis* [castor oil plant]) against clinical isolates of *S. typhi*. The leaves were all collected from homestead gardens in Osogbo Metropolis, Southwest Nigeria.

MATERIALS AND METHODS

Study Area and Study Design:

This research was a cross-sectional study carried out at the Osogbo Central Hospital, Oke-Baale, Osogbo, Osun State Nigeria (with coordinates 7.775058, 4.545022) (Fig. 1). A total of 280 in- and out-patients enrolled for the study after obtaining the Ethical approval. Parental consent was sought from parents/guardians for individual participants under the age of 18 years.

Collection and Processing of Samples:

Rectal swabs were collected from 280 participants. Sterile cotton-tipped applicators were inserted approximately one inch beyond the anal sphincter of each participant, then carefully rotated to sample the anal crypts, after which the applicator was withdrawn and properly labelled. All collected swabs were stored with ice packs in a box to maintain appropriate conditions and were promptly transported to the Microbiology Laboratory at Osun State University, Osogbo, for subsequent processing.

The rectal swabs collected were inoculated in test tubes containing 10mL each of sterile Selenite F broth and incubated for 24–48 hours at 37±2°C. Afterwards, a loopful of each culture was streaked out on solidified Salmonella-Shigella agar (SSA) plates and incubated for 24 hours at 37±2°C. The development of colonies with black centres on the plates denotes the presence of *Salmonella* species. Distinct colonies were sub-cultured on new SSA plates to acquire pure cultures, which were then stored in Tryptone soy broth (TSB) enriched with 15% glycerol at -20°C. The identity of isolates was confirmed by growth on Hi-Chrome agar, as well as the rapid biochemical tests by using Analytical Profile Index API 20E (bioMérieux, France).

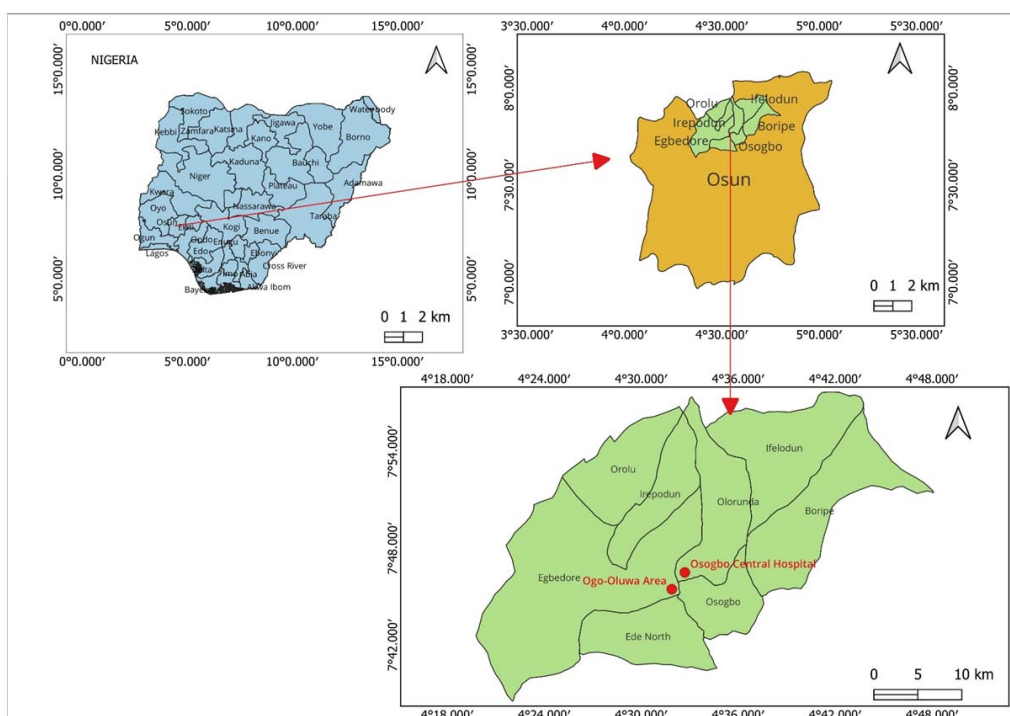


Fig. 1. The map of the study area showing patient sampling and leaf collection sites

Antibiotic Susceptibility Testing:

The recovered *Salmonella* isolates were assessed for antibiotic susceptibility using the agar disc diffusion techniques (Bauer *et al.*, 1966) against 8 selected antibiotics (Oxoid) in eight separate classes. These antibiotics include aztreonam (30µg), ticarcillin (75µg), trimethoprim-sulfamethoxazole (1.25-23.75), fosfomycin (50µg), gentamicin (10µg), levofloxacin (5µg), nitrofurantoin (100µg), and tigecycline (15µg). Sterile Mueller Hinton agar (MHA) plates were inoculated with the pure test isolate with a sterile cotton-tipped applicator to produce a lawn, and the antibiotic discs were aseptically placed using an Oxoid 8-place disc dispenser, then incubated at $37\pm 2^{\circ}\text{C}$ for 18–24 hours. Inhibition zones (if any) were visually observed, and the zone diameter was recorded (in mm). The results were interpreted using the EUCAST breakpoint guideline vs 14.0 (EUCAST, 2024). Each bacterial isolate's Multiple

Antibiotic Resistance Index (MARI) was calculated using the methods described earlier. (Krumperman, 1983).

Preparation of Leaf Extract:

Collection, Identification and Preparation of Plant Samples:

Fresh whole leaves of each of the five medicinal plants to be screened for antimicrobial activity against *S. typhi*—namely neem (*A. indica*), pawpaw (*C. papaya*), cotton (*G. hirsutum*), physic nut (*J. curcas*) and castor oil (*R. communis*)—were collected from Ogo-Oluwa area (with GPS coordinates 7.75765, 4.53154) in Osogbo, Osun State, Nigeria. Voucher specimens of leaves of each plant were collected and processed for confirmation and allocation of voucher numbers within the Department of Botany's herbarium at Obafemi Awolowo University, Ile-Ife, Nigeria (IFE Herbarium). (Table 1). In the laboratory, the

Table 1: Plant species used in *in-vitro* tests against *Salmonella typhi*

| S/No. | Name of Plant | Biological Name | Family | Voucher Number in IFE Herbarium |
|-------|------------------------|----------------------------------|---------------|---------------------------------|
| 1. | Neem /Indian lilac | <i>Azadirachta indica</i> A.Juss | Meliaceae | IFE – 18311 |
| 2. | Pawpaw | <i>Carica papaya</i> L. | Caricaceae | IFE – 18312 |
| 3. | Upland Cotton | <i>Gossypium hirsutum</i> L. | Malvaceae | IFE – 18313 |
| 4. | Physic /Barbados Nut | <i>Jatropha curcas</i> L. | Euphorbiaceae | IFE – 18314 |
| 5. | Castor bean/Castor oil | <i>Ricinus communis</i> L. | Euphorbiaceae | IFE – 18315 |

leaves of each plant species collected were thoroughly rinsed in clean water, air-dried, and later sun-dried for 2 days. The leaves were then pulverized using a sterile blender and stored in moisture-free containers for further use.

Aqueous Extraction in Cold and Hot Water:

Extracts of different concentrations were obtained by separately weighing 10g of each leaf powder sample into 50mL, 100mL, and 200mL of each of cold and hot water for 48 hours and two hours, respectively. A cocktail comprising a combination of the five samples was obtained by weighing 2g of each leaf powder to make up 10g and processed as described above in both cold and hot water. The suspensions were filtered, and the filtrates were kept until needed.

Aqueous Extraction by Soaking in Distilled Water:

Aqueous plant extracts of the five plant powders were obtained by weighing 5g of each pulverized leaf powder separately into 50mL of sterile distilled water for 120 hours (5 days). This was shaken intermittently using a shaker incubator, and then Whatman No. 1 filter paper was used to filter the filtrate, which was then collected in a beaker for future use.

Aqueous Decoction by Boiling for Ten Minutes:

The decoction of the plant extracts was made by weighing 5g of each leaf powder sample into 50mL of sterile distilled water. The mixture was boiled for 10 minutes and allowed to cool. The mixture was separated by filtration through Whatman No. 1 filter paper, and the liquid portion was preserved for subsequent testing.

Aqueous Extraction by Squeezing Fresh Leaves:

The fresh leaves of each plant were rinsed in clean water and 20g was weighed and squeezed. The extracts were filtered and kept in a beaker for further use.

In-vitro antimicrobial screening of crude leaf extracts against recovered *Salmonella typhi* isolates:

On sterile Mueller Hinton agar (MHA) plates, the agar well diffusion method was used to evaluate the crude leaf extracts' antibacterial activity against the test *S. typhi* isolates. (Adeyemi et al., 2023). Eight holes of uniform diameter and depth (approximately 6mm and 4mm, respectively), about 25mm apart were made on the solidified sterile MHA plates using a sterile cork-borer. A few colonies of an 18-to-24-hour culture of each test isolate, dispersed into 5mL sterile Ringer to form a suspension, and standardized to 0.5 McFarland Turbidity Standard (to attain an inoculum density $\approx 1 \times 10^8$ CFU/mL) were

homogenously swabbed on an agar plate with a sterile swab stick to produce a lawn. A 100 μ L aliquot of each of the six crude plant extract filtrates (extracted in different volumes of hot and cold water as described above) was dispensed into six of the wells with a micropipette and labelled appropriately. Ciprofloxacin and sterile distilled water (100 μ L each) were dispensed into the last two wells, as positive and negative controls respectively.

The antibacterial activity of the leaf extracts prepared via soaking, boiling, and squeezing was assessed as described above. Three separate MHA plates on which eight wells were bored were used for each test isolate, one each for the crude leaf extracts prepared by soaking, boiling, and squeezing. Five of the wells on each plate were dispensed with 100 μ L aliquot of each of the crude extracts of *A. indica*, *C. papaya*, *G. hirsutum*, *J. curcas*, and *R. communis*. A combination of the five samples was obtained by adding 0.5mL of each of the crude extracts of each plant prepared by soaking, boiling, and squeezing respectively, and 100 μ L of each mixture was added to the sixth well, while the last two wells were filled with positive and negative controls (Ciprofloxacin and sterile distilled water), respectively.

A reference sample obtained from a local herb seller comprising a combination of the powdered leaves of the five plants was also prepared as described above and screened against the *S. typhi* isolates using the agar diffusion method. All inoculated plates were incubated overnight at $37 \pm 2^\circ\text{C}$ and observed visually afterwards for clear zones indicating inhibition around each well. If present, the zone diameters were measured to the nearest mm, and the sizes were documented. Resistance was indicated by the complete absence of clearing around the wells.

Qualitative Phytochemical Screening of Crude Plant Extracts:

Qualitative phytochemical screening of the crude plant extracts of *A. indica*, *C. papaya*, *G. hirsutum*, *J. curcas* and *R. communis* for the presence of secondary metabolites (alkaloids, steroids, flavonoids, saponins, glycosides, tannins, reducing sugars, and terpenes) was conducted.

Qualitative test for alkaloids:

Each crude plant extract (3 mL) was combined with 1 mL of 1% HCl. The mixtures were heated in a water bath for 2 minutes with constant stirring, then cooled and subjected to filtration. A 1 mL sample of each filtrate was treated with 0.5 mL of Mayer-Wagner reagent. The appearance of a precipitate, ranging from cream to brown-red, confirmed the presence of alkaloids, as outlined by Solanki et al. (2019).

Qualitative test for steroids:

Approximately 0.5 g of each extract was mixed with 5 drops of acetic anhydride, followed by the addition of one drop of concentrated sulfuric acid (H₂SO₄). The resulting mixture was subjected to steam heating for one hour. After heating, the solution was neutralized using sodium hydroxide (NaOH). Subsequently, chloroform was added to the mixture. The formation of a blue-green coloration was observed, which served as an indicator for the presence of steroids, as described by Shaikh and Patil (2020).

Qualitative Test for Flavonoids:

The solution of NaOH (Two drops) were added to 1 ml of each plant extract and then AlCl₃ solution (Two drops) was added to the mixture, followed by the addition of concentrated H₂SO₄. The presence of flavonoids was confirmed when a bright yellow color that turned colorless when H₂SO₄ was added (Solanki et al., 2019).

Qualitative Test for Saponins:

The presence of saponins was confirmed by observing persistent foaming in a test tube after mixing each extract (0.5g) with water and foam persist even after heat (Silva et al., 1998; Solanki et al., 2019).

Qualitative Test for Glycosides:

The extracts (0.5g) were dissolved in chloroform and H₂SO₄ was added, carefully, to form a lower layer, revealing a reddish-brown color at the interface indicating the presence of a glycone portion of cardiac glycosides (Harbourne, 1984, Ben et al. 2013).

Qualitative Test for Tannins/Phenol:

The extract (0.5g) was stirred with distilled water (10mL) filtered. By adding 0.1% FeCl₃ reagents to the filtrate, the development of a blue-black or blue-green precipitate was considered the presence of tannins or phenolic. The extract (0.5g) was stirred with distilled water (10mL) filtered. By adding 0.1% FeCl₃ reagents to the filtrate, the development of a blue-black or blue-green precipitate was considered the presence of tannins or phenolics (Ben et al. 2013).

Qualitative Test for Reducing Sugar:

An aliquot of 5mL of equal volume of Fehling solutions A and B was added to 5mL of each extract and boiled for 5 minutes. The development of a red precipitate or rusty brown colour indicated

a positive result (Ben et al. 2013, Shaikh and Patil, 2020).

Qualitative Test for Terpenoids:

The presence of terpenoids was assessed by adding 2mL of each extract into 2mL of chloroform and evaporating until dry. Afterwards, 2mL of concentrated H₂SO₄ was added and heated for about 2 minutes. The development of a reddish-brown colour denoted the presence of terpenoids (Shaikh and Patil, 2020).

RESULTS**Information Relating to Subjects:**

Two hundred and eighty (280) rectal swabs were collected from participants enrolled in the study. These comprise 108 (38.6%) male and 172 (61.4%) female participants. The ages of the participants ranged between 6 months to 61 years. With regards to marital status, 166 participants were single, while ninety-one were married; the category "others" includes the divorced, separated, and widowed. Other socio-demographic information about the study participants is detailed in Fig. 2.

Antibiotic Resistance Profile of Recovered Isolates:

Altogether, a total of 57 (20.4%) *S. typhi* were recovered from the 280 samples. All the isolates were resistant to trimethoprim-sulfamethoxazole (100%), closely followed by ticarcillin and fosfomycin at 93.0% each (53/57), and then levofloxacin at 56.1% (32/57). The highest sensitivity was to nitrofurantoin (26.3%). Details are highlighted in Table 2 and Fig. 3.

All the isolates were multidrug-resistant (MDR) as six (10.5%) isolates were resistant to three antibiotic classes, eleven (19.3%) isolates to four classes, 19 (33.3%) were resistant to five classes while 21 (36.8%) were resistant to six antibiotic classes or more. The Multiple Antibiotic Resistance Index (MARI) was also determined for each of the 57 isolates. All the isolates had MARI ≥ 0.4 , with 4 of them having an absolute value of 1 (Fig. 4).

Qualitative Phytochemical Screening of Crude Plant Extracts:

The phytochemical analyses of five plant extracts revealed the presence of steroids, flavonoids, glycosides, and tannins, but no terpenoids were found. Saponins were found in *A. indica* and *J. curcas*, while alkaloids and reducing sugars were found in *G. hirsutum* and *A. indica*, respectively.

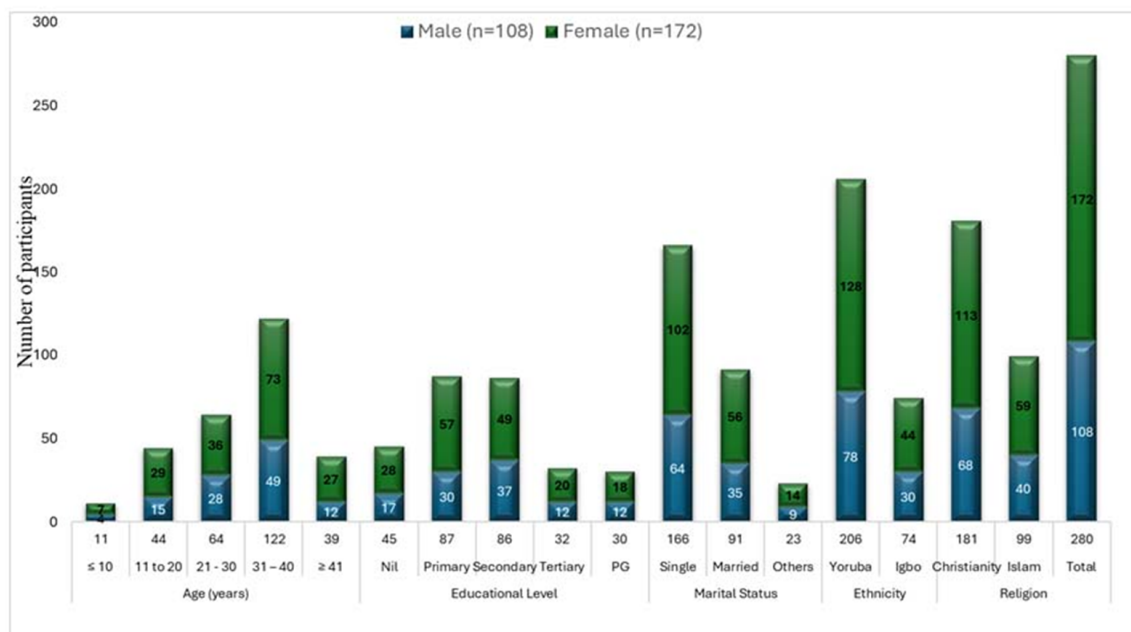


Fig. 2. Socio-demographic details of study participants

Table 2. Antibiotic resistance profiles of recovered *S. typhi* isolates

| Antibiotic class | Antibiotics | R (%) | I (%) | S (%) |
|-------------------------------|-------------------------------|------------|-----------|-----------|
| B-LACTAMS (Monobactam) | Aztreonam | 26 (45.6) | 9 (15.8) | 22 (38.6) |
| B-LACTAMS (Carboxypenicillin) | Ticarcillin | 53 (93.0) | 0 (0.0) | 4 (7.0) |
| FOLATE PATHWAY ANTAGONIST | Trimethoprim-sulfamethoxazole | 57 (100.0) | 0 (0.0) | 0 (0.0) |
| FOSFOMYCIN | Fosfomycin | 53 (93.0) | 2 (3.5) | 2 (3.5) |
| AMINOGLYCOSIDES | Gentamicin | 20 (35.1) | 8 (14.0) | 29 (50.9) |
| FLUOROQUINOLONE | Levofloxacin | 32 (56.1) | 10 (17.5) | 15 (26.3) |
| NITROFURANS | Nitrofurantoin | 15 (26.3) | 0 (0.0) | 42 (73.7) |
| GLYCYLCYCLINE | Tigecycline | 41 (71.9) | 12 (21.1) | 4 (7.0) |

Legend: R = Resistance, S = Susceptible and I = intermediate.

The phytochemical screening for secondary metabolites, such as alkaloids, steroids, flavonoids, saponins, glycosides, tannins, reducing sugars, and terpenes, of crude plant extracts from neem (*A. indica*), pawpaw (*C. papaya*), cotton (*G. hirsutum*), physic nut (*J. curcas*) and castor oil plant (*R. communis*) is described in Table 3.

Susceptibility profile of recovered isolates to crude aqueous leaf extracts:

The crude leaf extracts made by boiling, squeezing, and soaking in cold water were tested for antibacterial activity. None of the crude extracts displayed any activity against any of the isolates as no zone of inhibition was observed to any of the extracts by agar diffusion method.

Likewise, the reference sample collected from the local herb seller in Osogbo did not show any zone of inhibition throughout this study.

For the assessment of crude extract activity prepared in different volumes of hot and cold water giving different concentrations of the extract, there were no zones of inhibition observed with the three different concentrations of the aqueous leaf extracts of *C. papaya*, *G. hirsutum*, *J. curcas*, and *R. communis*. However, a particular concentration of *A. indica* (10g of extract in 50mL hot water) revealed zones of inhibition (ranging between 12mm and 7mm) against only 6 out of 57 isolates (10.5%). All six isolates were multidrug resistant

as they were resistant to ≥ 4 classes of antibiotics (≥ 4 and ≤ 6).

DISCUSSION

Medicinal plants are widely used globally to treat various types of bacterial diseases, as noted by Salim et al., 2019; Tabuti et al., 2003; Umair et al., 2019. In this study, the antibiotic susceptibility patterns of *S. typhi* clinical isolates were determined, and antimicrobial activities of five medicinal plants commonly used as a cocktail by traditional medicinal practitioners in Osogbo, Southwest Nigeria to treat typhoid fever were investigated.

All *S. typhi* isolates recovered in this study

revealed absolute resistance to trimethoprim-sulfamethoxazole followed by ticarcillin, fosfomicin, and levofloxacin. Multidrug resistance was observed in all the tested strains as all were resistant to three antibiotic classes or more. However, ciprofloxacin used as a positive control during the screening of the plant extracts revealed activity as all recovered isolates were susceptible to it.

Previous studies from various parts of the world have reported resistance rates varying between 5.2 to 100% to sulfonamides in *Salmonella* strains (Moe et al., 2017; Sodagari et al., 2015; Terentjeva et al., 2017; Xu et al., 2020; Zeng et al., 2019). Many of the resistant strains in these studies were

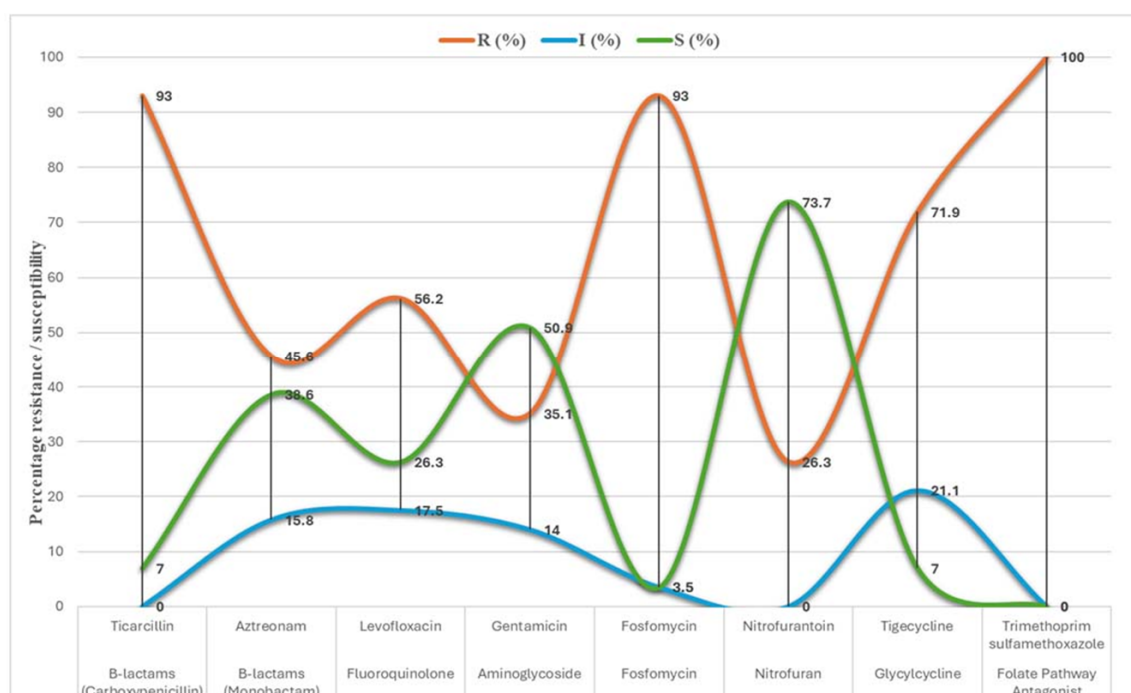


Fig. 3: The percentage resistance/susceptibility profile of the recovered *S. typhi* isolates to different antibiotics

Table 3. Phytochemical analysis of the five (5) medicinal plants

| | <i>Azadirachta indica</i> | <i>Carica papaya</i> | <i>Gossypium hirsutum</i> | <i>Jatropha curcas</i> | <i>Ricinus communis</i> |
|-------------------|---------------------------|----------------------|---------------------------|------------------------|-------------------------|
| Alkaloids | - | - | + | - | - |
| Steroids | + | + | + | + | + |
| Flavonoids | + | + | + | + | + |
| Saponins | + | - | - | + | - |
| Glycosides | + | + | + | + | + |
| Tannins | + | + | + | + | + |
| R/Sugar | + | - | - | - | - |
| Terpenes | - | - | - | - | - |

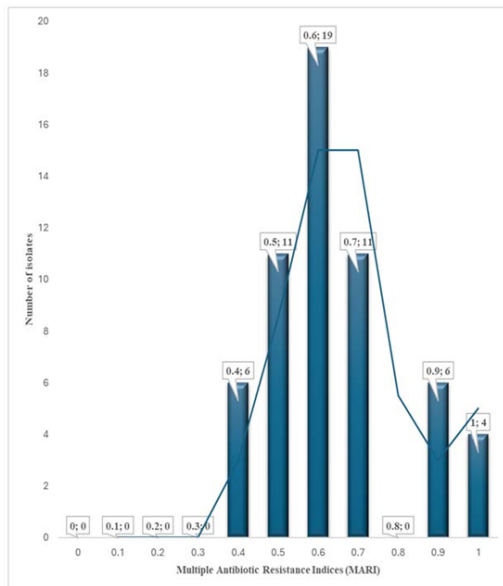


Fig. 4: Multiple antibiotic resistance indices (MARI) of *S. typhi* isolates

recovered from meat products—mainly chicken and pork. This could be adduced to the use of sulfonamides as growth enhancers in the food production chain, which could ultimately end up in humans as the transmission is mainly via contaminated food and water. This unwarranted use of sulfonamides results in their accumulation within the environment (Wang et al., 2014) via excretion into livestock wastewater (Chen and Xie, 2018), and their ability to resist breakdown during waste treatment (Felis et al., 2020).

The high resistance of the isolates to fosfomycin in this study (93.0%) contrasts sharply with the report of Humaira et al. (2017) who reported sensitivity to fosfomycin in 83.3% of *S. typhi* isolates. However, the emergence and subsequent spread of fosfomycin resistance in bacterial species, including *Salmonella* spp., have been reported (Ito et al., 2017). Monte et al. (2023) discovered an alarmingly high occurrence of fosfomycin resistance determinants in 26,165 strains of *Salmonella*, of which a whopping 26,144 (99.9%) were *Salmonella enterica* subsp *enterica* spanning 73 distinct serovars in 47 countries across six continents.

Nitrofurantoin was the most effective of the antibiotics used. This correlates with the report of Martínez-Pucholet et al. (2020), where nitrofurantoin was reported to have inhibitory effects against *Salmonella*. Another study by Mohakud et al. (2023) reported an effective dose-dependent inhibitory effect on *S. enterica* Typhimurium ms202 by nitrofurantoin, which came about due to the synergistic interaction between membrane damage, oxidative stress and genotoxicity.

The antimicrobial activity of the five medicinal plants evaluated *in-vitro* against the test *S. typhi* strains showed no antimicrobial activity except the extract of *A. indica* which demonstrated weak activities against only 6 strains of the target organism with zones of inhibition ranging between 7mm and 12mm. Ranjit et al. (2014) reported an inhibition zone of 10 mm to leaf and bark extracts of *A. indica* extract by *S. typhi*, a finding which corresponds to the values reported in our study. However, in another study (Marcelin et al., 2016), an aqueous extract of *A. indica* showed no activity against *S. typhi*. At variance nonetheless, Adetutu et al. (2021) explored and reported that anti-*Salmonella* capabilities of *A. indica* can be found in the aqueous and chloroform soluble fractions.

A recent study (Obboh et al., 2023) reported higher anti-*Salmonella* activity with ethanolic (inhibition zone 10/32 mm at 75/100% concentration) than aqueous extract of *C. papaya* seeds (inhibition zone 4/9mm at 75/100% concentration), insinuating the higher efficacy of ethanolic than aqueous extracts. This may be due to the phyto-components being less hydrophilic and more readily soluble in organic solvents. Another study on the antimicrobial activity of papaya leaves also revealed that the ethanol extract of papaya leaves has antibacterial activity against *S. typhi* (Muhammad et al., 2022). In contrast with our findings however, Ogunjobi and Ogunjobi (2011) reported that both aqueous and ethanolic leaf extracts of *C. papaya* inhibited the growth of *S. typhi*.

Many studies have reported a different range of activities for various extracts of *J. curcas* (Ajayi et al. 2016; Rahu et al., 2021; Rampadarath et al., 2016). The study by Ajayi et al. (2016) described the antibacterial activity of the leaf extract of *J. curcas* against *S. typhi* and *S. typhimurium* isolates. Conversely, Rahu et al. (2021) stated that the aqueous extract of the plant leaf had no inhibitory effect on *S. typhimurium*, while the study of Singh and Patidar (2017) recorded excellent antibacterial activity by the leaf extract of *J. curcas* against all tested bacterial species except for *S. typhi* against which no plant part extract was active, aligning with our finding in the present study.

Recently, iron and silver nanoparticles of *R. communis* were found to be active against *S. typhi* (Linima et al., 2023), while Emoruwa et al. (2023) drew attention to the inhibitory potentials of *G. hirsutum* against *S. typhi*. In line with our study nonetheless, other authors have recorded zero activity against *S. typhi* by the aqueous leaf extract of *R. communis* while methanol and hexane extracts revealed excellent activity (Mathur et al., 2011).

The antimicrobial activity of plant extracts largely depends on the plant species and plant parts explored, the type of solvent and extraction method, the concentration of the extract and the microorganism tested (Balandrin et al., 1985; Kalimuthu, et al., 2010). The results of this study correspond with several previous research that aqueous extracts of plants generally show little or no antibacterial activities compared with extracts of organic compounds (Ashafa et al., 2008; Koduru, et al., 2006; Nyembo et al., 2012). In most plants, this could be a result of the deactivation or denaturing of the antimicrobial components in the plants during the drying process, and the solubility of the phyto-components in organic or aqueous media. Conventionally, the dried powder preparations of these leaf extracts, generally called “àgúnmu” in southwest Nigeria, are traditionally taken with porridge (usually pap or “àkàmù”) which is an aqueous media for the oral administration of the herbs. The aqueous mode of administration makes it most unlikely that the bioactive components may be fully extracted from the herbs for the intended purpose of inhibiting *S. typhi*.

The results obtained confirm the presence of phytochemicals which are known to have medicinal and physiological activities (de Lima et al., 2021; Sharma and Singh, 2012; Singh and Patidar, 2017). Phytochemicals are plant-based, non-nutritive bioactive chemicals produced by plants for their protection but have been found to protect humans against diseases (Kumar et al., 2023). These constituents include steroids, flavonoids, glycosides, and tannins in all five selected plants.

In a study by Doğan et al. (2017), the authors reported that steroids did not seem to possess extensive antibacterial properties as they were only able to deter the growth of a few organisms, and their activities do not appear to be better than those of conventional antibiotics.

Flavonoids and tannins, present in all the plant extracts in this present study, are polyphenolic compounds that are secondary plant metabolites commonly found in nature (Moura de Melo et al., 2023; Shamsudin et al., 2022). Flavonoids are known to be produced in response to plant microbial infections and have been extensively studied and found to have antimicrobial activity against an array of microorganisms *in-vitro*, sometimes including *S. typhi* in various studies (Farooq et al., 2020; Linden et al., 2020; Ortega-Vidal et al., 2022; Shamsudin et al., 2022; Song et al., 2021). Their activity has been ascribed to several mechanisms, notably suppression of nucleic acid synthesis, energy metabolism and plasma membrane function (Wu et al., 2013; Gómiak et al., 2019; Biharee et al., 2020; Donadio

et al., 2021). They are also reported to limit adhesion and biofilm formation, cell membrane porins, membrane permeability, and bacterial pathogenicity (Gómiak et al., 2019; Biharee et al., 2020; Donadio et al., 2021), undo antibiotic resistance and enhance antibiotic efficacy (Song et al., 2021). Antimicrobial activity in tannins has been attributed to their capacity to denature proteins (Moura de Melo et al., 2023), chelate iron, suppress cell wall synthesis, and damage cell membranes (Farha et al., 2020), in addition to anti-biofilm potentials (Villanueva et al., 2023).

The nature and concentration of phyto-components in the leaves of various plants vary widely and may be influenced by crop type and variety, soil type, and the growth environment or season (Kumar et al., 2023). Several studies on plant phytochemicals have reported varying findings to the one presently recorded in this study (Singh and Patidar, 2017; Nyembo et al., 2012). The fact that, regardless of the previous reports of inhibitory activities in the five plants assessed, none of the crude aqueous extracts (either singly or as a cocktail) exhibited any significant antibacterial activity against all the 57 *S. typhi* strains in this study is quite worrisome.

In conclusion, this study assessed the antibacterial activity of a local “herbal cocktail” of the leaves of five plants against *Salmonella typhi* from clinical sources. Despite the widespread local use of these herbs, this study revealed no activity except from the extracts of *A. indica* which exhibited only a little activity against just a few isolates. There is an urgent need to curtail the regular dispensing of these local herbs by traditional herb sellers until further studies to validate their efficacy and the methods of administration for optimum effect are established. Necessary interventions to control the distribution of unvalidated herbs within the community should be implemented and strictly enforced by the appropriate health authorities. Proper testing and subsequent treatment by qualified medical professionals should be advocated. It is recommended that further studies on extracts (such as ethanolic, methanolic, chloroform and acetone extracts) should be conducted.

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