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Ethnobotanical Survey and Evaluation of Anti-Salmonella Potentials of Commonly Used Plants for Typhoid Treatment in Ogbomoso, Oyo State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author AA designed the work. Authors TDO and TMA carried out the experiment, analyzed the results. Authors AA and TDO wrote the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

This study assessed the sensitivity of *S. typhi* to extracts of commonly used plants in treatment of typhoid in Ogbomoso, Oyo State, Nigeria. Ethnobotanical survey was conducted using a structured questionnaire. Powdered leaves of plants were extracted by maceration in distilled water. *In vitro* sensitivity of *S. typhi* to the extracts were assessed using agar well diffusion method. Groups of rats were infected orally with 0.5 McFarland suspension of *S. typhi*. Bacteremia in the animals was monitored by plating on Salmonella-Shigellar agar. Biochemical parameters were determined spectrophotometrically. Histological analysis was performed using H&E staining method. *S. typhi* was sensitive only to *P. guajava* and *A. indica* leaf extracts. *V. amygladina* (90.31%) and *C. papaya* (92.26%) showed highest percentage inhibition of *S. typhi* comparable with ciprofloxacin (99.86%). Haematological parameters varied significantly ($p<0.05$) among the groups. Antioxidant status of rats, total protein, albumin and the corresponding globulin level increased significantly ($p<0.05$) in the groups treated with *P. guajava* and *A. indica*. *C. papaya* and *C. citratus* ameliorated

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the histopathological damages observed in the liver and intestine of *S. typhi* infected rats. The studied plants have direct activities against *S. typhi*, thus reflecting the reason for their combination in traditional system of medicine.

Keywords: S. Typhi; susceptibility; bacteremia; immunomodulatory; antioxidant.

1. INTRODUCTION

Typhoid fever is a common and sometimes fatal infection of both adults and children that is responsible for bacteraemia and inflammatory destruction of the intestine and other organs. The disease account for about 12.5 million new cases worldwide [1] with children mostly affected [2,3]. The risk of death may be as high as 20% without treatment [3]. Typhoid fever is caused by *Salmonella enteric* serovar Typhi, (*S. typhi*), a water-borne gram negative aerobe that is associated with poor sanitation and faecal contamination of water and food supplies [4]. Symptoms such as fever, abdominal pain, constipation, severe headaches, diarrheal, vomiting, oss of appetite, rose spot, anorexia and hepato-slpenomegaly (Mweu and English, 2008) [5] characterize salmonella infection. These symptoms may vary from mild to severe and usually begin six to thirty days after exposure (Ochiai, 2015).

Treatment of typhoid fever involves the use of ciprofloxacin, ampicillin, chloramphenicol, and trimethoprim sulfamethoxazole. However, the widespread emergence of multi-drug resistant *S.* Typhi has impeded the effectives of these antibiotics, therefore necessitating the search for other therapeutic options [6,7]. Residents of Ogbomoso, Oyo state, Nigeria has relied on the use of herbs for management and treatment of various diseases [8]. The use of *Psidium guajava, Azadirachta indica, Carica papaya* and *Venonia amygdalina* in traditional management of typhoid in other parts of Nigeria has also been reported (Fadimu et al., 2014) [9,10,11]. In addition, Adjanohoun et al. [12] investigated the activity of *Carica papaya* in combination with *Anacardium occidentale*, *Psidium guajava*, and *Azadirachta indica* against typhoid fever. Therefore, this present study was undertaken to identify the plants used in management of typhoid fever in Ogbomoso, Oyo state (Nigeria), and conduct a preliminary and independent investigation of the anti-salmonella activity of each of the selected plant extracts both *in-vitro* and *in-vivo*. This will help identify the plant with effective anti-salmonella activity for further study.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant samples

Leaves of *P. guajava, A. indica, V. amygdalina, C. papaya* and *C. cymbopogon* were collected from the Ladoke Akintola University of Technology, Ogbomoso, Oyo State Nigeria, in February 2018. The plants were identified and authenticated by a taxonomist at the herbarium unit of the Department of Pure and Applied Biology of same institution.

2.1.2 Salmonella enterica serovar typhi

Clinical isolate of *S. typhi* was obtained from University of Ilorin Teaching Hospital, and maintained on Salmonella-Shigellar agar throughout the study.

2.1.3 Chemicals and reagents

Agars and broth were obtained from Hi-Media laboratories, India. Assay kits (ALT, AST, Total protein, Albumin, Globulin, LDH, ALP, Bilirubin, and SOD) were products of Randox Laboratories Ltd., United Kingdom. Other reagents used were of analytical grade and were prepared in glass apparatus using distilled water. MDA were assayed for using Oxford Biomedical research kit (Oxford, USA). Other reagents used were of analytical grade.

2.2 Methods

2.2.1 Ethnobotanical survey and selection of plants

Information were collected from Ogbomoso area of Oyo State, Nigeria on the popular plants used in the management of typhoid fever in the area using a semi-structured questionnaire. Five of those plants were selected for this preliminary study based on availability of those plants in the immediate community.

2.2.2 Preparation of plant Samples

Leaves were air-dried in the laboratory until constant weight was obtained and ground to powder using electric blender. The aqueous extraction of the samples was carried out according to Jamil et al., [13]. 100 g of plant samples were soaked in 500 ml of distilled water separately for 72 hours with intermittent shaking. After 72 hours, the mixtures were filtered using Whatman No 1 filter paper. The residues were discarded and the filtrate concentrated with a freeze dryer. The extracts obtained were stored at 4°C in refrigerator.

2.2.3 Preparation of Inoculum

A few colony of *S. typhi* from an overnight Salmonella-Shigella Agar (SSA) culture was introduced into freshly prepared Mueller Hinton Broth (MHB) and the turbidity adjusted to 5McFaland standard (containing approximately 1.5 X 10^8 CFU/mL).

2.2.4 In vitro sensitivity study

Agar well diffusion method was used to determine the *in vitro* sensitivity of *S,* Typhi to the plant extracts. Freshly prepared Mueller Hinton Agar (MHA) was allowed to cool to about 45^0C, poured into sterile petri dishes (about 4mm high) and left to solidify at room temperature. Inoculum was aseptically swabbed on the surface of agar plate using a sterile swab stick, and was allowed some minutes to pre-diffuse. Wells of 6mm in diameter were bored into the plates using a sterile cork borer. Fifty microliter (50µL) of aqueous extract of each plant (100 mg/mL) was added into each well, distilled water serve as negative control while 10mg/ml of chloramphenicol and ciprofloxacin were positive controls. These were then left on the bench for a while for adequate diffusion of the extracts and thereafter incubated at 37°C for 18 hours. After incubation, the diameter of the zones of inhibition around each well were measured to the nearest millimeters. The tests were performed in triplicate, and the mean was then calculated [14].

2.2.5 Experimental animals

Forty-five (45) Male albino rats (80-100g) were purchased from a private animal breeder, Ogbomoso, Oyo state. The animals were acclimatized for a period of two weeks and fed with standard animal feed with free access to clean water. Animals were maintained under standard laboratory conditions of light, humidity, and temperature. The handling of the animals was in compliance with the animal management and care and the general guideline for animal experimentation.

2.2.6 Infection of experimental animals with *S. typhi* and grouping

Prior to the infection, animals were fasted overnight. They were then given 1 mL of 0.5McFarland standard suspension of *S. typhi* via oral route using oral cannula. Forty-eight (48) hours after infection, blood were collected from the tail of the animals into test tubes containing 1 mL of normal saline and plated on SSA. The growth of *S. typhi* on the agar established infection and only infected animals were used in the study.

The rats were assigned into nine groups of five animals each as shown in Table 1. Animals were treated for a period of 7days, starting from 72 hours post- infection.

Treatment lasted for 7 days. Bacteraemia was monitored by plating animal blood samples on SSA.

Table 1. Grouping of animals with treatments administered

Group	Treatment
Normal Control	Received only distilled water
Negative Control	Infected + distilled water
Positive Control CIP	Infected + Ciprofloxacin (14.28 mg/kgbw)
Positive Control CHL	Infected + chloramphenicol (7.14 mg/kgbw)
Psidium guajava	Infected + 500mg/kg body weight *of P guajava*
Azadirachta indica	Infected + 500mg/kg body weight of *A. indica*
Vernonia amygdalina	Infected + 500mg/kg body weight of *V. amygdalina*
Carica Papaya	Infected + 500mg/kg body weight of *C. papaya*
Cymboopogon citratus	Infected + 500mg/kg body weight of *C. citratus*

Infected: Orally administered with 1.5 x10^8 cfu of S. typhi

2.2.7 Collection and preparation of samples

Twenty four hours after the last treatment, animals were sacrificed by cervical dislocation, and blood samples were collected through cardiac puncture using sterilized needles into EDTA bottles and plain bottles for the haematological test and biochemical assays respectively.

The blood sample in the plain bottle was centrifuged at 4000 rpm for 5 minutes. Pellet was discarded and supernatant was stored at 4^0C until used.

For histological studies, portions of liver and intestine were removed, rinsed and fixed in 10% formalin, while for antioxidant assay liver was rinsed and placed in iced cold 0.25 M sucrose solution.

A portion of the liver (0.5 g) was homogenized in 5 mL of phosphate buffer saline (PBS) using tissue homogenizer. The homogenate was centrifuged at 4°C using cold centrifuge at 3000 rpm for 15 minutes. The supernatants were collected and used for biochemical assays.

2.2.8 Haematological parameters

Blood collected into EDTA bottles were analyzed for WBC, RBC, HBG, HCT, PLT, LYM using Sysmex Automated Hematology Diagnostic Machine (XP-300), Mundelein, USA.

2.2.9 Biochemical analyses

All biochemical analysis was carried out using standard procedure as provided in the manufacturer guide of kits used.

2.2.10 Histological study

The tissue was allowed to fix in 10% formal saline for 48 hours, it was then grossed and cut into smaller pieces of 3 mm thick in pre- labelled tissue cassette, and processed using automatic tissue processor (LEICA TP1020). Thereafter, it was passed through alcohol of ascending concentrations dehydration. Then it was passed through two changes of Xylene and three changes of molten Paraffin wax set at 65°C and was processed for 12 hours.

The tissue was embedded in Paraffin wax and sectioned at 4 microns using rotary microtome (LEICA RT2115). Staining was done using Haematoxylin and Eosin staining technique.

2.2.11 Statistical analysis

Data were expressed as mean ± SEM and analyzed by one-way analysis of variance (ANOVA) test using Statistics Package for Social Sciences (SPSS) version 21.0. The difference between mean were analysed by Duncan's multiple range test (DMRT) at level $p<0.05$.

3. RESULTS

3.1 Ethnobotanical Survey Report

A report of the Ethnobotanical survey conducted within Ogbomoso, Oyo State is presented in Table 2. A total of 36 plants species belonging to 26 families were reported to be among the plants used in the local management of typhoid fever. Leaves, stem barks, roots, whole fruits, seeds and cloves are all utilized in 45, 40, 22.5,5,5 and 2.5% of plant parts used respectively.

3.2 *In-vitro* Sensitivity of *S. typhi* to Aqueous Extracts of Selected Plants

Only *P. guajava* and *A. indica* exhibited activity against *S. typhi* in-vitro. The plant extracts exhibited lower activity when compared with standard ciprofloxacin, whereas, *S. typhi* showed no sensitivity to the a*queous* extracts of *V. amygdalina, C. papaya* and *C. citratus* as shown in Table 3.

3.3 *In-vivo* Anti-salmonella Activity of Plants Extracts

3.3.1 Bacteraemia of *S. typhi*-infected rats administered plant extracts

There were reduction in the amount of bacteria in the blood of experimental animals in all groups as depicted in Figure1. The percentage inhibition of *S.* Typhi is presented in Table 4. The inhibition of bacterial multiplication observed in the groups treated with the aqueous extracts of *V. amygdalina* and *C. papaya* compare not significantly ($p<0.05$) different from those of ciprofloxacin-treated animals. Aqueous extracts of *A. indica* and *C. citratus* showed percentage inhibition comparable with chloramphenicol while there was only 30.1% inhibition of *S.* Typhi in the negative control group at day 7.

Plate 1. Leaves of selected plants A-*Psidium guajava;* B-*Azadirachta indica;* C-*Vernonia amygdalina;* D- *Carica papaya;* E- *Cymbopogon citratus*

Table 2. Report of ethnobotanical survey on commonly used plants in management of typhoid fever in Ogbomoso, Oyo State

S/No	Scientific name	Family	Common name(s)	Local (yoruba) name(s)	Parts used	Frequency
1	*Azadirachta indica*	Meliaceae	Neem	Dongoyaro,	leaves, stem bark	11
2	*Carica papaya*	Caricaceae	Pawpaw	Eresile ibepe	Fallen leaves, unripe fruit	9
3	*Psidium guajava*	Myrtaceae	Guava	Girofa	Leaves	8
4	*Vernonia amygdalina*	Asteraceae	bitter leaves	ewuro	Leaves	7
5	*Cymbopogon citratus*	Poaceae	lemon grass	kooko oba	Leaves	7

S/No	Scientific name	Family	Common name(s)	Local (yoruba) name(s)	Parts used	Frequency
6	*Morinda lucida*	Rubiaceae	Brimestone	oruwo	root. Leaves	6
7	*Brysocarpus coccineus*	Conniraceae	Crimson thyme	amuje	leaves, stem bark	5
8	*Magnifera indica*	Anacardiaceae	Mango	Mangoro	leaves, stem bark	4
9	*Alstonia boonei*	Apocynaceae	Alstonia	Eepo wawon/ahun	leaves, stem bark	4
10	*Sphenocenturm jollyanum*	Menispertmaer	Sphenocentum	akerejupon	roots, stem bark	4
11	*Nauclea latifolia*	Rubiaceae	African peach	egbesi	root, stem bark	4
12	*Citrus aurantifolia*	Rutaceae	Lime	osan wewe	leaves, whole fruits	3
13	*Xylopia aethipica*	Annonaceae	Ethiopian pepper	Eru	Root, Leaves	2
14	*Kigelia Africana*	Bignoniaceae	Sausage tree	pandoro	Seeds	2
15	*Garcinia kola*	Clusiaceae	bitter kola	orogbo	stem bark	2
16	*Caesalpinia Bonduc*	Leguminosae	Ricker nut	Omo ayo	Seeds	2
17	*Lawsonia inermis*	Lythraceae	Henna plant	Laali	Leaves	2
18	*Allium sativum*	Alliaceae	Garlic	aayu	Cloves	1
19	*Anacadium occidentalis*	Anacardiaceae	Cashew	kasu	Leaves	1
20	*Enantia chlorantha*	Annonaceae	Lettuce leaves	Awopa	Leaves	1
21	*Rauvolfia vumitoria*	Apocynaceae	Indian snack root/ African rauivofia	asofeyeje	root	1
22	*Ocimum grattissimum*	Labiatae	Scent leaves	Efirin,		1
23	*Gossypium arboreum*	Malvaceae	Cotton	Owu	Leaves	1
24	*Ficus exasperata*	Moraceae	Sand papper tree	Ipin	Root	1
25	*Ficus carica*	Moraceae	Fig tree	opoto	Leaves	1
26	*Cajanus cajan*	Papilionaceae	Pigeon pea	otili		1
27	*Piper guineense*	Piperaceae	West African black pepper	iyeye	Leaves	1
28	*Nicotiana tobacum*	Solanaceae	Tobacco	taba	Leaves	1
29	*Cola millenii*	Sterculiaceae	monkey kola	obi edun	Leaves	1
30	*Theobroama cacao*	Sterculiaceae	Cocoa	koko	stem bark	1
31	*Curcuma longa*	Zingiberaceae	Turmeric	ata ile pupa	Leaves	1
33	*Phyllantus amarus*	Phyllanthaceae	Sleeping plant	eyin olobe	Leaves	1

Table 3. *In-vitro* sensitivity of *S. typhi* to selected plant extract

Sample	Zone of Inhibition (mm)*
Psidium guajava (100mg/ml)	17.67±0.58
Azadirachta indica (100mg/ml)	14.00±2.00
Venonia amygdalina (100mg/ml)	NI
Carica Papaya (100mg/ml)	NI
Cymboopogon citratus (100mg/ml)	NI
Chloramphenicol (10mg/ml)	26±0.50
Ciprofloxacin (10mg/ml)	28±1.00

**Values are mean ±S.E.M (n=3); NI- no inhibition*

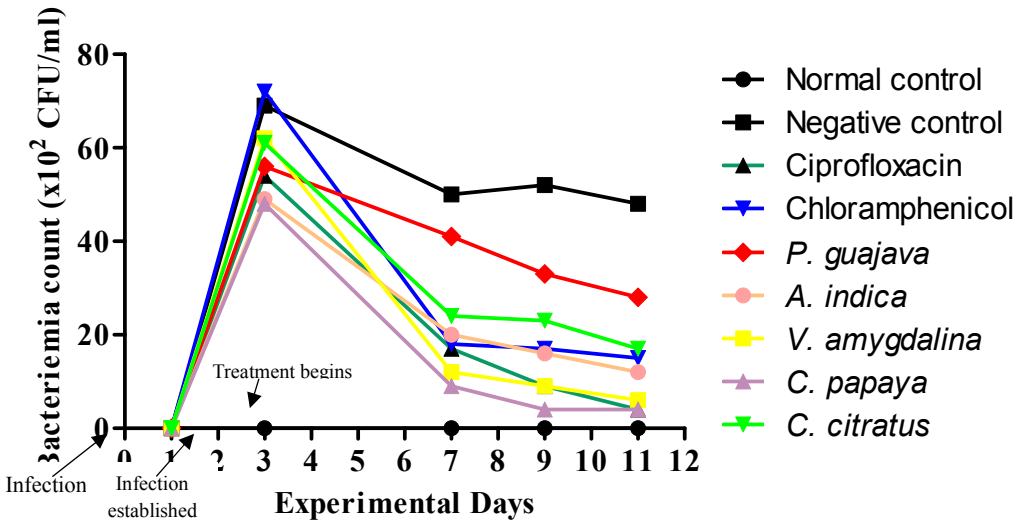

Fig. 1. Effect of extract administration on bacteraemia in *S. typhi*-infected rats

Table 4. Percentage inhibition of *S. typhi* in infected rats by the plants extracts

Sample	Percentage Inhibition	
	Day 5	Day 7
Negative control	27.8	30.1
Ciprofloxacin	69.97	99.86
Chloramphenicol	74.41	79.34
Psidium guajava	26.72	50.52
Azadirachta indica	60.1	74.93
Vernonia amygdalina	80.86	90.31
Carica Papaya	82.20	92.26
Cymboopogon citratus	64.01	72.53

3.3.2 Effects of extracts on haematological parameters of *S. typhi* infected rats

Table 5 showed the haematological parameters of *S. typhi* infected rats treated with different plant extracts and the control rats. *S. typhi* infection caused a significant ($p<0.05$) increase in the WBC count of animals across infected groups except the group treated with *V. amygdalina*-treated group which was not significantly different ($p<0.05$) from normal control. The insignificant reduction in RBC occasioned by infection was reverted by all extracts except *V. amygdalina*. Haemoglobin concentration raised significantly ($p<0.05$) by *S. typhi* infection was lowered in all treatment

groups except *P. guajava*-treated group when compared with the normal control group. Significant ($p<0.05$) decrease was only observed in the PCV of the untreated, Ciprofloxacin, Chloramphenicol and *V. amygdalina* treated groups. Platelet counts was significantly ($p<0.05$) increased in infection but was lowered comparably with normal control in all treatment groups. Only *V. amygdalina* and *C. citratus* raised the relative lymphocyte significantly ($p<0.05$) reduced by infection.

3.3.3 Effects of extracts on selected serum biochemical parameters of *S. typhi* infected rats

Presented in Table 6 is the serum biochemical parameters of rats across the various groups. Serum proteins (albumin and globulin) decreased significantly ($p<0.05$) in the *S. typhi*-infected (and untreated) animals. Ciprofloxacin-, Chloramphenicol-treated animals had total protein and globulin levels not significantly ($p<0.05$) different from those of negative control. *P, guajava* and *A. indica* improved the level of serum total protein and globulins significantly *P, guajava* and *A. indica* more than in normal control group while *V, amygdalina* and *C. papaya* improved the two parameters insignificantly ($p<0.05$). Serum total protein and globulin in *C. citratus* treated group was significantly lower than that of negative control. Serum albumin level was significantly lowered in infection but revert in all treatment groups. Significant increase ($p<0.05$) was observed in serum LDH activity of the untreated. Only treatment with ciprofloxacin lowered the activity significantly. Serum LDH in chloramphenicol, *V. amygdalida* and *C. citratus* treatment groups were the highest.

3.3.4 Effects of extracts on the antioxidant status of *S. typhi* infected rats

Concentrations of reduced glutathione (GSH) and malondialdehyde (MDA) in the livers of experimental animals are presented in Fig. 2. Although there was no significant rise level GSH upon infection, there were significant ($p<0.05$) increase in *P. guajava* and *A. indica* treatment groups. MDA concentration increased significantly ($p<0.05$) in the untreated group relative to normal control. There were no significant difference in MDA concentration in all treatment groups compared with those of normal control.

3.3.5 Histological presentation of the distal ileum of rats infected with *S. typhi* and treated with plant extracts

Results in Fig. 3 show the representative photomicrograph of Haematoxylin and Eosin (H and E) stained section of the intestine of rats infected with *S. typhi* and treated with different plant extracts with the control. The figure revealed the four layers of the gut:mucosa (M), submucosa (SM), muscularis (ME), lamina propria (LP), Villi (V), and Brunner's glands (BG).

Distal ileum of normal control animal had undisturbed cytoarchitecture while ciprofloxacin-, chloramphenicol-, and *C. papaya*-treated groups did not show significant histopathological presentation. There are distinct hypertrophy of the muscular layer (black and yellow arrow head), considerably distorted mucosa layer, mild cellular fragmentations and vacuolation in the submucosal layer as well as condensation of Brunner's glands due to *S. typhi* infection in the untreated group. Notable in groups treated with *P. guajava*, *V. amygdalina* and *C. citratus* is the state of the distorted mucosa layer, mild cellular fragmentations and vacuolation of the submucosal layer. There is also condensation of Brunner's glands (BG) especially in *P. guajava*- and *V. amygdalina*-treated animals.

3.3.6 Histological presentation of the liver of rats infected with *S. typhi* and treated with plant extracts

Representative photomicrograph of Haematoxylin and Eosin (H and E) stained liver section of rats infected with *S. typhi* and treated with various treatments across the group are presented in Fig. 4. The figure showed the portal triad (PT), hepatic vein (HV), hepatic artery (HA) and the biliary duct (BD). The photomicrograph showed normal morphological presentation for normal control, positive controls and *C. papaya*-treated groups. The negative control, *P. guajava*-, *A. indica*-, and *V. amygdalina*-treated groups had marked degenerative cyto-architecture characterized with fragmented hepatocytes, some fibrosis and heavy haemorrhage from the walls of the hepatic vessels (black and red arrows). The group E showed mild histopathological presentation.

Table 5. Haematological parameters of rats infected with *S. typhi* and treated with various plant extracts and the control rats

GROUPS	WBC ($X10^3$)/µL	RBC ($X10^6$)/µL	HGB (g/dL)	PCV (%)	PLT ($X10^3$)/µL	LYMP%
Normal control	8.37±2.04^{b}	7.04±0.41^{a}	10.40±0.82^{a}	42.8±1.51^{b}	362.67±167.89^{a}	83.03±3.47^{d}
Negative control	10.90±2.10^{c}	5.76±2.99^{a}	12.50±1.20^{b}	37.05±1.48^{a}	1098.50±85.5^{b}	73.65±0.35^{b}
Ciprofloxacin	10.67±1.38^{c}	6.11±0.03^{a}	9.73±0.82^{a}	37.63±0.37^{a}	452.00±198.00^{a}	80.30±1.22^{cd}
Chloramphenicol	10.25±1.75^{c}	6.12±1.50^{a}	11.05±0.15^{a}	38.10±7.80^{a}	334.50±67.50^{a}	85.5±2.50^{d}
P, guajava	11.55±2.09^{c}	6.66±0.18^{a}	12.25±1.06^{b}	48.30±0.81^{c}	639.00±1.41^{a}	62.40±0.71^{a}
A. indica	5.57±0.33^{a}	6.31±1.17^{a}	10.17±0.98^{a}	40.13±8.53^{b}	655.00±285.77^{a}	69.57±3.02^{ab}
V. amygdalina	7.85±0.85^{b}	5.50±1.89^{a}	11.15±0.25^{a}	37.65±9.25^{a}	319.00±69.00^{a}	81.35±0.15^{cd}
C. Papaya	10.67±1.38^{c}	7.28±0.19^{a}	11.00±0.20^{a}	43.65±0.95^{b}	493.00±86.00^{a}	72.7±3.80^{bc}
C. citratus	11.00±0.70^{c}	6.86±0.45^{a}	10.60±0.40^{a}	43.95±0.95^{b}	506.50±73.50^{a}	85.55±0.15^{d}

Values were expressed a mean ± SEM and considered significant at P value <0.05. Different alphabet represent significant different among the samples

Table 6. Serum biochemical parameters of rats infected with *S. typhi* and treated with plant extracts and the control rats

Groups	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	LDH (U/L)
Normal control	3.05±0.14^{b}	0.65±0.08^{ab}	2.40±0.45^{bc}	64.55±11.64^{a}
Negative control	2.02±0.24^{a}	0.36±0.33^{a}	1.67±0.57^{b}	229.14±75.89^{ab}
Ciprofloxacin	2.28±0.40^{a}	0.68±0.10^{ab}	1.59±0.31^{b}	77.46±11.18^{a}
Chloramphenicol	2.34±0.39^{a}	0.87±0.04^{b}	1.48±0.41^{b}	464.74±29.58^{c}
P. guajava	9.03±1.02^{c}	1.50±1.11^{c}	7.54±1.11^{d}	193.64±14.79^{ab}
A. indica	8.69±1.04^{c}	1.46±0.20^{c}	7.23±1.19^{d}	246.89±2.79^{b}
V. amygdalina	2.75±0.39^{ab}	0.86±0.08^{b}	1.88±0.37^{b}	413.10±17.97^{c}
C.Papaya	2.83±0.15^{ab}	0.87±0.05^{b}	1.96±0.12^{b}	190.41±64.30^{ab}
C. citratus	1.42±0.19^{a}	0.93±0.06^{b}	0.52±0.20^{a}	490.56±48.56^{c}

Values were expressed a mean ± SEM and considered significant at P value <0.05. Different alphabet represent significant different among the samples

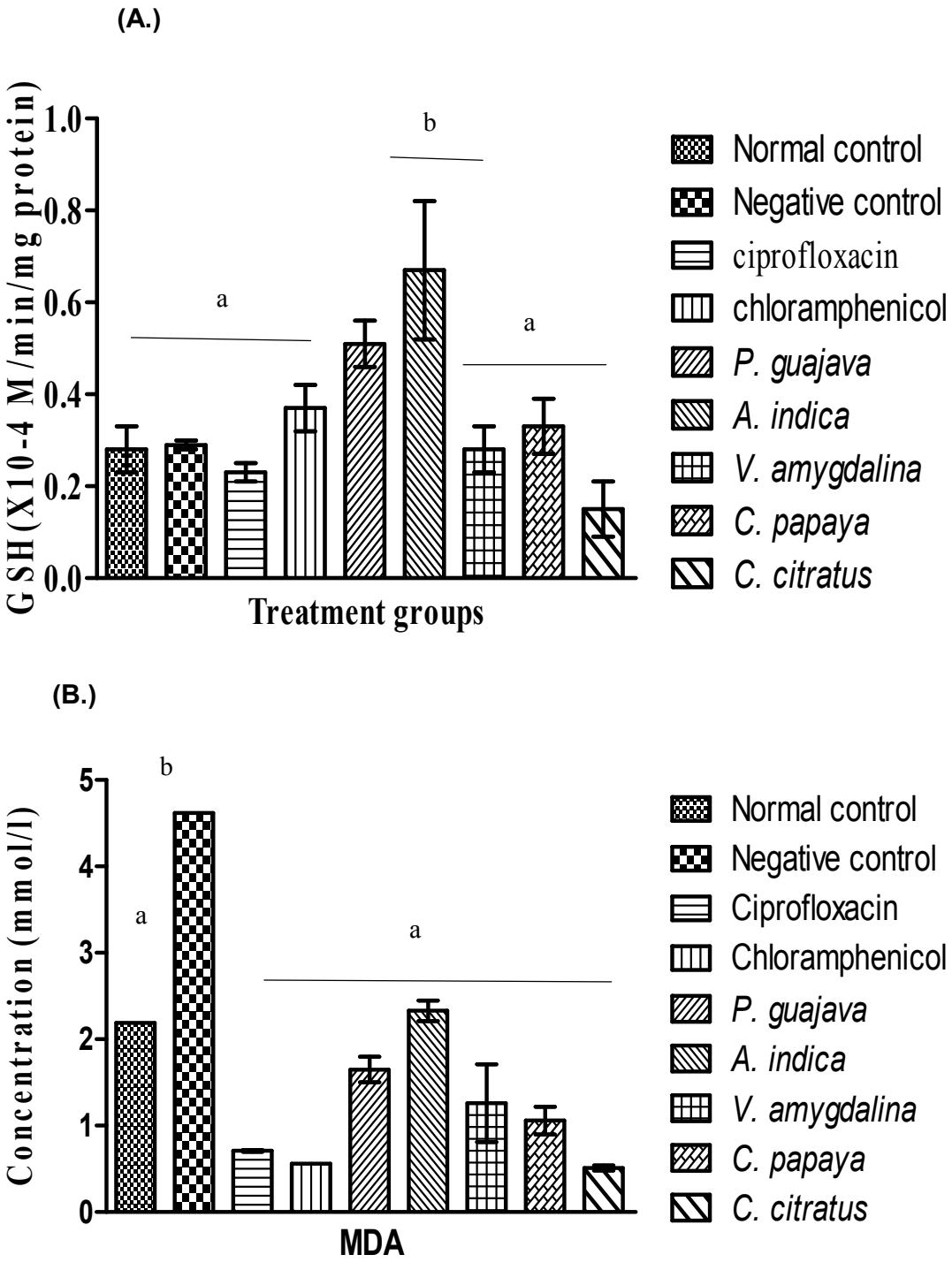

Fig. 2. Oxidative stress markers: (A.) Reduced glutathione, GSH and (B.) malondialdehyde, MDA of *S. typhi*-infected rats administered selected plant extracts

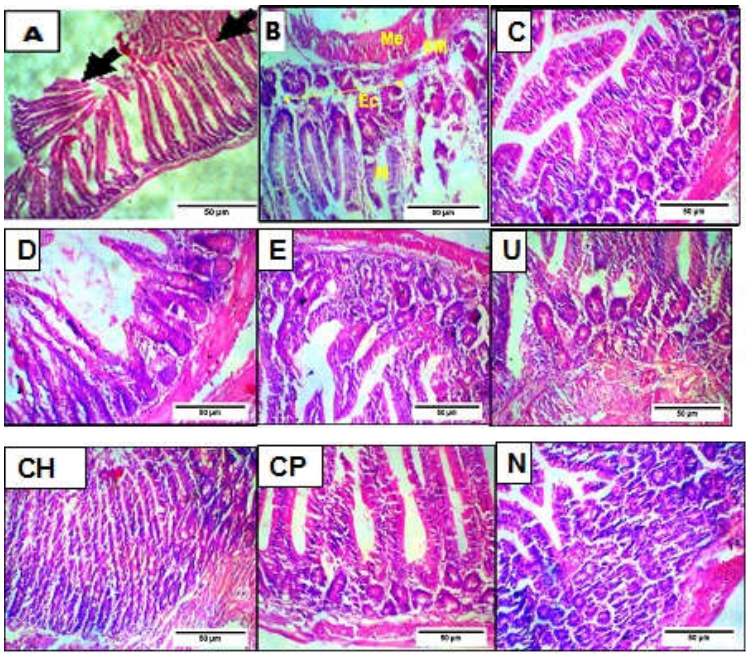

Fig 3. Representative photomicrograph of intestine of *S. typhi* infected rats treated with plant extracts. MG X100, H&E stain

A- Psidium guajava treated group; B- Azadirachta indica treated group; C- Vernonia amygdalina treated group; D- Carica Papaya treated group; E- Cymboopogon citratus treated group; N- Normal control group; CP- Ciprofloxacin treated group; CH- Chloramphenicol treated group; U- Untreated group

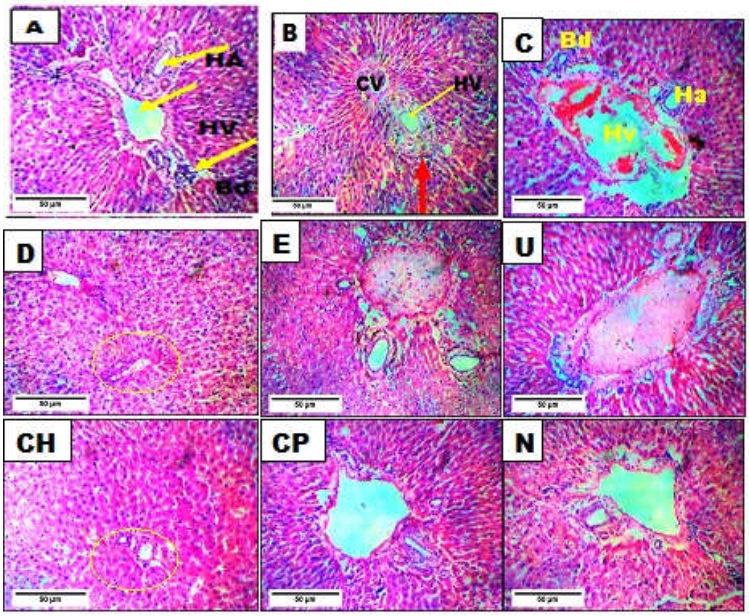

Fig 4. Representative photomicrograph of liver of *S. typhi* infected rats treated with plant extracts. MG X100, H&E stain

A-Psidium guajava treated group; B-Azadirachta indica treated group; C-Vernonia amygdalina treated group; D-Carica Papaya treated group; E-Cymboopogon citratus treated group; N- Normal control group; CP- Ciprofloxacin treated group; CH-Chloramphenicol treated group; U-Untreated group

4. DISCUSSION

Due to cost and not-so-easy accessibility to modern antibiotics, many people (about 80%) African nations still depend on local plants for management of many infectious diseases [15]. The local people of Ogbomoso, Oyo State (Nigeria) are no exception. In this study, aqueous leave extracts of each of the selected plant were investigated individually against *S.* Typhi both *in vitro* and *in vivo*.

The *in vitro* antibacterial assessment is a qualitative method used clinically to measure antibiotic resistance [16]. The *in vitro* activity observed for *P. guajava* and *A. indica* is in agreement with previous report [17,18,19]. Antimicrobial activities of *P. guajava* and *A. indica* has been linked to various phytochemicals present in their extracts. Flavonoids present in *P. guajava* leaves have been reported to have strong antibacterial activity [20]. Whereas the insensitivity of Salmonella bacilli to aqueous extracts of *C. papaya* leaves has been reported [21,22]. Chikwendu et al. [23] also concluded that aqueous extract of roots, stem and leaves of *V. amygdalina* has no anti-salmonolla effects.

Contrary to the observation in the *in vitro* study, drastic reduction in percentage bacteremia in experimental animals established the acclaimed therapeutic efficacy of the selected plant extracts and supported their folkloric use in the management of typhoid fever [12,24,25]. The activities observed for *V. amygdalina, C. papaya* and *C. citratus in vivo* could be due to immune system modulation as depicted in rise in total white blood cell count or their antioxidant properties. During salmonella infection, there is leukopenia, which is a decrease in the number of circulating white blood cells, with eosinopenia and relative lymphocytosis [26]. The higher lymphocyte count in the groups treated with *C. papaya*, *V. amygdalina* and *C. citratus* relative to the untreated and other extract groups maybe due to immune system stimulatory effects of the plant extracts and might be responsible for the clearance of the bacteria in the animals. Immuno-modulatory properties of *P. guajava* [27], *V. amygdalina* [28], *C. papaya* [29] have previously been reported. Other haematological changes associated with *Salmonella* infection include erythropenia and thrombocytopenia. These changes can be associated with the ability of *S. typhi* to invade the bone marrow with consequent depressed bone marrow function [30]. Such depressed bone marrow function may not have occurred in extracts-treated animals due to prompt intervention of the extracts.

Liver function is of great importance in typhoid fever [31]. Hundreds of proteins are dissolved in the plasma. Measuring the concentration of these proteins gives information regarding disease states [32]. Increased serum globulin and total protein in typhoid conditions as well as decreased albumin concentration was related to humoral response and inflammation respectively [33]. In this study, the increased globulin level observed in the groups treated with *P. guajava* and *A. indica* might be a consequence of improved immune function.

Cytokines produced by activated macrophages are important sources of reactive oxygen species which are capable of inducing cellular necrosis with accompanied rise in serum levels of cytoplasmic enzymes. Reduced glutathione (GSH) plays a crucial role in quenching theses metabolites (i.e ROS) [34]. The significant increase in the level of GSH in *P. guajava*- and *A. indica*- treated animals is indicative of the antioxidant properties of these plants and could be one of the mechanisms of the anti-salmonella activities observed in this study. Malondialdehyde (MDA) another marker of free radical damage to cells is a decomposition product of lipid hydroperoxides (Yeou-*Li* et al., 1999), and marker of salmonella-mediated cell injury. Prevention of elevation of MDA concentration in extracted-treated animals is indicative of their capacity to break chains of lipid peroxidation reactions associated with salmonella infection [35].

Hepatic injury in typhoid has several underlying mechanisms including local or systemic effects of specific endotoxin, non- specific reactive inflammation in response to intestinal ulceration and cytotoxin produced by *S. typhi* that have infected kuffer cells [33]. Hepathomegaly is usually observed in enteric fever [36]. The enlargement of liver in typhoid is caused by hypertrophy and hyperplasia of kupmffer's cells with characteristic inflammation of the liver. In this study, similar hepatic damages were observed with salmonella infection; however, treatments with *C. papaya* and *C. citratus* ameliorated this damage and restored the liver cytoarchitecture to near normal. The organ protective effects of the plant could be attributed to antioxidant flavonoids and phenolics [37,38].

Intestinal perforation is a serious complication of typhoid fever [39], associated with high morbidity

and mortality [40,41]. The high incidence of perforation in most developing countries has been attributed to late diagnosis and the emergence of multidrug resistant and virulent strains of *S. typhi* [42]. The intestinal damage observed in this study was ameliorated when treated with *C. papaya* with near normal restoration of the intestinal integrity.

5. CONCLUSION

This study revealed that each plant extracts function in different ways to relieve the burden of *Salmonella* infection in the experimental rats, thus reflecting the reason for their combination in traditional system of medicine. The results suggest immune-modulatory and antioxidant effects as mechanism of their anti-salmonella activities. It could be concluded that each extracts contributes different pharmacological functions when combine for the management of typhoid.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICS APPROVAL

Ethical approval was granted by FBMS, LAUTECH for the animal experiment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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