The Journal of Phytopharmacology 2018; 7(4): 392-398
Online at: www.phytopharmajournal.com

The Journal of Phytopharmacology
(Pharmacognosy and phytomedicine Research)

Research Article

ISSN 2320-480X
JPHYTO 2018; 7(4): 392-398
July- August
Received: 08-07-2018
Accepted: 09-08-2018
© 2018, All rights reserved

**Yunana B.T.**
Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria

**Guiyi J.C.**
Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria

**Bukar B.B.**
Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria

**Correspondence:**
**Yunana Toba Bitrus**
Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria
Email: bitrus2toba[at]gmail.com

# *In vitro* and *in vivo* evaluation of antibacterial activity of *Bridelia ferrugine* extracts on some clinical isolates

Yunana B.T.*, Guiyi J.C., Bukar B.B.

## ABSTRACT

The ethanol extracts of root, bark and leaf of *Bridelia ferrugineawas* investigated for antibacterial activity against clinical isolate of *Staphylococcus aureus* and *Escherichia coli*. The extracts had significant antibacterial activity in vitro at concentration of 25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml and in vivo at dose of 50 mg/kg and 100 mg/kg. The root extract *in vitro* had the highest zone of inhibition, followed by the bark extract for both *Staphylococcus aureus* and *Escherichia coli.* The concentration of 200 mg/ml had the highest zone of inhibition in vitro. The minimum inhibitory concentration (MIC) showed a decreasing inhibitory effect of the plant extracts for both *Staphylococcus aureus* and *Escherichia coli* as the concentration decreases with root having 3.125 mg/ml, bark having 6.25 mg/ml and leaf having 25 mg/ml for *Staphylococcus aureus* and *Escherichia coli*. Likewise, the minimum bactericidal concentration (MBC) showed decreasing bactericide effects with decrease concentration with root having 12.5 mg/ml, bark having 12.5 mg/ml and leaf having 25 mg/ml for *Escherichia coli* while root had 6.25mg/ml, bark had 12.5mg/ml and leaf had 25mg/ml for *Staphylococcus aureus*. The *in vivo* investigation showed that the root and bark extract exhibited antibacterial activity on both *Staphylococcus aureus* and *Escherichia coli* at doses of 100mg/kg and 50mg/kg; the root extract had higher activity than the bark and root/bark combined. The dose of 100 mg/kg had the highest colonies reduction for *Staphylococcus aureus* and *Escherichia coli in vivo*. Preliminary phytochemical screening of root, bark and leaves of *Bridelia ferruginea* revealed the presence of tannins, flavonoids, carbohydrates, cardiac glycoside (root, bark and leaves), saponins (root and bark). The presence of tannins, saponins, flavonoid, cardiac glycoside and carbohydrate in the bark and root extracts of the plant indicates that the bark and root extracts were pharmacological importance.

**Keywords:** *Bridelia ferruginea,* Antibacterial, *Staphylococcus aureus, Escherichia coli*.

## INTRODUCTION

*Bridelia ferruginea* is a native medicinal plant in Nigeria, commonly found in the savannah region of the country. It is a shrub of 6-15meter high which may sometime attain the size of a tree in favourable condition, it belongs to the family *Euphorbiaceae* and genus *Bridelia*. *Bridelia ferruginea* in Nigeria commonly called Kirni, Kizni in Hausa language; Mirehi in Fulani language; Iralodan in Yoruba language and Ola in Igbo language. *Bridelia ferruginea* root, bark and leaf are commonly sold in Nigeria markets and shop as traditional medicine for various purpose including treatment of infectious diseases. *Bridelia ferruginea* has been used traditionally for diverse purpose in Nigeria and other African countries. The roots bark and leaves are constituents of Yoruba (in Nigeria) in fusions, primarily given to children; the same use is made of the root decoction in Ivory Coast [1]. The plant bark and infusion (bright red) from it are frequently sold in Nigeria markets and shops for use as mouth-wash and to redress thrust in children [2]. The bark decoction has been used for toothache in Congo and for dysentery, diarrhea or as a laxative in Ivory Coast [3]. The plant bark has also been used as antidote against poison and arrow poison [1]. According to Irobi, *et al.,* 1994 the root decoction is used by local medicine men for the treatment of gonorrhea infection [4]. Cimanga, *et al.,* 1999, reported that the leaves decoction has been used to treat diabetes, and as a purgative and a vermifuge [5]. Iwu,1984 also reported that a leaf extract in saline solution is reported is produce marked reduction of blood sugar in laboratory rats and clinical trials have given a drop from 250mg% to the normal less than 120mg% after eight weeks of daily treatment [6]. The roots have been reportedly used as chewing stick and root bark used for treatment of intestinal and bladder disorders and as remedy for skin disease [7]. The bark preparation has been shown to be useful for immunity against arrow poison and syphilis [2], likewise the bark extract was reported to be beneficiary for milk coagulation as well as lime juice for the formulation of traditional gargle "Ogunefu" [8].

Some reported activity of the plant extract included antimicrobial[9]; trypanocidal potential of methanolic extract[10]; Molluscidal activity[11]; Anti-inflammatory activity[12]; Antimicrobial properties of stem bark

against facultative gram negative organisms[13]; Positive effect on gram positive bacteria (Sarcina lutea and staphylococcus aureus)[14]; Bark extract of *Bridelia ferruginea* mix with the stem of costus afer for the treatment of minor epilepsy[15]; Water treatment potential [16]; Analgesic and antipyretic properties of stem bark[17].

The antimicrobial activities of the leaves and stem bark of *Birdelia ferruginea* against some potential pathogenic organism have been extensively investigated *in vitro* [2, 4]. However *in vitro* investigation on the activities of the root extracts of *Bridelia ferruginea* and *in vivo* evaluation of the leaves bark and root extracts have not been extensive. This current study was designed to investigate if *Bridelia ferruginea* plant extract (leaves, bark and roots) have antibacterial activity *in vitro* and *in vivo* and with a view of validating the plant's enthnomedicinal treatment of infectious diseases.

Also known as antibacterial are a group of agents called antimicrobial drugs effective in treatment and prevention of bacterial caused infections [18]. These agents may either kill or inhibit bacterial growth. The American Society of Health System Pharmacists in 2015 reported that a few numbers of antibiotics also have antiprotozoal activity [19]. Alexander Fleming identified penicillin in 1928, a chemical substance with antibiotic properties [20]. Since after then antibiotics have completely changed modern medicine and have saved lives of million patients [18, 21]

The drug antibiotic did not only save millions of patients' lives, but have played a key role in accomplishing major development in medicine and surgery [20]. It has positively stop and managed infections that emerge in patients who are on chemotherapy treatment, patient with chronic disease like rheumatoid arthritis, renal diseases, diabetes or patient who had complex surgery like organ transplant, joint replacement or cardiac surgery [18,20,21,22 &23]. In under developed country such as Nigeria where hygienic measures or recycling waste is still poor, antibiotics have been reported to reduce the morbidity and mortality that occur as a result of food borne infections and other poverty related infections [23]. In developed country like the United States, antibiotics have contributed to increase life span expectancy by altering the state of bacterial infections [23, 24]. Congressional Research Service reported that people in the United State were expected to live only to the age of 56.4 years old in 1920, but currently the average life span expectancy of people in the United State is approximately 80 years.

In 1928, the contemporary era of antibiotics began with the discovery of penicillin by Sir Alexander Fleming [24, 25]. Following the discovery of penicillin, antibiotics have transformed contemporary medicine and rescued millions of patients' lives [18, 21]. Antibiotics were initially prescribed to treat weighty infections in the 1940s [18]. Penicillin was effective in restraining bacterial infections among World War II Soldiers, nevertheless briefly after that; penicillin resistance befitted a substantial clinical problem. In response to penicillin resistance a new beta-lactam antibiotic were uncovered, developed and added to re-establishing trust [25. 26]. However, the first case of methicillin resistant Staphylococcus aureus (MRSA) was established in the same decennium in the United Kingdom (1962) and the United State (1968)[18, 26]

According to Centers for Disease Control and Prevention, office of infectious Disease. Antibiotics resistance has unfortunately been seen to closely all antibiotics developed [18]. In the year 1972, Vancomycin was announced into clinical practice for treatment of methicillin staphylococcus aureus (MRSA) and coagulase-negative staphylococci[18, 25]. It had been very hard to produce vancomycin resistance, notwithstanding cases of vancomycin resistance had been reported in coagulase-negative *Staphylococci* in the year 1979 and 1985[18]. Spellbery and Gilbert, 2014 reported that Pharmaceutical industry had introduced many newer antibiotics from the late 1960s to the early 1980s to tackle the resistance difficulty associated with antibiotics, yet thereafter, the antibiotics pipeline started to dry up and fewer new drugs were added. As a matter of fact, in 2015, resistance to antibiotics is still observed after many years of patients treated with antibiotics; bacterial infections have again become a threat [20, 26].

## MATERIALS AND METHODS

### Plant Materials Collection

Bark, Roots and leaves of *Bridelia ferruginea* (Voucher Number: FHJ236) were collected from the Herbarium unit of Department of forestry technology, Federal College of Forestry Jos Nigeria. The plant and its parts were authenticated by Mr. Joseph Jeffrey Azila of the department of Forestry Technology, Federal College of Forestry Jos Nigeria.

### Preparation of Extracts:

The roots bark and leaves of the plant were chopped into smaller parts and dried at $25^0$C in the laboratory for four weeks. The dried samples were pulverized and grounded into powder using blender machine and the powders were labeled and stored in an air tight container for further use. 100g of each powdered sample of root, bark and leaves was weighted into 500ml conical flasks each and soaked in 70% ethanol and Then allowed to stand overnight (24 hours) and shook for three hours on a mechanical shaker. The content was filtered using a non-absorbent cotton wool on a Buchner funnel-flask using a vacuum pump. The root, bark and leaves residue were subjected to several parts of rinsing and filtration with fresh solvents to attain some level of elusive maceration (extraction). The root, bark and leaves filtrates were evaporated to dryness using a rotary evaporator and a drying cabinet. The percentage yield was determined (Root=19%, Bark=14.2% and Leaves=18.9%) and the extract transferred into stirrer sample container properly labeled and preserved in the refrigerator for further use [27. 28].

### Phytochemical Analysis:

The phytochemical evaluation was performed to ascertain the presence of active metabolite which is of pharmacological importance in the extracts. The phytochemical analysis for flavonoids, cardiac glycosides, alkaloids, tannins, anthraquinones, steroids and carbohydrates, saponins were carried out based on standard procedures [27, 28].

### Test Organisms

The clinical isolates used in this study were *Escherichia coli* and *Staphylococcus aureus.* They were obtained from the faculty of Pharmaceutical Sciences, Department of Pharmaceutical Microbiology University of Jos, Plateau State of Nigeria. Biochemical and identification test were carried out for detection and identification of the test organisms.

Indole test was carried out for detection of *Escherichia coli*. Test organism was inoculated into a sterile bottle containing 3ml of sterile

tryptone water then incubate at 35-37 ºC for 48hours then 0.5ml of Kovac's reagents was added and shake gently a red colour in the surface layer within 10minutes indicated *Escherichia coli;* identification test for *Escherichia coli* was carried out by culturing test organism on MacConkey agar then observed for rose pink after incubating for 18-24hours.

Catalyst test was carried out to detect *staphylococcus aureus*. Hydrogen perioxde was placed on a slide and test organism inoculated, bubble sign indicated *Staphylococcusaureus;* Identification test for *Staphylococcus aureus* was carried out by culturing test organism on a mannitol salt agar then observed for a change from red to yellowish colour after incubating for 18-24hours.

***In Vitro* Assay**

**Antibacterial Sensitivity Test**

The antibacterial sensitivity test was determined using the agar well diffusion method [29]. The bacteria isolates were cultured in nutrient broth for 18hours before use and standardized to $10^6$cfu/ml *Staphylococcus auerus* and $10^6$cfu/ml *Escherichia coli* respectively.

The medium employed was nutrient agar solution, which was prepared according to manufacturer's standard of 38g of Mueller Hinton Agar was dissolved in 1000ml of distilled water. The agar was heated gently to dissolve completely. 20mls was then dispensed into sterile bottles and autoclaved at 121 ºC for 15minutes. The agar was allowed to cool at room temperature. Broth cultures of test isolates (0.1ml) containing $10^6$ cfu/ml organisms was introduced into the bottles containing 20ml of molten Mueller Hinton Agar, the content was thoroughly mixed and poured into labeled sterile petri-dish and allowed to set firm. The plates were surface dried at 35 ºC for 15minutes, then 6mm diameter well were made with a sterile cork borer on the surface of the inoculated agar, then equal volumes of plant extracts (1000µL) and gentamycin (control) were transferred into well with the aid of micropipette from extracts concentration of (200, 100, 50 and 25) mg/ml prepared by serial dilution and 20µg/ml of gentamycin as control. Plates were then kept to stand for sixty minutes to allow for pre-diffusion of the extracts [30]. Plates were then incubated for 18-24hours. The plates were observed for zone of inhibition in millimeters (mm). The experiment was carried out in triplicates.

**Minimum Inhibitory Concentration (MIC)**

The Minimum Inhibitory Concentration (MIC) was determined according to the methods of Ndukwe *et al*., 2007 [31]. The medium used was the nutrient agar solution which was prepared according to manufacturer's specification of 38g/1000ml. Double strength was prepared by dissolving 38g in 500ml of distilled water which was heated to allow for complete dissolution of medium. After which 10ml of the medium was dispensed in to sterile bottles and autoclaved at 121 ºC for 15minutes. The agar was allowed to cool at room temperature. Each graded solution was then added and mixed gently with the molten double strength nutrient agar and was poured into the labeled sterile petri-dish and allowed to set firm for 1hour. Extracts concentrations of (200,100, 50, 25, 12.5, 6.25 and 3.125) mg/ml were prepared by serial dilution. The plates were surfaced dried at 35 ºC for 5minutes and plates were inoculated with test isolates at 37 ºC for 18-24hours. At the end of incubation, plates were observed for growth or death of test organisms. The lowest concentration inhibiting growths were taken to be the minimum inhibitory concentration (MIC). Experiments were carried out in triplicates.

**Minimum Bactericidal Concentration (MBC)**

This test determines the lowest concentrations at which an antibacterial agent will kill a particular microorganism [31]. A portion from each plate that exhibited no growth were taken from the MIC test and then sub-cultured on a Mueller Hinton agar (nutrient agar) and was incubated at 37 ºC for 24hours. The lowest concentration that revealed no visible bacterial growth was taken as the minimum bactericidal concentration.

***In Vivo* Assay**

**Animal**

Male and female mice aged between 6 and 8weeks (15-25g) obtained from random bred at National Veterinary Research Institute (NVIR), Vom Plateau State Nigeria, were used for this investigation. Animals were housed in the animal house of university of Jos in temperature-controlled room under 12hours light and 12-hour dark cycle. Animals had free access to food and water and were acclimatized for more than a week prior to experiments.

**Evaluation of Antibacterial Activity**

The determination of the antibacterial activity was carried out *in vivo* according to Hosseinzadeh *et al.,* 2007 [32] with little modifications. 48 mice were used, male and female mice aged between 6 and 8weeks (15-25g) and grouped into eight groups of six animals each. In each group, three mice were infected with

0.1ml ($10^6$cfu/ml) of *Staphylococcus aureus*and three mice with 0.1ml ($10^6$cfu/ml) *Escherichia coli* suspension intraperitoneally then placed in different cages. Twenty-four hours later specimen (blood) was taken from the animal's tail and culture on a specific medium for *Staphylococcus aureus* (Mannitol salt agar) and *Escherichia coli* (MacConkey agar) respectively. Colonies growth was observed for both *Staphylococcus aureus* and *Escherichia coli* indicating that animals are already infected with the test organisms. Twenty-four hours later each group received different treatments. Groups were designed as follow:

**Negative Control.**Group 1 received 0.4ml of normal saline against *Staphylococcus aureus* and *Escherichia coli.*

**Positive Control.** Group 2 received 4mg/kg gentamycin against *Staphylococcus aureus* and *Escherichia coli*

**Root Extract.** Group 3 received 100mg/kg Root Extracts against *Staphylococcus aureus* and against *Escherichia coli.*

**Root Extract.** Group 4 received 50mg/kg Root Extract against *Staphylococcus aureus* and against *Escherichia coli.*

**Bark Extract.** Group 5 received 100mg/kg Bark extract against *Staphylococcus aureus* and against *Escherichia coli.*

**Bark Extract.** Group 6 received 50mg/kg Bark extract against *Staphylococcus aureus* and against *Escherichia coli*.

**Root/Bark Extract.** Group 7 received 100mg/kg Root/Bark extract against *Staphylococcus aureus* and against *Escherichia coli.*

**Root/Bark Extract.** Group 8 received 50mg/kg Root/Bark extract against *Staphylococcus aureus* and against *Escherihia coli.*

After 72hours aspirated specimen from mice tail were collected and cultured on Mannitol salt agar for *Staphylococcu saureus* and MacConkey agar for *Escherichia coli*. Plates were then incubated overnight at 35 ºC and the number of colonies formed on each plate counted and the mean value for each group compared with the negative control and percentage activity calculated. This procedure was repeated for 48hours, 72hours and 144hours respectively until there was no colony growth.

**Statistical Analysis**

All experiments were performed in triplicate for validity of statistical analysis. Results were expressed as mean ± SD. Two ways ANOVA were performed on the data sets generated, differences were considered significant for P-values < 0.05. Analyzed data/results were presented as tables.

## RESULTS AND DISCUSSION

Preliminary phytohemical investigation of root, bark and leaves of *Bridelia ferruginea* revealed the presence of tannins, flavonoids, carbohydrates, cardiac glycoside (root, bark and leaves), saponins (root and bark) as indicate in Table 1. The availability of flavonoid, tannins, cardiac glycoside, saponins and carbohydrate in the extracts (root and bark) of the plant reveals that the extracts were of pharmacological importance [13]. The presence of saponins and tannins enhanced antimicrobial activity as reported by Ndukwe *et al.,* 2005 [13]. The root extract exhibited higher activity against *Staphylococcus aureus* and *Escherichia coli* at concentration of 25, 50, 100 and 200mg/ml *in vitro*. But the leaf extract exhibited lesser activity compared to root and bark. The *in vitro* and *in vivo* Antibacterial investigation shows that root and bark extracts had significant antibacterial activities than that of the leaf extract. This could be attributed to the presence of active substance which could be higher in root and bark and are probably different from that of the leaf. According to Adetunji, 1999 *Bridelia ferruginea* has been used traditionally in the treatment of typhoid fever and various stomach related problems [33]. There were no significant different when the root extract was compared with the control at P < 0.05, also in this study the extract root and bark inhibit growth of *Eschrerichia coli* and *staphylococcus aureus* to a high degree. The relative high zone of inhibition shown by the root extracts on *staphylococcus aureus* and *Eschrerichia coli* suggest, the plant can be used for the treatment of infectious diseases primarily associated to microorganisms as reported by Owoseni *et al.,* 2010[2].The concentration of 200mg/ml had the highest zone of inhibition for *staphylococcus aureus* and *Eschrerichia coli* this suggest that as the concentration of the extract was increased the antibacterial activity increased, this could be attributed to the fact that antimicrobial activities of substance is concentration dependent[34], and that antimicrobial activity is a function of active ingredient reaching an organism. Minimum inhibitory concentration (MIC) as shown in Table 3a & 3b indicates a decreasing inhibitory effect of the plant extracts as concentration decreases for all the extracts (root, bark and leaf) this implies that the antibacterial activities are concentration dependent [34]. The root and bark extracts had minimum inhibitory concentration of 6.25 and 12.5 mg/ml for both *staphylococcus aureus* and *Eschrerichia coli* respectively, while the leaf had minimum inhibitory concentration of 25mg/ml for *staphylococcus aureus* and *Eschrerichia coli.*

**Table 1:** Phytochemical constituents of bark root and leave of *Bridelia ferruginea*

| Constituents | Root | Bark | Leaves |
|---|---|---|---|
| Alkaloids | - | - | - |
| Tannins | +++ | ++ | +++ |
| Flavonoids | ++ | ++ | +++ |
| Saponins | + | + | - |
| Steroids | - | - | - |
| Anthraquinones | - | - | - |
| Carbohydrates | +++ | +++ | ++ |
| **Cardiac glycosides** | + | + | ++ |

Key: -: Negative, +: Positive

**Table 2a:** *In vitro* antibacterial activities of ethanol extracts of *Bridelia ferruginea* against *Staphylococcus aureus*.

| | | TREATMENT GROUPS | | | |
|---|---|---|---|---|---|
| **Extracts** | **Control (Gentamycin)** | **25mg/ml** | **50mg/ml** | **100mg/ml** | **200mg/ml** |
| **Root** | 25.3±0.3 | 16.5±0.29*≠ | 18.7±0.3*≠ | 21±0.3*≠ | 22.5±0.3* |
| **Bark** | 25.5±0.3 | 11.7±0.9*T≠ | 13.3±0.7*T≠ | 15.5±0.7*T≠ | 17.8±0.2*T |
| **Leaves** | 25.3±0.3 | 9.5±0.3*T≠ | 10.5±0.3*T≠ | 12.5±0.3*T≠ | 14.8±0.2*T |

*P<0.05 when compared to control (gentamycin)
TP<0.05 when compared to root extract.
≠P<0.05 when compared to 200mg/ml concentration

**Table 2b:** *In vitro* antibacterial activities of ethanol extracts of *Bridelia ferruginea* against *Escherichia coli.*

| | | TREATMENT GROUPS | | | |
|---|---|---|---|---|---|
| **Extracts** | **Control (Gentamycin)** | **25mg/ml** | **50mg/ml** | **100mg/ml** | **200mg/ml** |
| **Root** | 25.7±0.3 | 19.3±0.3*≠ | 21.6±0.3*≠ | 23.5±0.3*≠ | 25.7±0.3* |
| **Bark** | 26.5±0.4 | 15.3±0.4*T≠ | 17.8±0.8*T≠ | 19.7±0.4*T≠ | 21.5±0.4*T |
| **Leaves** | 26.2±0.2 | 11.2±0.6*T≠ | 12.8±0.4*T≠ | 11.2±0.6*T≠ | 26.2±0.16*T |

*P<0.05 when compared to control (gentamycin)
TP<0.05 when compared to root extract.
≠P<0.05 when compared to 200mg/ml concentration

**Table 3a:** Minimum inhibitory concentration (MIC) of ethanol extracts of *Bridelia ferruginea* against *Staphylococcus aureus*.

| | Minimum inhibitory concentrations (mg/ml) | | |
|---|---|---|---|
| Concentrations of extracts (mg/ml) | Root | Bark | Leaves |
| 200 | – | – | – |
| 100 | – | – | – |
| 50 | – | – | – |
| 25 | – | – | + |
| 12.5 | – | – | + |
| 6.25 | – | + | + |
| 3.125 | + | + | + |

**Key:** ─: No growth, +: Growth

**Table 3b:** Minimum inhibitory concentration (MIC) of ethanol extracts of *Bridelia ferruginea* against *Escherichia coli.*

| | Minimum inhibitory concentrations (mg/ml) | | |
|---|---|---|---|
| Concentrations of extracts (mg/ml) | Root | Bark | Leaves |
| 200 | – | – | – |
| 100 | – | – | – |
| 50 | – | – | – |
| 25 | – | – | + |
| 12.5 | – | – | + |
| 6.25 | – | + | + |
| 3.125 | + | + | + |

**Key:** ─: No growth, +: Growth

**Table 4a:** Minimum bactericidal concentration (MBC) of ethanol extracts of *Bridelia ferruginea* against *Staphylococcus aureus.*

| | Minimum bactericidal concentrations (mg/ml) | | |
|---|---|---|---|
| Concentrations of extracts (mg/ml) | Root | Bark | Leaves |
| 200 | – | – | – |
| 100 | – | – | – |
| 50 | – | – | – |
| 25 | – | – | + |
| 12.5 | – | + | + |
| 6.25 | + | + | NT |
| 3.125 | NT | NT | NT |

**Key:** ─: No growth, +: Growth, NT: Not tested

**Table 4b:** Minimum bactericidal concentration (MBC) of ethanol extracts of *Bridelia ferruginea* against *Escherichia coli.*

| | Minimum bactericidal concentrations (mg/ml) | | |
|---|---|---|---|
| Concentrations of extracts (mg/ml) | Root | Bark | Leaves |
| 200 | – | – | – |
| 100 | – | – | – |
| 50 | – | – | – |
| 25 | – | – | + |
| 12.5 | + | + | + |
| 6.25 | + | NT | NT |
| 3.125 | NT | NT | NT |

**Key: —**: No growth, +: Growth, NT: Not tested

The minimum bactericidal concentration (MBC) illustrates a decreasing inhibitory effect as shown in Table 4a & 4b with concentration dependent. The root, bark and leaf extracts exhibited a bactericidal activity at 12.5, 25 and 50 mg/ml respectively. The root extract showed a better minimum bactericidal concentration when compared with the bark and leaves extract this suggest that the root extract could be bactericidal agent (kill organisms).

The *in vivo* assay showed that the root extract had higher activity than the bark and root/bark combined. The dose of 100mg/kg had the highest colonies reduction for *Staphylococcus aureus* and *Eschrerichia coli* this suggest that as the dose of the extracts was increased the numbers of colonies were reducing until there was a clear plate, but there was no significant difference when concentrations used was compared, this could be attributed to the fact that antimicrobial activities of substance is a function of active ingredient reaching an organism [34]. When compared with positive control there were no significant difference between the treatment groups and positive control, this may suggest that the plant extract exhibits antibacterial activity and could be employed in the treatment of infectious diseases. In the same vein when treatment groups were compared with negative control, there was significant difference between the treatment groups this suggests, that plant extracts may have antibacterial activity. The antibacterial screening of crude ethanol extract carried out *in vivo* revealed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* at concentration of 50mg/kg and 100mg/kg. The root extract exhibited higher bactericidal activity than the bark extract. The combination of root and bark extracts showed synergetic activity against *Staphylococcus aureus* and *Escherichia coli.* The extracts inhibited bacterial colony completely after treatment for 114 hours.

## CONCLUSION

The antibacterial activities exhibited by *Bridelia ferruginea* in this study validate its use in ethnomedicinal treatment of infectious diseases. This study and previous reports show that *Bridelia ferruginea*has effective antibacterial activities *in vitro*. The *in vivo* study reveals that the extracts of *Bridelia ferruginea*also exhibited effective antibacterial activity against Gram positive *(Staphylococcus aureus*) and Gram negative (*Eschrerichia coli)* organisms. Its shows that the plant extracts possess activity against Gram positive and Gram-negative organisms.

This study shows that the plant extract (root) contained phytochemical constituents that are of pharmacological importance and exhibit effective antibacterial activity when compared to the bark and leaves *in vitro* and *in vivo.*

**Conflicts of Interest**

No conflict of interest among authors.

**Acknowledgement**

Our appreciation first goes to the Almighty God for His love, mercy, guidance, protection and provision throughout this work, without whom we could not have come this far. We always remain forever grateful to Technical assistance given by Mr. Thomas Philip Yakubu and Mrs. Josephine Department of Pharmacognosy University of Jos, Mr. Adah Peter Omoha, Mrs. MashorUmbule, Mr. LamsiNanpon Department of Pharmaceutics and Pharmaceutical Microbiology University of Jos, Mr. Luka Wazoh, Mr. Luka GampyalGarba Animal House Pharmacology Department University of Josand Mr. J.J. Azila Department of Forestry Technology, Federal College of Forestry Jos, Nigeria.

## REFERENCES

1. Burkil HM. The Useful Plants of West Tropical Africa. The Royal Botanic Garden, Reviews.1994; 2: 636.
2. Abimbola AO, Tolulope O. Antibacterial effects of thre selected chewing sticks extracts on Lactobacillus sp. Int. J. Trop. Med. 2006;1(3):103-6.
3. Gill LS. Ethnomedical uses of plants in Nigeria. Benin: Uniben Press ix. 1992.
4. Irobi ON, Moo-Young M, Anderson WA, Daramola SO. Antimicrobial activity of bark extracts of Bridelia ferruginea (Euphorbiaceae). Journal of Ethnopharmacology. 1994 Jul 22;43(3):185-90.
5. Cimanga K, De Bruyne T, Apers S, Pieters L, Totté J, Kambu K, Tona L, Bakana P, van Ufford LQ, Beukelman C, Labadie R. Complement-inhibiting constituents of Bridelia ferruginea stem bark. Planta medica. 1999 Apr;65(03):213-7.
6. Iwu MM. Antidiabetic properties of Bridelia ferruginea.plantaMedica. 1980; 39:247.
7. Bruyne TD, Cimanga K, Pieters L, Claeys M, Dommisse R, Vlietinck A. Gallocatechin–(4′→ 0→ 7)-epigallocatechin, a new biflavonoid isolated from Bridelia Ferruginea. Natural Product Letters. 1997 Dec 1;11(1):47-52.
8. Orafidiya LO, Lamikanra A, Adediji JA. Coagulation of milk as an index of astringency of the bark extract of Bridelia ferruginea benth and lime juice for the formulation of a traditional gargle 'Ogun Efu'. Phytotherapy Research. 1990 Oct;4(5):189-94.
9. Adeoye GC, Abaeli AM, Owowumi CJ, Olukoye DK. Antimicrobial activity of Bridelia ferruginea in Book of abstract of the Symposium on drug production from natural products. Drug Research and Production Unit, Obafemi Awolowo University Ile-Ife. 1988; 24
10. Ekanem JT, Kolawole OM, Abbah OC. Trypanocidal potential of methanolic extract of Bridelia ferruginea benth bark in Rattus novergicus. African Journal of Biochemistry Research. 2008 Feb 28;2(2):045-50.
11. Iwu MM. In proceedings of 4th Annual Conference of Nigeria Society of Pharmacognosy, University of Nigeria, Nsukka. The

state of Medicinal Plant Research in Nigeria. Edited by Abayomi sofowora. 1984:57.

12. Olajide OA, Makinde JM, Awe SO. Effects of the aqueous extract of Bridelia ferruginea stem bark on carrageenan-induced oedema and granuloma tissue formation in rats and mice. Journal of Ethnopharmacology. 1999 Jul 1;66(1):113-7.
13. Ndukwe KC, Okeke IN, Lamikanra A, Adesina SK, Aboderin O. Antibacterial activity of aqueous extracts of selected chewing sticks. J. Contemp. Dent. Pract. 2005 Aug 15;6(3):086-94.
14. Malcolm SA, Sofowora EA. Antimicrobial activity of selected Niegerian folk remedies and their constituent plants. Lloydia. 1969 Dec;32(4):512.
15. Akubue PI, Mittal GC. Clinical evaluation of a traditional herbal practice in Nigeria: a preliminary report. Journal of Ethnopharmacology. 1982 Nov 1;6(3):355-9.
16. Kolawole OM, Oguntoye SO, Agbede O, Olayemi AB. Studies on the efficacy of Bridelia ferruginea benth bark extract for domestic wastewater treatment. Bulletin of the Chemical Society of Ethiopia. 2007;21(2):205-11.
17. Akuodor GC, Mbah CC, Anyalewechi NA, TC IU, Osunkwo UA. Pharmacological profile of aqueous extract of Bridelia ferruginea stem bark in the relief of pain and fever. Journal of Medicinal Plants Research. 2011 Oct 16;5(22):5366-9.
18. Centers for Disease Control and Prevention. Office of Infectious Disease. Antibiotic resistance threats in the United States, 2013. April 2013. Accessed January. 2015;28.
19. The American Society of health system pharmacist. 2015.
20. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and Therapeutics. 2015 Apr;40(4):277.
21. Gould IM, Bal AM. New antibiotic agents in the pipeline and how they can help overcome microbial resistance. Virulence. 2013 Feb 15;4(2):185-91.
22. Wright GD. Something old, something new: revisiting natural products in antibiotic drug discovery. Canadian journal of microbiology. 2014 Jan 22;60(3):147-54.
23. Rossolini GM, Arena F, Pecile P, Pollini S. Update on the antibiotic resistance crisis. Current opinion in pharmacology. 2014 Oct 1;18:56-60.
24. Piddock LJ. The crisis of no new antibiotics—what is the way forward?. The Lancet infectious diseases. 2012 Mar 1;12(3):249-53.
25. Sengupta S, Chattopadhyay MK, Grossart HP. The multifaceted roles of antibiotics and antibiotic resistance in nature. Frontiers in microbiology. 2013 Mar 12;4:47.
26. Spellberg B, Gilbert DN. The future of antibiotics and resistance: a tribute to a career of leadership by John Bartlett. Clinical infectious diseases. 2014 Sep 15;59(suppl_2):S71-5.
27. Trease GE, Evan WC, Pharmacognosy 14th edition, published by Brown Publication. 1983.
28. Harbourne JB, Phytochemical methods: A Guide to modern technique of Plants analysis, Chapman and Hall Ltd London. 1983.
29. Osadebe PO, Ukwueze SE. A comparative study of the phytochemical and anti-microbial properties of the Eastern Nigerian specie of African Mistletoe (Loranthus micranthus) sourced from different host trees. Bio-research. 2004;2(1):18-23.
30. Esimone CO, Adikwu MU, Okonta JM. Preliminary antimicrobial screening of the ethanolic extract from the lichen Usnea subfloridans (L). J. Pharm. Res. Dev. 1998;3(2):99-101.
31. Ndukwe IG, Amupitan JO, Isah Y, Adegoke KS. Phytochemical and antimicrobial screening of the crude extracts from the root, stem bark and leaves of Vitellaria paradoxa (GAERTN. F). African journal of Biotechnology. 2007;6(16):1905-09.
32. Hosseinzadeh H, Bazzaz F, Haghi MM. Antibacterial activity of total extracts and essential oil of Nigella sativa L. seeds in mice.Pharmacologyonline, 2; 2007: 429-435.
33. Adetunji LK. Great Secret of Nature. Natural Links Centre. 1999:19-23.
34. Oboh PA, Abulu EO. The antimicrobial activities of extracts of Sidium guajava and Citrus aurantifolia. Nigerian journal of Biotechnology. 1997;8(1):25-9.



**HOW TO CITE THIS ARTICLE**

Yunana BT, Guiyi JC, Bukar BB. *In vitro* and *in vivo* evaluation of antibacterial activity of *Bridelia ferrugine* extracts on some clinical isolates. J Phytopharmacol 2018; 7(4):392-398.