Journal of Advances in Medical and Pharmaceutical Sciences

22(5): 10-20, 2020; Article no.JAMPS.58099
ISSN: 2394-1111

In-vivo Anti-typhoid Activities of Ethanol Stem Bark Extract of *Bridelia ferruginea* (Wild) in Albino Rats Infected with *Salmonella typhi*

Ebenezer Oluyemi Dada[1] and Busayo Temitope Akinyele[1*]

[1]Department of Microbiology, School of Sciences, Federal University of Technology, Akure, P.O. Box 704, Ondo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author EOD designed the study and wrote the protocol. Author BTA performed the statistical analysis, wrote the first draft of the manuscript, managed the analyses of the study and managed the literature searches. Author EOD guided in the entire research and edited the final draft of the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2020/v22i530170
Editor(s):
(1) Dr. Erich Cosmi, University of Padua, Italy.
Reviewers:
(1) Erza Genatrika, Universitas Muhammadiyah Purwokerto, Indonesia.
(2) Abrar Hussain Mian, Hazara University Mansehra, Pakistan.
Complete Peer review History: http://www.sdiarticle4.com/review-history/58099

Original Research Article

Received 03 April 2020
Accepted 10 June 2020
Published 16 June 2020

ABSTRACT

Aims: To study the *In-vivo* anti-typhoid activities of ethanol stem bark extract of *Bridelia ferruginea* in albino rats infected with *Salmonella typhi.*
Study Design: Experimental design.
Place and Duration of Study: Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria. Between January, 2019 and June, 2019.
Methodology: Fresh stem bark of *Bridelia ferruginea* were collected, dried, powered and extracted using 70% ethanol. Twenty – seven rats of same age between 90 -120 g in weight were selected and divided into 9 groups containing three each. The infectivity dose (ID) was determined with the clinical *S. typhi*. After which the rats were infected and orally administered various standard doses of the *B. ferruginea* stem bark extract (50-5000 mg/kg) accordingly for 7 days. During the treatment period, the fecal samples were collected to monitor the ability of the extract to reduce the fecal

**Corresponding author: E-mail: akintope709@gmail.com;*

shedding of *S. typhi*. Also, the rats were weighed daily to establish the effect of treatment on their metabolism.
Results: Ethanol extract of *B. ferruginea* Stem bark at concentrations of 50 mg/ml – 100 mg/ml, didn't produce any zone of inhibition but from 300-5000mg/ml produce zone of inhibition (ZI) at 2.00-18.33 mm (P< 0.05) on culture of clinical *S. typhi* isolate and on typed *S. typhi* isolate zones of inhibitions were seen at concentrations 50mg/ml-5000mg/ml, it produced ZI of 8.00 – 26.19 mm(P< 0.05). Minimum inhibitory concentration of the ethanol extract on the clinical *S. typhi*is 300mg/ml while on the typed isolate the minimum inhibitory concentration of the ethanol extract was 2600 mg/ml on ethanol extract respectively. The MBC for the two *S. typhi* isolates were 300 mg/ml and 1000 mg/ml respectively. The *in-vivo* investigation showed the ethanol extract of *B. ferruginea* stem bark on *S. typhi* colony forming units per ml (cfu/ml) of suspensions of faeces of infected rats and treated with the ethanol extract of *B. ferruginea* stem bark decreased significantly (P< 0.05) as the days of the treatment increased while the cfu/ml of the infected but untreated group significantly (P< 0.05) increased. There were no significant (P< 0.05) difference between weights of *S. typhi* un-infected, infected rats treated with Ciprofloxacin and ethanol extract of *B. ferruginea* stem bark but weight of the untreated group significantly (P< 0.05) decreased. Preliminary phytochemical screening of stem bark of *Bridelia ferruginea* ethanol extract revealed the presence of saponins, tannins, flavonoids, glycosides and terpenoid were pharmacological importance.
Conclusion: The *in-vivo* anti- typhoid activity of stem-bark ethanol extract of *Bridelia ferruginea* was found to be relatively safe against *Salmonella typhi*.

Keywords: Bridelia ferruginea; in-vivo; ethanol; stem-bark.

1. INTRODUCTION

Human societies have been in close contact with their environments since the beginning of their formation and used the ingredients of the environment to obtain food and medicine, therefore, medicinal plants are used as a medical resource in almost all cultures and a great number of tree and shrubs have great nutritional and medicinal potentials [1].

Bridelia ferruginea (W) is referred to as Ira which belong to family Euphorbiaceae. It a wood shrub that grows in the Savannah or rain forests of Africa [2]. [3] stated that the stem bark extract is used for milk coagulation and also in lime juice for the formulation of traditional gargle (ogun efu). The bark extract of the plant possesses antimicrobial activities against some micro-organisms known to cause enteric and secondary upper respiratory water treatment [4]. Plants synthesize hundreds of chemical compounds for functions including defense against insects, fungi, diseases and herbivorous animals. The ethanol extract of the stem bark of this plant contains tannins, alkaloids, sterols, terpenes flavonoids and saponins [5]. According to World Health Organization [6], most of the world's population still depends on the usage of plants for their healthcare. The stem bark (bright red infusion) is commonly sold in Nigeria markets and shops for use as a mouth –wash and remedy for thrush in children as reported [4]. The bark is used as antidote against poison and arrow poison [2]. The plant has diverse traditional uses some of which have been verified scientifically: the anti-inflammatory effects, antipyretic, immunomodulatory etc. [7].

The test organism *Salmonella typhi,* it also called *Salmonella entrica typhi* is a pathogenic gram-negative enteric bacillus which belong to the family Enterobacteriaceae (all organisms that belongs to this family ferment glucose, reduce nitrate and are oxidase negative, motile, rod-shaped, facultative anaerobes that is susceptible to various antibiotics [8]. *S. typhi* causes enteric fever which is a major public health problem, both in developing as well as developed economics [9]. It is solely a human pathogen predominately found in the intestinal lumen. Its toxicity is due to an outer membrane consisting largely of lipopolysaccharide (LPS) which protect the bacteria from the environment. The bacteria infect by coming in direct contact with the phagocytic cells. This contact involves the formation of appendages formed which are shorter than flagella but thicker than both flagella and pili. [10] reported that *S. typhi* cells enters the epithelial cells lining the intestine they cause host cell ruffing which temporarily damages the microvilli on the surface of the cell. This causes a rush of white blood cells into the mucosa, which throws off the ratios between absorption and secretion, lead to diarrhea. However, typhoid *Salmonella* species have increasingly become

resistant to conventional antibiotics such as ampicillin, chloramphenicol, cotrimoxazole and fluoroquinolones in developing countries [11].

Documentation on *B. ferruginea* has been proven to possess saponins, tannins, glycosides and flavonoids as the major constituents in the plant which contribute greatly to the bioactivity of *B. ferruginea* and its usage in treating various diseases [12]. However, little attention has been paid to *in-vivo* anti-typhoidial activities of ethanol stem bark extract of *B. ferruginea*. Hence, the present study was aimed at investigating the *in-vivo* anti-typhoid activities of ethanol stem bark extract of *B. ferruginea* against *Salmonella typhi*.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

Fresh stem bark of *Bridelia ferruginea* W. were collected from a garden at Igbara – Odo in Ekiti State. The stem bark were identified and authenticated by using the herbarium specimens of the Department of Crop, Soil and Pest management, Federal University of Technology, Akure (FUTA).

2.2 Source and Preservation of Bacteria

Pure clinical and typed isolates of *Salmonella typhi* were obtained from the stock culture of The University of Ibadan Teaching Hospital, Ibadan Oyo State and typed isolate of *S. typhi* ATCC19214 was obtained from Pathology and Clinical Laboratory Pathcare of Lagos University Teaching Hospital (LUTH) Lagos State, Nigeria. The bacteria isolates were kept on already prepared nutrient agar slants and transported immediately to the microbiology laboratory of the Federal University of Technology, Akure, Ondo State for further analysis.

2.3 Extraction of the Bioactive Constituents of *B. ferruginea* Stem Bark

The methods of [13] were employed. The fresh stem bark of the plant were washed and air-dried at room temperature for the period of four weeks. The stem barks were coarsely powered using a sterile mortar and pestle and were further pulverized to powered using an electric blender (Marlex Electrolyn IS:4250). The powered was transferred into grease-free closed airtight containers to avoid absorption of moisture. Three hundred grams(300 g) of the powdered samples were respectively soaked in two thousand, five hundred millimeter(2500 ml) of 70% ethanol and water as solvents to extract the bioactive compounds. The container was labeled appropriately and left for 72 hours (3 days). Thereafter the crude extracts were sieved respectively using muslin cloth and then filtered using 0.45µm micropore filter. The filtrates were vapourised to dryness using rotary evaporator and subsequently lyophilized to remove the extracting solvent. The weight of the dried extracts was measured respectively and reported as percentage recovery.

Percentage Recovery = (Weight of extract recovered after extraction × 100%)/Initial weight of plant part.

Then, the ethanol extract was reconstituted with 30% dimethlysulphoxide (DMSO) to obtain varying concentrations [13].

2.4 Phytochemical Screening of *B. ferruginea* Stem Bark

The phytochemical evaluation was preformed to ascertain the presence of bio-active metabolite which is of pharmacological importance in the extracts. The phytochemical analysis for saponins, flavonoids, tannins, steroids, glycosides and alkaloids were carried out based on standard procedures [13].

2.5 Anti-typhoid Sensitivity Test

The anti-typhoid sensitivity test was determined using agar well diffusion method [7]. The bacterial isolates were cultured in nutrient broth for 18 hours before use and standardized to 10^6cfu/ml respectively. 38 g of Mueller Hinton Agar (MHA, Difco, USA) was dissolved in 1000ml of distilled water and has been prepared according to manufacturer's standard. Small volume of bacterial suspensions was swabbed on each prepared plates by the means cotton swab making sure they are evenly distributed on the surface of the agar plates. The plates were allowed to stand for 40 minutes for the inoculated bacteria to be established in the medium. 6mm diameter well were made with sterile cork borer on the surface of the inoculated agar at allowance of 30mm between opposite wells and the edges of the petri – dishes. A 0.1 ml of the different concentrations of the ethanol extracts was then introduced into each well in the plates using sterile pipette. A separated plate was used for positive control (Ciprofloxacin). The plates were incubated at 37^oC for 24 hours. The

resulting zones of inhibition were measured in millimeters(mm). The experiment was carried out in triplicates.

2.6 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Bridelia ferruginea* Stem Bark Extract

The MIC and MBC of the ethanol extract was determined according to the methods [14]. 1 ml of graded concentrations (5000 mg/ml, 2600 mg/ml, 1000 mg/ml, 300 mg/ml, 100 mg/ml and 50 mg/ml) of the ethanol extract of *Bridelia ferruginea* stem bark was used.10 ml of 24hrs Mueller-Hinton broth was added and a loopful of test organisms previously diluted was introduced into the tubes. Ciprofloxacin was introduced as positive control and distilled water as negative control in different tubes. A tube containing only nutrient broth was seeded with test organism to serve as positive control while a tube that was not inoculated served as the negative control. All the broth cultures were incubated at 37^{o}C for 24hrs. At the end of incubation, tubes were observed for using a spectrophotometer (Beckman model 35). Growth inhibition was revealed by low turbidity. The concentration at which there was no growth as indicated by clear broth is taken as the minimum inhibitory concentration while the MBC was determined by taking a loopful of broth collected from tubes that do not show any visible growth, was incubated at 37^{o}C for 24hrs. after incubation, the least concentration that showed no growth was recorded as the minimum bactericidal concentration (MBC).

2.7 Animals

The method described by [14], twenty-seven (27) Swiss albino rats (90-120 g) were obtained from Department of Animal Production and Health, Federal University of Technology, Akure, Ondo State, Nigeria. The rats were fed with standard rat pellets (Livestock Feeds, Ikeja, Lagos State) and water *ad libitum*. The animals were housed under standard laboratory conditions and were acclimatized for 7 days before the treatment started. The experimental procedures involving animals were conducted in conformity with International National and Institutional Guidelines [4].

2.7.1 Preparation of standard inoculum of *S. typhi* for *In-vivo* assay

About 0.238 g of sodium hydrogen phosphate was dissolved in 0.019 g of potassium dihydrogen phosphate and sodium chloride respectively. The mixture was made up to 100ml with distilled water and pH was adjusted to neutral pH as described by [15]. Then standard inoculum of *Salmonella typhi* was inoculated into 1000ml of nutrient broth and incubated for 24hours. After incubation, the cells were centrifuged at 2000 rpm for 10 minutes and the supernatant was discarded. Pellets were re-suspended in Phosphate buffered saline (PBS) and centrifuged again for four times. The final cell button was re-suspended in PBS and serially diluted 10^{1} to 10^{6} [16].

2.7.2 Determination of Infectious Dose (ID)

The method described by [17] was adopted. Twenty- seven healthy Swiss albino rats were used to determine the Infectious Dose of *S. typhi*. The rats were divided into nine groups of three rats. Each group was infected with different concentrations of *Salmonella typhi* suspension. The groups were closely monitored for Five days. The concentration of *S. typhi* suspension that produces the signs like unformed stool, feverish condition, temperature rises, weak, scattered fur, falling of hairs, stool with mucous and weight loss in animals was 3.2 ml (10^{2}cfu/ml) and was given as the infectivity dose (ID_{50}) of *S. typhi*. Also, corresponding colony-forming units per millilitres (cfu/ml) of the bacterial dilutions was determined using plate count method on Salmonella-Shigella agar (SSA).

2.7.3 *In-vivo* assay

The method described by [17,16] was adopted. The Swiss albino rats had free access to feeds and water and they were acclimatized for more than a week prior to experiment. Twenty-seven Swiss albino rats having average weight between 90-120 g of 8-12 weeks was infected with *Salmonella typhi* suspension orogastrically and then placed in nine (9) different cages containing three(3) in each for 7 days to determine the anti-typhoid activities using ethanol extracts of *Bridelia ferruginea* stem bark. Group 1a was not infected, group 2a was infected but treated with Ciprofloxacin, group 3a was infected but not treated, group 4a was infected and treated with 50 mg/kg of ethanol extract, group 5a was infected and treated with 100 mg/kg of ethanol extract, group 6a was infected and treated with 300 mg/kg of ethanol extract, group 7a was infected and treated with 1000 mg/kg of ethanol extract, group 8a was infected with 2600 mg/kg of ethanol extract and group 9a was infected

with 5000 mg/kg of ethanol extract. All the experimental rats in groups 1, 2,3,4,5,6,7,8 and 9 were done in triplicates and observed for signs of infection before and after being treated.

2.7.4 Effect of the extract on faecal shedding of *S. typhi* in Swiss albino rats

Samples of the stool/faecal were collected before and during infection for monitoring. One gram (1g) of the sample was serially diluted in physiological saline, plated in triplicates on *Salmonella Shigella* Agar (Difco, USA), and then incubated at 37^{o}C for 24 hours. Typical colonies of *S. typhi* (clear colonies with pinpoint black centres) were then counted on plates that contained between 30 and 300 colonies as reported by [13].

2.7.5 Determination of rat's body weight

[13] method was employed. Each group of rats was fed time a day with standard rat chow (Livestock feeds, Ikeja) and given 20ml of water *ad libitum* two times daily. The body weight of each rat was weighed using a sensitive weighing balance before and during the period of infection and treatment to determine the effect of observed daily for general signs of toxicity and mortality.

2.7.6 Statistical analysis

Data obtained were subjected to One Way Analysis of Variance (ANOVA) while the mean values of replicates were compared with Duncan's Multiple Range Test at 95% confidence interval using SPSS 23.0. Differences were considered significant at ($p \leq 0.05$).

3. RESULTS

3.1 Percentage Recovery of Ethanol Stem Bark Extract of *B. ferruginea*

The percentage recovery of the stem bark extract of *B. ferruginea* after extraction was revealed in Table 1. The table showed that the extracted solvent recovered is 14.0% its viscosity was low and the colour is light brown respectively.

Table 1. Percentage recovery of stem bark ethanol extract of *B. ferruginea*

Parameters	Results
Colour	Light brown
Viscosity	Low
Weight of extract(g)	250
Weight of extracted (g)	35.0
Percentage yield (%)	14.0%

3.2 Phytochemical Screening

The phytochemical screening of the ethanol extract of *B. ferruginea* stem bark is presented in Table 2. The results revealed that saponins, tannins, flavonoids, terpenoids, alkaloids and cardiac glycosides.

Table 2. Qualitative phytochemical screening of ethanol stem bark extract of *B. ferruginea*

Phytochemical constituents	Ethanol
Saponin	+
Tannin	+
Phlobatannin	-
Flavonoid	+
Steroid	-
Terpenoid	+
Alkaloid	+
Anthraquinone	-
Cardiac glycosides	+
Legal test	+
Keller kiliani test	+
Salkwoski test	+
Lieberman test	+

Legend: - Absent + Present

Table 3 revealed the quantitative phytochemical (mg/100g) composition of ethanol crude extracts obtained from the stem bark extract of *B. ferruginea*. The result showed that alkaloids, terpenoids, cardiac glycosides and tannins had the highest values.

Table 3. Quantitative phytochemical screening of ethanol stem bark extract of *B. ferruginea*

Phytochemical composition	Ethanol extract
Alkaloid(%)	9.740^{e}± 0.19
Tannins (mg/g)	$6.081^{b,c}$± 0.28
Flavonoids (mg/g)	3.323^{a}±0.00
Cardiac glycosides (mg/g)	$7.304^{c,d}$±0.67
Terpenoids (mg/g)	7.657^{d}± 0.04
Saponins (mg/g)	3.569^{a}±0.09
Steroids (mg/g)	$0.000^{a,}$±0.00

3.3 Anti-typhoid Sensitivity Test

The anti-typhoid sensitivity pattern of clinical and typed isolates of *S. typhi* to ethanol extract of *B. ferruginea* stem bark showed that the degree of susceptibility as indicated by zones of inhibition varied from one concentration to the other. It was observed that in clinical isolate, there was no zones of inhibition from 50-100 mg/ml but from 300-5000 mg/ml in clinical isolate, there are zones of inhibition. Typed isolates inhibited

various zones of inhibitions concentrations 50-5000 mg/ml (Table 4). The MIC values for clinical and typed *S.typhi* were 300 and 2600 mg/ml respectively while the MBCs values for the clinical and typed isolate were 300 and 1000 mg/ml for the clinical and typed isolates respectively.

Table 4. Inhibition zone diameters (mm) of the ethanol extract of *B. ferruginea* stem bark against clinical and typed *Salmonella typhi*

Extract (mg/ml)	*S. typhi* clinical	*S. typhi* typed
50	0.00^{a}± 0.00	8.00^{a}±1.00
100	0.00^{a}± 0.00	10.00^{a}±0.50
300	2.20^{a}±0.50	13.83^{a}±0.76
1000	10.17^{a}±1.26	19.17^{a}±0.29
2600	14.83^{a}± 0.76	23.17^{a}±0.29
5000	18.33^{a}±0.58	26.17^{a}±0.76

Data are represented as mean ± S.D. mean values with same superscript are not significantly different. Values are considered significantly different at P≤0.05

The *Salmonella typhi* dosage that produced infection signs like weakness, scattered fur, feverish condition, stool with mucous and weight loss in the experimental rats (infectious Dose- ID) was 3.2 ml (10^{2}cfu/ml). The result of faecal shedding of *S.typhi* before and during treatment revealed that all groups did not shed *S. typhi* in their faeces on day 0(before treatment). Similarly, *S. typhi* was not isolated in stool sample of control examined throughout the treatment period. However, infected but untreated group showed increasing *Salmonella* shed as against decreasing shed in groups treated with extract and conventional antibiotics (Table 5). Table 6, showed the weight (g) of rats in their respective treatment groups were monitored. There was no significant change in weight of rats in all the groups before and after treatment except infected but untreated group where there was significant decrease in weight till the fifth day.

4. DISCUSSION

The work tested the *in-vivo* anti-typhoid activities of ethanol stem bark extract of *Bridelia ferruginea* in albino rats infected with *Salmonella typhi*. Traditional medicine involving the use of plant parts with medicinal or healing effect has been the mainstay of meeting most primary health care needs in rural parts of Nigeria and even in a few metropolitan cities. The highest percentage recovery of ethanol could be due to its ability to dissolve more of the active components of the plant than water [18]. [19] also reported that polar solvents have been shown to be more effective in extracting organic and inorganic materials from plants. The study of bioactive compounds present in *B. ferruginea* have a broad range of biological activities. The bioactive components include saponins, tannins, flavonoid, terpenoid, alkaloids, cardiac glycosides and steroids. This is in agreement with the work of Vinha and Soares [20], reported saponins to have anti-inflammatory effects. Kunle and Egharevba [21], reported that the bioactive compounds to possess a wide range of pharmacological and biological activities such as anticancer, anti- malarial, anti-bacterial, antiviral, antifungal, antioxidant. The phytochemical screening results of the extract are consistent with the results reported by [22] [23,24], where authors mentioned the presence of tannins, phenols, alkaloids, glycosides, saponins, terpenoids and steroid in screened *Bridelia ferruginea.* The result of the anti-typhoid activity test (Table 4) revealed that the extract of stem bark of *B. ferruginea* possess antimicrobial activity against clinical and typed *S. typhi* used in this study though at higher concentration. Previous reports have shown that when solvents like ethanol are used to extract plants, such plants were able to exhibit inhibitory effect on both gram positive and gram negative bacteria [25]. In this study, there were variation in the inhibitory activities demonstrated by the ethanol stem bark extract of *B. ferruginea* against clinical and typed isolates. [26], also reported that *Bridelia ferruginea* exerts bactericidal effects on pathogenic organisms. The concentration of 5000mg/ml had the highest zone of inhibition for both clinical and typed isolates of *S. typhi* this suggested that as the concentration of the extract increases, the anti-typhoid activities increases, this could be due to the fact that antimicrobial activities of substance is concentration dependent [12]. In addition to this observation, the non-inhibition observed at 50-100mg/ml concentrations of the ethanol extract on clinical isolate in this study could be that the ethanol used as solvent did not sufficiently extract the bioactive components capable of causing effective inhibition. [27] stated that different solvents have diverse solubility capacities for different phytoconstituents. [13] also stated inhibition observed at higher concentration might be accounted for by the presence of other bioactive components such as tannins, saponin and flavonoid in the ethanol extract According to Adetunji, 1999 *Bridelia ferruginea* has been used traditionally in the treatment of typhoid fever and various stomach related problem [28]. It was also noted in this study that the zone of inhibition on clinical isolate

Table 5. Effect of Extract of *B. ferruginea* treatment on faecal shedding of clinical *Salmonella typhi* (cfu/ml) using plate method

Treatment	0 day	Day 1	Day 2	Day 3	Day 4	Day 5
Not infected	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Infected but treated Ciprofloxacin	0.00±0.00^{a}	20.00±0.57^{c}	11.67±0.33^{b}	3.66±0.33^{b}	0.00±0.00^{a}	0.00±0.00^{a}
Infected but not treated	0.00±0.00^{a}	36.00±0.57^{e}	50.00±0.57^{d}	59.00±0.57^{e}	65.00±1.15^{d}	75.00±1.15^{c}
50 mg/kg ethanol extracts	0.00±0.00^{a}	25.67±0.33^{d}	13.73±0.06^{c}	17.00±0.57^{d}	10.67±0.33^{c}	3.00±0.00^{b}
100 mg/kg ethanol extracts	0.00±0.00^{a}	22.00±1.15^{cd}	12.33±0.66^{c}	7.00±0.00^{d}	3.00±0.00^{b}	0.00±0.00^{a}
300 mg/kg ethanol extracts	0.00±0.00^{a}	24.00±0.57^{cd}	13.67±0.33^{c}	7.67±0.33^{c}	2.00±0.00^{b}	0.00±0.00^{a}
1000 mg/kg ethanol extracts	0.00±0.00^{a}	20.67±0.33^{bc}	6.67±0.33^{b}	3.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}
2600 mg/kg ethanol extracts	0.00±0.00^{a}	17.00±0.57^{b}	7.67±0.3^{b}	2.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}
5000 mg/kg ethanol extracts	0.00±0.00^{a}	15.00±0.57^{b}	5.67±0.33^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Data are represented as mean ± S.D. mean values with same superscript are not significantly different. Values are considered significantly different at P≤0.05

is significantly lower to the zones of inhibition of typed isolate (i.e highest zone of inhibition was observed in typed isolate than clinical isolate). This may be due to the fact that the patients from which the isolate was obtained from has been exposed to commonly used antibiotics. This opinion differed from the reports of [29] who stated that antibiotic resistance does not interfere with antibacterial action of plant extracts and that the extracts might have different modes of action on test organism. Therefore, the demonstration of antibacterial activity of stem bark of *Bridelia ferruginea* extract against clinical and typed isolates used in this study provides a scientific basis for its use in the treatment of typhoid infection as also stated by [13].

The MIC values for clinical and typed isolates were 300 and 2600 mg/ml respectively while the MBCs for clinical and typed isolates were 300 and 1000 mg/ml respectively. According to what was observed in this study, MIC is the lowest concentration of the extract needed to inhibit the growth of the test organism while MBC is the lowest concentration of the extract that kills the test organism (*S. typhi)*. This revealed that in this study, that the plant extract used can be bacteriostatic and bactericidal.

The reduction/decrease in the *S. typhi* shed (Table 5) observed in groups treated with conventional antibiotics(Ciprofloxacin) and plants extract is also similar to the findings of [13] who reported significant ($p< 0.05$) drop in the *S. typhi* load with time in groups of experimental rats treated with ethanol extract of *A. muricata* and conventional antibiotics(Ciprofloxacin). Similarly, [30] reported significantly ($p< 0.05$) drop in the *S.typhi* load with time in groups of experimental rats administered with methanol leave extract of *Paullinia pinnata* and conventional antibiotics (ciprofloxacin and oxytetracycline). However, the continuous increase in *Salmonella typhi* shed in the infected but not treated group is also similar to [13] who observed that the *Salmonella* discharged in the stool remained relatively high in the untreated group. The decrease observed in *S. typhi* shed in group treated with all concentrations of ethanol extract of *B. ferruginea* stem bark will be effective in combating *Salmonella* related infection and which could be useful when treating such infection. Also, the decrease observed in the quantity of stool passed by rats infected but treated with extract confirmed the use of *Bridelia ferruginea* stem bark as an anti-diarrheal agent [31].

The decrease observed in the ethanol extract, however competed favourably with that was observed in the group treated with Ciprofloxacin as a positive control in the study.

Table 6 showed the weight changes which were observed to be insignificant and this is in agreement with the findings of [13] who observed in his report that the insignificant difference observed in body weight of rats given extract in his study. Similarly, [32] found out in his study that all groups of experimental rats treated with leaf extract of *M. charantia* recorded steady

Table 6. Effect of treatment on weight of experimental rats(g) before and after infection with *Salmonella typhi*

Treatment	Initial weight	Day 1	Day 2	Day 3	Day 4	Day 5
Not infected	115.30 ±0.57^{c}	115.60±0.57^{c}	115.10±0.57^{c}	115.80±0.57^{c}	115.60±0.57^{c}	115.20±0.57^{c}
Infected but treated Ciprofloxacin	120.10±1.15^{d}	121.67±0.88^{d}	126.40±0.57^{d}	127.43±1.20^{d}	129.90±0.57^{e}	130.30±0.57^{e}
Infected but not treated	97.65±0.57^{a}	96.53±0.88^{a}	93.33±0.88^{a}	92.80±0.57^{a}	91.30±0.57^{a}	90.09±0.57^{a}
50mg/kg ethanol extracts	101.32±0.88^{b}	102.56±0.88^{b}	104.33±0.88^{b}	104.45±1.15^{b}	104.31±1.15^{b}	106.14±1.15^{b}
100mg/kg ethanol extracts	101.33±0.88^{b}	103.53±0.88^{b}	106.00±1.15^{b}	107.90±1.15^{b}	108.45±1.15^{c}	108.67± 0.88^{b}
300mg/kg ethanol extracts	112.03±0.88^{c}	112.33±0.88^{c}	113.20±1.15^{c}	114.18±1.15^{c}	116.05±1.15^{d}	119.46±0.88^{d}
1000mg/kg ethanol extracts	103.30±0.57^{b}	105.04±0.57^{b}	106.30±0.57^{b}	107.23±0.57^{b}	108.16±0.88^{c}	111.13±0.88^{c}
2600mg/kg ethanol extracts	120.67±0.88^{d}	122.78±0.88^{d}	124.20±1.15^{d}	126.67±0.88^{d}	128.67±0.88^{e}	131.67±0.88^{e}
5000mg/kg ethanol extracts	118.16±0.88^{d}	122.10±1.15^{d}	123.47±1.15^{d}	126.14±0.57^{d}	128.89±0.57^{e}	132.67±0.88^{e}

increase in body weight. The expected significant decrease ($p < 0.05$) observed in group infected but untreated might be due to the establishment of infection in the rats leading to loss of weight. However, on the first to fifth day for concentration of 2600-5000 mg/ml, it was observed that there was high increase in the weight of the rats which can be compared to group treated with Ciprofloxacin can be due to their recovery as a results they both results in the fighting activity of white blood cells differentials. This observation is similar to a case reported by [33] in his own findings of a human case in which some victims of *E.coli* 0157:H7 infection recovered without treatment within 5-10 days. Therefore, the body weight change serves as an indicator of the general health status of animals and it will be significant if the body weight loss that occurred is more than 10% from the initial weight [34]. Hence, the insignificant difference observed in body weight of rats given extract in this study indicates that the ethanol extract of *Bridelia ferruginea* stem bark does not interfere with the metabolism of the rats.

5. CONCLUSION

The present study which was demonstrated to show the*in-vivo* anti-typhoid activity of ethanol stem bark extract of *Bridelia ferruginea* on clinical and typed isolates of *Salmonella typhi*. The reduction which occurred in the faecal shedding of *S. typhi* in group of rats treated with the stem bark ethanol extract of *B. ferrruginea* provides scientific basis for its use as a treatment of typhoid infection. The study also revealed that the plant extract used, can be bacteriostatic and bactericidal. When applied in the low concentrations the ethanol extract of *B. ferruginea* stem bark were unable to performed as expected. Therefore, it is recommended that further research should be carried out using other solvents that is different from the solvent used for this study so as to know the best isolate active component of the stem bark extract.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

ACKNOWLEDGEMENT

All glory be to Almighty God. I also wish to appreciate the good people God has used to bless me morally and financially during this journal write-up like Dcn. and Mrs. K.S Adeyeye c/o Monarch Model College, Ibadan, Nigeria and my Parents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fatemeh Jamshidi-Kia, Zahra Lorigooini, Hossein Amini-Khoei. Medicinal plants: Past history and future perpective. Journal Herbmed Pharmacol. 2018;7(1):1-7.
2. Burkil HM. The useful plants of West Tropical Africa. The Royal Botanic Garden, Kew.1994;2:636.
3. Bakoma B, Eklu-Gadegbeku K, Berké BA, Agbonon A, Aklikokou K, Gbeassor M. Chemical components and effect on streptozotocin induced diabetes of *Bridelia ferruginea* benth root bark ethyl acetate fraction. International Journal of Pharma and Bio Sciences. 2015;6(1):843-853.
4. Adeleye AO, Oloyede OI, Bolarinwa TO, Adebayo OA. Inhibitory effects of aqueous extract of *Brideliaferruginea* stem bark on Iron (II) Sulphate - Induced Oxidative Stress in Brain and Liver of Albino Wistar Rats. Journal of Applied Life Sciences International. 2017;14(2):1-7.
5. Ahn K. The worldwide trend of using botanical drugs and strategies for development global drugs. BMB Reports. 2017;50(3):111–116.
6. World Health Organisation, Food Agricultural Organisation of the United Nations and World Organisation for Animal Health – W.H.O/FAO/OIE. Expert Workshop on Nonhuman Antimicrobial Usage and Antimicrobial Resistance: Scientific Assessment. 2003;1-5.
7. Kwesi Prah Thomford, Ronald Yeboah, Ama Kyeraa Thomford, Dominic Adotey Edoh, Merlin Lincoln Kwao Mensah, Alfred Ampomah Appiah. A Retrospective Clinical Study on the Effectiveness of the Aqueous

Leaf Extract of *Brideliaferrugenia* (Benth) in the Management of Diabetes Mellitus. International Journal of Herbal Medicine. 2015;2(5):43-45.

8. Ong SY, Pratap CB, Wan X, Hou S, Rahman AYA, Saito JA, Alam M. The genomic blueprint of Salmonella enterica subspecies entericaserovar Typhi Pstx-12. Standards in Genomic Sciences. 2013;7: 483-496.
9. Alba S, Bakker MI, Hatta M, Scheelbeek PFD, Dwiyanti R, Usman R, et al. Risk factors of typhoid infection in the Indonesian archipelago. PLoS One. 2016; 13(3):1-9.
10. Darton TC, Zhou L, Blohmke CJ, Jones C, Waddington CS, Baker S, et al. Blood culture - PCR to optimise typhoid fever diagnosis after controlled human infection identifies frequent asymptomatic cases and evidence of primary bacteraemia. J Infect. 2017;74:358–366.
11. Dahiya S, Sharma P, Kumari B, Pandey S, Malik R, Manral N, et al. Characterization of ntimicrobial resistance in Salmonellae during 2014–2015 from four centres across IndiaAn ICMR antimicrobial resistance surveillance network report. Indian J Med Microbiol. 2017;35:61.
12. Yunana BT, Guiyi JC, Bukar BB. In vitro and in vivo evaluation of antibacterial activity of *Brideliaferrugine* extracts on some clinical isolates. J. Phytopharmacol. 2018;7(4):392-398.
13. Faleye OS, Dada EO. Effects of ethanol extract of unripe *Annona muricata* (l.) fruits onthe haematological and histopathological parameters in Swiss albino rats infected with *Salmonella typhi*. British Journal of Pharmaceutical Research. 2016;9(1):1-13.
14. Cheesbrough M. District laboratory practice in Tropical countries. 2nd edition, Cambridge University Press, UK. 2014; 434.
15. Khanam F, Sayeed MA, Choudhury FK, Sheikh A, Ahmed D, Goswami D, Hossain ML, Brooks A, Calderwood SB, Charles RC, Cravioto A, Ryan ET, Qadri F. Typhoid fever in young children in Bangladesh: Clinical findings, antibiotic susceptibility pattern and immune responses. PLoS. Negl. Trop. Dis. 2015;9(4):e0003619. DOI: 10.1371/journal.pntd.0003619 8
16. Reddy YRR, Lokanatha O, Ratnam KSVP, Reddy CS, Naga Raju I, Reddy CD. Acute and sub acute toxicity of *Moringa oleifera* stem bark extract in Swiss albino mice. Intl. J. Life Sci. Biotechnol. & Pharma Res. 2013;2(4):73-84.
17. Dada EO, Komolafe OI. *In vivo* evaluation of the inhibitory effects of *Ocimum gratissimum* on Salmonella typhi. Intl. J. Pharm. & Biol. Res. 2013;4(4):185-191
18. Aina VO, Abdulsalami MS, Ibrahim MB, Adejo GO, Audu G. Comparative antibacterial study of aqueous and ethanolic leaf extracts of *Annona muricata.* Journal of Natural Sciences Research. 2016;6(3):141-146.
19. Harbourne N, Marete E, Jacquier JC, O 'Riordan D. stability of phytochemicals as sources of anti-inflammatory, nutraceuticals in beverages. A review. Food Research International Journal. 2013;50(16):481-487.
20. Vinha AF, Soares MO. Phytochemical characterization and radical scavenging activity of aqueous extracts of medicinal plants from Portugal. European Journal of Medicinal Plants. 2012;2(4):335-347.
21. Kunle Oluyemisi Folasade, Egharevba Henry Omoregie. Chemical consistuents and biological activity of medicinal plants used for the managements of sickle cell disease: A review. Journal of Medicinal Plants Research. 2013;7(48):3452-3476.
22. Dada-Adegbola HO, Oluwatoba OA, Adebiyi OE, Odikagbue AN. *In-vitro* evidence of anti-infective activity of crude aqueos extract obtained by boiling ripe stem bark of *Bridelia ferruginea* Benth. Joural of Pharmacognosy and Phytotherapy. 2010;2(4):43-48.
23. Owoseni AA, Ayanbamji TA, Ajayi YO, Ewegbenro IB. Antimicrobial and phytochemical analysis of leaves and bark extracts from *Bridelia ferruginea.* African Journal of Biotechnology.2010;9(7):1031-1036.
24. Ndam IM, Mih AM, Fongid AG, Tening AS, Tonjock RK, Enang JE, Fujii Y. Phytochemical screening of the bioactive compounds in twenty Cameroonian medicinal plants. International Journal of Current Microbiology and Applied Sciences. 2014;3(12):768-778.
25. Shyamala Gowri S, Vasantha K. Phytochemical screening and antibacterial activity of *Syzygium cumini* L. (Myrtaceae) leaves extracts. International Journal of Phramaceutical Research. 2010;2(5):1569-1573.

26. Olanipekun Mary K, Adedeji Damilola E, Kayode Joshua, David O. Moses, Adewusi Damilare. Phytochemical Analysis and Anti- Microbial Effects of *Alstonia boonei* (De Wild), *Bridelia ferruginea* (Benth), *Eucalyptus tereticornis* (Dehnh), *Terminalia schimperiana* (Hochst) and *Polyalthia longifolia* (Sonn) Thwaites. International Journal of Biomedical Papers. 2016;1:5-12.
27. Alabi OA, Haruna MT, Anokwuru CO, Jegede T, Abia H, Okegbe VU, Esan BE. Comparative studies on antimicrobial properties of extract of fresh and dried leaves of *Carica papaya* (L.) on clinical bacterial and fungal isolates from clinical samples in Riyadh area. Europ. Sci. J. 2015;11(6):136-143.
28. Adetunji LK. Great Secret of Nature. Natural Links Centre. 1999;19-23.
29. Oskay M, Oskay D, Kalyoncu. Activity of some plant extracts against multi-drug resistant human pathogens. Iranian J. Pharma. Res. 2009;8(4):293-300.
30. Adeyi AO, Jinadu AM, Arojojoye OA, Alao OO, Ighodaro OM, Adeyi OE. *In-vivo* and *in-vitro* antibacterial activities of *Momordica charantia* on *Salmonella typhi* and its effect on liver function in typhoid-infected rats. J. Pharmacog and Phytother. 2013;5(11): 183-188.
31. Taylor L. Technical data report for graviola (*A. muricata*) In: The healing power of rainforest herbs. Sage Press Inc. Austin; 2005.
32. Patel D, Desai S, Devkar R, Ramachandran AV. Acute and sub-chronic toxicological evaluation of hydromethanolic extract of *Coriandrum sativum* L. Seeds. EXCLI Journal. 2012;11:566575.
33. Centre for Disease Control and Prevention. Questions and Answers: Sickness caused by *E. coli* 0157: H7; 2006. Available:https:www.cdc.gov/ncidod/dbmd/ disease info/*Escherichia coli*-g.htm
34. Nweke CN, Ibiam OFA. Pre and post harvest fungi associated with the soft rot of the fruit of *Annona muricata* and their effects on the nutrient content of the pulp. Am. J. Food & Nutri. 2012;2(4):78-85.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/58099