The Study of Antimicrobial Activity of Partially Purified Ethyl acetate Extracts of *Bridelia ferruginea* on Clinical Isolates

Osuntokun Oluadare Temitope\(^1\), O. A. Fasusi\(^2\), T. C. Odeluyi\(^3\), A. O. Rotowa\(^4\)

\(^1\)Department of Microbiology, Faculty of Science, Adekunle Ajasin University, AkungbaAkoko, P.M.B 001, Ondo state, Nigeria

\(^2,4\)Department of Chemistry, Faculty of Science, Adekunle Ajasin University, AkungbaAkoko, P.M.B 001, Ondo state, Nigeria.

*Corresponding Author:
Osuntokun Oluadare Temitope
Email: osuntokun4m@yahoo.com

**Abstract:** *Bridelia ferruginea* is commonly grown in Western Tropical Africa. The purpose of this research work is to determine the antimicrobial activities, qualitative and quantitative phytochemical screening, elemental composition, proximate analysis and anti-nutrient composition of purified fraction of *Bridelia ferruginea* extracts. The organisms used for this research are *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 35659, *Pseudomonas aeruginosa* ATCC 25619, and *Candida albican* ATCC 90029. The leaf and bark were extracted using ethyl acetate as the extracting solvent. The plant extracts were partially purified using Column chromatography method of analysis, to separate the plant extracts to different fractions and various eluting solvent namely N-hexane, ethyl acetate and ethanol were used during Column chromatograph. The antimicrobial activity of partially purified fractions *Bridelia ferruginea* were determined by agar dilution method. The result revealed that fraction of ethyl acetate extracts of *Bridelia ferruginea* leaf and bark eluting with N-hexane, ethyl acetate and ethanol were effective against some of the clinical isolates. In fraction one (f\(_1\)), fraction two (f\(_2\)) and fraction three (f\(_3\)) of purified ethyl acetate leaf extracts, using ethanol, ethyl acetate and n-hexane as the eluting solvent. *Klebsiella pneumonia* ATCC 35659, *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were the most susceptible isolate at 6.0mm, 8.0mm and 7.0mm in 20mg/ml concentration of *Bridelia ferruginea* respectively. Fraction four (f\(_4\)), fraction five (f\(_5\)) and fraction six (f\(_6\)) of purified ethyl acetate bark extracts, using ethanol, ethyl acetate and n-hexane as the eluting solvent shows that, *Klebsiella pneumonia* ATCC 35659 and *Escherichia coli* ATCC 25922, has the highest inhibition of 3.0mm in 20mg/ml concentration in ethanol elute, *Klebsiella pneumonia* ATCC 35659 has the most susceptible ratio of 6.0mm in 20mg/ml concentration in ethyl acetate elute and *Staphylococcus aureus* ATCC 29213 has the highest inhibition ratio of 8.0mm in 20mg/ml concentration of *Bridelia ferruginea* using N-hexane as the eluting solvent. The phytochemical result shows that the *Bridelia ferruginea* bark and leaf extracts contain the saponins, tannin, flavonoid, phenol, alkaloids and oxalate. The proximate analysis were also determined and it was observed that *Bridelia ferruginea* contains carbohydrate, crude protein, fat, moisture content and fibre, at appreciable quantity. *Bridelia ferruginea* also contain elements such as Sodium, Calcium, Potassium, Magnesium, Zinc, Iron, Copper and Manganese. Thus, *Bridelia ferruginea* leaf and bark possesses a lot of potential as an additional source of antimicrobial agents, to fight against and inhibits the important pathogens that has been a menace to man over the years. This plant can also be a very important ingredient in drug discovery and production.

**Keywords:** Antimicrobial activity, Purified fraction, phytochemical activity, proximate composition, elemental constituent.

**INTRODUCTION**

The entire dependence of man on plants and plants products directly for his basic needs as food, clothing and shelter and indirectly for their beneficial influence on the climate and maintenance of his immediate and remote environment make plants vital to his survival and the basis of his continued existence [1]. Some plants serve as source of medicines, which are useful in treatment of various categories of human ailment and conditions. The world health organisation WHO has estimated that up to 80% of the world’s population relies on plants for their primary health care, while in Nigeria, in 1985 WHO survey estimated that up to 75% of the population patronizes traditional medicine [2].

Medicinal plants are the backbone of traditional medicine, and finding healing power in
plants is an ancient idea. In other words, medicinal plants in any forms have used in ancient times for trado-
medicinal practices in health care. A peep into the pre
historic era revealed that there is a great wealth of
traditional medicine, at present time, accepted as an
alternative form of health care in conjunction with the
western medicinal practise in many countries.

Traditional African medicine (TAM) is a
shorthand reference to indigenous forms of healing that
are practiced all over Africa. *Bridelia ferruginea*, family
(Euphorbiaceae) is a shrub which is employed in for
treating arthritis and as an embrocation for the treatment
of bruises, boils, dislocation, and burns. Tea made from
the pulped bark is used for fevers, headaches, stiffness,
and rheumatic pains and as a local application for
treating oedemas [3].

*Bridelia ferruginea* is a shrub growing up to 8
meters tall, or a straggly tree with a crooked bole that
can grow up to 15 meters tall. It sometimes has spiny
branches. The tree is much utilized from the wild by
local people, who use it for medicine and many other
commodities. Medicinal preparations are sometimes
sold in local market. They are found in Western tropical
Africa Sierra Leone, east to Central Africa Republic,
south to Angola and Zambia. It can reach a height of
8m. It hangs in numerous clusters of more than dozen
on the trees. The bark is sometimes added to palm-wine
to strengthen it and enhance fermentation.

The medicinal uses of *Bridelia ferruginea* are
as follows . The leaf-extract in saline solution has been
shown to produce a marked reduction of blood-sugar in
laboratory trials. Decoctions of the leaves, leafy twigs
and bark are commonly used in the treatment of urethral
discharges; dysentery and diarrhea; fever and rheumatic
pains [4]. The grated bark may be taken mixed with
tapioca flour to treat dysentery and the bark, and the
bright red infusion from it, are commonly used as a
mouth-wash and remedy for thrush in children. The
bark has a great reputation as an antidote against
poisons. The bark is chewed and then applied to a
wound caused by a poisoned arrow, after which the
wound is sucked to remove any more poison [5].

The non - medicinal uses of *Bridelia ferruginea* should be mention in this write up the roots
are used by the Yoruba as chew-sticks, while the
Maninka of the Upper Niger (Guinea) grind the wood to
a fine powder for use as a dentifrice. The wood is
brown. It is said to be termite-proof, and is used for this
reason in the Soudan and Guinean region to make
granaries. In Sierra Leone it is used as a primary
structural timber. In the Central African Republic, it is recognized as a good firewood, indeed as
a ‘woman’s firewood that is one good for the hearth and
cooking place, long-lasting while the housewife is away
on other chores, and picking up quickly from sleeping
embers with a hot flame and minimal amount of smoke
[6].

*Bridelia ferruginea* are commonly used as a
mouth-wash and remedy for thrush in children and to
treat skin diseases, infections and eruptions [7]. A tea of
the leaves is taken to relieve stomach ache, various
inflammatory condition and wound healing [8].

**MATERIALS AND METHOD**

The leaf and stem bark from *Bridelia ferruginea* plants were collected in the tropical rain
forest Oshogbo Osun State, Nigeria, in the morning
time of around 6.35am on 20th of January, 2016. The
plant specimen was identified and authenticated by the
Department of Plant Science and Biotechnology,
Adekunle Ajasin University Akungba Akoko, Nigeria
where the voucher specimens was kept on record. Voucher number AAU-2200 was recorded for the plant
extract for future reference.

**Preparation Of Extract**

The *Bridelia ferruginea* plant were washed and
dried at room temperature and then reduced to coarse
powder by slicing and grinding into almost powdery
form. A 400g of dried grinded leaves and bark were
separately extracted with ethyl acetate solvent of
1200ml each. This is done in a sterile rubber bottle for
easy extraction. Each bottle was labeled as regard what
it contains. The mixtures was allowed to soak for about
9 days at ratio 1:3. The mixture was shaken thoroughly
throughout these nine days of soaking. It was kept in a
cool dry place. After the 9th day, the *Bridelia ferruginea*
plant was filtered using the funnel and a filter paper.
Extracts of each mixture were collected into conical
flask and labeled properly.

**Test Organisms**

Test organisms used in the experiment were
collected from Department of Microbiology, Faculty of
Science, Obafemi Awolowo University Ile Ife Osun
State, Nigeria. They include: *Candida albican* ATCC
90029, *Klebsiella pneumonia* ATCC 35659, *Staphylococcus aureus* ATCC 29213, *Escherichia coli*
ATCC 25922.

**Partial Purification Of Extracts**

The ethyl acetate extract is partially purified using the
column chromatography techniques

**Column Chromatography**

Apparatus/solvents: column chromatography
glass wares, silica gel, N–hexane, ethanol, ethyl
acetate, cotton wool, stopcock, glass rod.

**Procedure:**

The column was clamped vertically.

The most active crude extract (Ethyl acetate extract) was subjected to silica gel (70–230 mesh) column
chromatography. Briefly, silica gel (500 g) was mixed

Available Online: [http://scholarsmepub.com/sjpm/](http://scholarsmepub.com/sjpm/)
with n-hexane to form a homogenous suspension/slurry and stirred using a glass-stirring rod to remove bubbles. The silica gel slurry was then poured into a glass column. The sample to load on the column was prepared by dissolving 15 g of the ethyl acetate extract in 100 ml of methanol. To the solution, 30 g of silica was added and mixed by stirring with a glass rod. The mixture was allowed to dry at room temperature. The dried silica extract mixture was layered on the column layer bed. The column was first eluted with n-hexane as the mobile phase with the polarity increasing by 5% increments of ethyl acetate. After getting to 100% ethyl acetate, the polarity was further increased by 5% increments of methanol. For each eluent system, two liter volumes were used and 250 ml fractions collected in 250 ml glass beakers. The collected fractions were concentrated to dryness using a rotary evaporator at 40°C [9].

Test for the antimicrobial activity and minimum inhibitory concentration of leaf and bark

The antimicrobial activities of the purified extracts were evaluated by Agar dilution method. After purification the extracts were used on the test organisms. The glass wares were sterilized using the oven at 160°C for 2 hours. Serial dilution of the extract was made using 8 concentrations (20g/ml, 10g/ml, 5g/ml, 2.5g/ml, 1.25g/ml, 0.625g/ml, 0.3125g/ml and 0.15625g/ml) of the purified extract of Bridelia ferruginea were prepared. 1ml or gram per dry mass after calculations using the $C_\text{V}_1 = C_\text{V}_2$ formula (Clinical Laboratory Science Institute) [10]. Exactly 4ml sterile distilled water was pipetted from the first test tube to the second and repeated for the 8 tubes, thereby the last tube containing 8ml. After making serials of the extracts in the tubes labelled respectively, 1ml was drawn using the graduated needle and syringe and dispensed into 19ml of prepared Mueller Hilton agar in a universal bottle. It was shaken properly and dispense in the sterilized plates respectively and allow to solidify [10]. In a sterile environment the plate was divided into four compartments, the organisms were inoculated using sterile inoculating loop in each part of the divided plate, labelled appropriately. The procedure was repeated for all samples obtained from the purified extracts. It was incubated at 37°C for 24 hours inverted. The compartment in the plate with the lowest dilution with no detectable growth on the naked eyes was considered as the MIC value.

Determination of Phytochemical Screening of Bridelia ferruginea

Qualitative Method of Analyses

(i) Test for Alkaloids

About 0.2gram was warmed with 2% of H$_2$SO$_4$ for two minutes, it was filtered and few drops of Dragendoff's reagent were added. Orange red precipitate indicates the present of Alkaloids [11].

(ii) Test for Tannins

One milliliter of the filtrate was mixed with 2ml of Fec$_1$, A dark green colour indicated a positive test for the tannins [12].

(iii) Test for Saponin

One milliliter of the plant filtrate was diluted with 2 ml of distilled water; the mixture were vigorously shaken and left to stand for 10minutes, during which time, the development of foam on the surface of the mixture lasting for more than 10minutes, indicates the presence of Saponins [12].

(iv) Test for Anthraquinones

One milliliter of the plant filtrate was shaken with 10ml of benzene; the mixture was filtered and 5 ml of 10% (v/v) ammonia were added, then shaken and observed. A pinkish solution indicates a positive test [12].

(v) Test for Flavonoid

About 5 mL of each aqueous extracts was added with 1% NH3 solution. A positive test result was confirmed by the formation of a yellow coloration or turbidity [2].

(vi) Test for Cardiac Glycoside

About 5 ml of the extract was mixed with 2 ml of glacial acetic acid containing one drop ferric chloride solution. To this, 1 ml of concentrated sulphuric acid was slowly underplayed to the sample mixture. A positive test result was confirmed by the presence of a brown ring at the Interface [2].

(vii) Test for steroids

10 ml of each ethanol extract are evaporated to insipient dryness over a steam bath and cooled to room temperature. It was then defatted repeatedly with hexane. The defatted aqueous layer was then warmed over a steam bath to remove the residual hexane. To this, 3 ml of Fec$_1$ reagent was added and 1 ml of concentrated sulfuric acid was then slowly added. A positive test was evident when a reddish brown coloration occurred [1].

(viii) Total Phenol (Spectrophotometric Methods)

2 g of each sample, 1 ml of diethyl ether was added for defatting. The fat free samples were boiled with 50 ml of ether for 15 min to obtain the phenolic components which were measured at 505 nm following the standard method [13].

Available Online: http://scholarsmepub.com/sjpm/
ml volume separately (water bath temperature was 90°C). Diethyl ether (20 ml) was used for extraction. The process was repeated three times. The ether layer was removed and 60 ml of n-butanol was added to the water layer. Butanol extract was washed with 5% NaCl aqueous solution. After evaporation, the samples were dried in oven to a constant weight; the saponin content was calculated as percentage of the starting material [1, 14].

(ii) Flavonoids
About 10 g of the plant sample were extracted repeatedly with 100 ml of 80% aqueous methanol, at room temperature. The whole solution was filtered through Whatman filter paper No 42. The filtrates were later transferred into a crucible and evaporated to dryness over a water bath. The dried extracts were weighed and the test procedure defined by Mahato and Sen 1997 was followed [15]

(iii) Tannins
About 500 mg of the plant sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the marked level. Then, 5 ml of the filtrate was transferred into a test tube and mixed with 2 ml of 0.1 M FeCl₃ in 0.1 M HCl and 0.008 M potassium ferricyanide. The absorbance was measured at 550 nm within 10 minutes. The tannins content was calculated using a standard curve of extract [15].

(v) Alkaloids
Five grams of the plant sample was weighed into a 250 ml beaker and 200ml of 10% acetic acid in ethanol was then be added, the reaction mixture was covered and allowed to stand for 4 hour. These were filtered and the extract will be concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation is complete. The whole solution was allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue being the alkaloid, which was dried and weighed to a constant mass [5]

Elemental Analysis of Bridelia ferruginea
The major elements comprising calcium, sodium, potassium and trace elements (Fe and Zn) were determined according to the standard method with slight modification [10]. The ground samples were sieved with a 2 mm rubber sieve and 2 g of each of the plant samples were subjected to dry ashing in porcelain crucible at 550°C in a muffle furnace. The resultant ash was dissolved in 5 ml of HNO₃ /H₂O₂ (1:1) and heated gently on hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionized water was added and heated until a colourless solution was obtained. The mineral solution in each crucible was transferred into a 100 ml volumetric flask by filtration through a Whatman filter paper and the volume was made to mark with deionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer (AAS). Concentration of each element was calculated on percentage of dry matter [16].

Determination of Proximate Analysis of bridelia ferruginea
The proximate parameters (moisture, dry matter, ash, crude fats, proteins and fibers, nitrogen, carbohydrates and energy values) were determined using Association of Official Analytical Chemists Methods.
1. Determination of moisture content was done by drying samples in oven (WiseVen, WON-50, Korea) at 110 °C until constant weight was attained [17].
2. Nitrogen estimation was carried out by the micro-Kjeldhal (BUCHI, KjelFlex K-360, and Switzerland) method with some modification [18].
3. The crude proteins were subsequently calculated by multiplying the nitrogen content by a factor of 6.25 [18]. The energy value estimation was done by summing the multiplied values for crude protein,
4. Crude fat and carbohydrate respectively at Water Factors (4, 9 and 4). Crude fats were determined by Soxhlet apparatus using n-hexane as a solvent.
5. The ash values were obtained by heating samples at 550 °C in a muffle furnace (Wise Them, FHP-03, Korea) for 3 h [18].
6. The carbohydrate content was determined by subtracting the total crude protein, crude fiber, ash content and crude fat from the total dry matter [17].
7. Crude fiber was estimated by acid-base digestion with 1.25% H₂SO₄ and 1.25% NAOH solutions [19].

RESULTS
Table 1 shows fraction one-antimicrobial activity of the purified ethyl acetate extracts of leaf using ethanol is the eluting solvent.It was observed that Klebsiella, pneumonia and Staphylococcus aureus has the highest zone of inhibition at concentrations 20mg/ml, 10mg/ml, and 5mg/ml of purified ethyl acetate leaf extracts of Bridelia ferruginea with 6.0mm, 4.0mm and 3.0mm respectively while Escherichia coli has the lowest value at the same concentration. At concentration 1.25, 0.625 and 0.3125mg/ml, Klebsiella pneumonia and Candida albicans has the same highest value of 1.0mm while Staphylococcus aureus and Escherichia coli has the lowest value of 0.0mm respectively. Only Klebsiella pneumonia were susceptible at the concentration of 0.1562mg/ml of purified ethyl acetate leaf extracts of Bridelia ferruginea.

Table 2 shows the fraction two of the antimicrobial activity of the purified ethyl acetate leaf extracts of Bridelia ferruginea.

Available Online: http://scholarsmepub.com/sjpm/
extracts using ethyl acetate as the eluting solvent. At concentrations of 20mg/ml of purified ethyl acetate leaf extracts of *Bridelia ferruginea*, *Klebsiella pneumonia* and *Staphylococcus aureus* has the highest zone of inhibition at 8.0mm, followed by *Candida albicans* at 6.0mm in the same concentration. At concentrations 10mg/ml, all microorganisms tested has the same zones of inhibition at 5.0mm respectively. At concentration 5.0, 2.5 and 1.25mg/ml, *Candida albicans*, *Klebsiella pneumonia* and *Escherichia coli* has the highest zone of inhibition of 4.0mm, 3.0mm and 2.0mm while *Staphylococcus aureus* has the lowest value of 3.0mm, 2.0mm and 1.0mm. At concentration of 0.625mg/ml, *Klebsiella pneumonia* has the highest zones of inhibition of 2.0mm while *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli* has the lowest value of 1.0mm respectively. At 0.3125mg/ml, all the isolates have the same value of zone of inhibition of 1.0mm. At concentration of 0.1562mg/ml *Candida albicans* has the highest zones of inhibition of 2.0mm while *Klebsiella pneumonia* has the lowest value of 0.0mm.

Table 3 shows the fraction three of the antimicrobial activity of the purified ethyl acetate leaf extracts of *Bridelia ferruginea* using n-hexane as the eluting solvent. it was observed that *Escherichia coli* has the highest zone of inhibition of 7.0mm and 5.0mm at concentrations 20mg/ml and 10mg/ml of purified ethyl acetate leaf extracts, followed by *Candida albicans* with 5.0mm and 3.0mm, then *Staphylococcus aureus* with 5.0mm and 2.0mm while *Klebsiella pneumonia* has the lowest value of 2.0mm at that concentrations. At concentration 5mg/ml of purified ethyl acetate leaf extracts of *Bridelia ferruginea*. At concentration 1.25mg/ml, *Candida albicans* and *Escherichia coli* have the same highest value of 1.0mm while *Klebsiella pneumonia* and *Staphylococcus aureus* is the lowest with 0.0mm. At concentration 0.625mg/ml only *Candida albicans* show growth while others did not show growth. The MIC value is at 0.3125 and 0.1563mg/ml concentrations of purified ethyl acetate leaf extracts of *Bridelia ferruginea*.

Table 4 shows the fraction four of the antimicrobial activity of the purified ethyl acetate bark extracts of *Bridelia ferruginea*, using ethanol as the eluting solvent. It was observed that *Escherichia coli* and *Klebsiella pneumonia* has the highest zone of inhibition of 3.0mm, followed by *Candida albicans* with 2.0mm and *Staphylococcus aureus* having the least value of 0.0mm at concentration 20mg/ml of purified ethyl acetate bark extracts of *Bridelia ferruginea*. At concentrations 2.5mg/ml *Escherichia coli* has the highest value of 1.0mm while *Candida albicans*, *Staphylococcus aureus* and *Klebsiella pneumonia* has the same value of 0.0mm. The MIC is at concentration 1.25, 0.625, 0.3125 and 0.1563mg/ml of purified ethyl acetate bark extracts of *Bridelia ferruginea*.

Table 5 shows the fraction five of the antimicrobial activity of the purified ethyl acetate bark extracts of *Bridelia ferruginea*, using ethyl acetate as the eluting solvent. *Klebsiella pneumonia* has the highest zone of inhibition at concentrations 20mg/ml at 6.0mm, followed by *Candida albicans* with 5.0mm while *Staphylococcus aureus* and *Escherichia coli* have the lowest value of 4.0mm at concentration of 20mg/ml purified ethyl acetate bark extracts of *Bridelia ferruginea*. At concentrations 5mg/ml *Candida albicans* has the highest value of 4.0mm while *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus* having the same value of 3.0mm respectively. At concentrations of 2.5mg/ml *Candida albicans* and *Escherichia coli* has the highest value of 3.0mm while *Staphylococcus aureus* and *Klebsiella pneumonia* have the same lowest value of 2.0mm. At concentrations of 1.25mg/ml *Candida albicans* have the highest value of 3.0mm, *Klebsiella pneumonia* has the value of 2.0mm while *Staphylococcus aureus* and *Escherichia coli* have the lowest value at the same concentrations with 0.0mm. At concentration 0.625 and 0.3125mg/ml *Candida albicans* have the highest value of 2.0mm, *Klebsiella pneumonia* have value of 1.0mm, while *Staphylococcus aureus* and *Escherichia coli* has the same value of 0.0mm.

Table 6 shows the fraction six of the antimicrobial activity of the purified ethanol bark extracts using n-hexane as the eluting solvent. At concentrations of 20mg/ml of purified ethanol bark extracts, *Staphylococcus aureus* has the highest zone of inhibition of 8.0mm, *Klebsiella pneumonia* has the value of 7.0mm, *Candida albicans* and *Escherichia coli* while has the least value of 6.0mm. At concentration 10mg/ml, *Staphylococcus aureus* has the highest zone of inhibition of 6.0mm, *Klebsiella pneumonia* has the value of 5.0mm while *Candida albicans* and *Escherichia coli* while has the least value of 4.0mm. At concentration 5mg/ml *Candida albicans*, *Staphylococcus aureus* and *Klebsiella pneumonia* has a highest value of 4.0mm while *Escherichia coli* has the lowest value of 3.0mm. At concentration 2.5mg/ml, *Klebsiella pneumonia* has the highest value while *Staphylococcus aureus*, *Candida albicans* and *Escherichia coli* have the lowest zone of inhibition. At concentrations 1.25mg/ml, *Klebsiella pneumonia* has the highest value of 3.0mm, *Staphylococcus aureus* and *Candida albicans* have the same value of 2.0mm while *Escherichia coli* have the lowest zone of inhibition of 1.0. At concentration 0.625, 0.3125 and 0.1563mg/ml of purified ethanol bark extracts. *Staphylococcus aureus* has the value of zone of inhibition of 2.0, 1.0, 0.0mm. *Candida albicans* and *Escherichia coli* have the value of 1.0mm in all the three concentrations while *Klebsiella pneumonia* have the lowest value of 0.0mm respectively.
Table 7 shows the phytochemical screening of *Bridelia ferruginea*, cardiac glycosides, steroids, anthraquinone, phenol, tannins, and Saponins are present while Flavonoids, alkaloids, anthraquinone and Saponins are not present in the leaf extracts while cardiac glycosides, steroids, phenol and tannins are present while Flavonoids, alkaloids, Saponins, anthraquinone and Saponin are not present in the bark of *Bridelia ferruginea*.

Table 8 shows the qualitative Analysis of the Phytochemical Screening of *Bridelia ferruginea*. Table 8 shows the elemental composition of *Bridelia ferruginea*. Sodium (Na), Potassium (K), Magnesium (Mg), Zinc (Zn), lead (Pb), Iron (Fe), Copper (Cu) and Manganese were all determined in both bark and leaf of *Bridelia ferruginea*. Magnesium has the highest value of 29.34 grams in bark extracts of *Bridelia ferruginea* and 32.98 grams in leaf extracts of the same plant extract. while Copper has the least value of 2.16 grams in the bark extracts and 0.02 grams in the leaf extracts.

Table 9 shows the ant-nutrients present in the *Bridelia ferruginea* extracts in percentages. They includes; tannin, phenol, phylate, oxalate, Flavonoids and alkaid. All were all determined in both bark and leaf. Oxalate has the highest value in leaf with 15.68% bark with 15.79%. The least value occur in phenol of both bark and leaf of the plant.

Table 10 shows the proximate nutrient present in *Bridelia ferruginea* in (%). They are: ash, crude, protein, fibre, fat, moisture content and carbohydrate. Carbohydrate has the highest value of 60.90 grams in bark and 60.55 grams in leaf. The least proximate nutrient is moisture content has 3.79 grams in barks and 3.74 grams in leaf of *Bridelia ferruginea*.

**ETHYL ACETATE LEAF EXTRACTS OF Bridelia ferruginea**

Table 1: Fraction one-antimicrobial activity of the purified ethyl acetate leaf extracts of *Bridelia ferruginea*, using ethanol is the eluting solvent

<table>
<thead>
<tr>
<th>Conc. mg/ml</th>
<th>Candida albican ATCC 90029</th>
<th>Klebsiella pneumonia ATCC 35659</th>
<th>Staphylococcus aureus ATCC 29213</th>
<th>Escherichia coli ATCC 25922</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>5.0</td>
<td>6.0</td>
<td>6.0</td>
<td>4.0</td>
</tr>
<tr>
<td>10</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>2.0</td>
</tr>
<tr>
<td>5</td>
<td>2.0</td>
<td>3.0</td>
<td>3.0</td>
<td>2.0</td>
</tr>
<tr>
<td>2.5</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>1.25</td>
<td>1.0</td>
<td>1.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.625</td>
<td>1.0</td>
<td>1.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.3125</td>
<td>1.0</td>
<td>1.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.1563</td>
<td>0.0</td>
<td>2.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Antimicrobial with interpretative criteria using clinical laboratory international standard (CLSI). Unit of inhibition –mm, susceptible ≤ 4µg/ml or 20mm

Table 2: Fraction two-antimicrobial activity of the purified ethyl acetate leaf extracts of *Bridelia ferruginea* using ethyl acetate as the eluting solvent

<table>
<thead>
<tr>
<th>Conc. mg/ml</th>
<th>Candida albican ATCC 90029</th>
<th>Klebsiella pneumonia ATCC 35659</th>
<th>Staphylococcus aureus ATCC 29213</th>
<th>Escherichia coli ATCC 25922</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>6.0</td>
<td>8.0</td>
<td>8.0</td>
<td>7.0</td>
</tr>
<tr>
<td>10</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>5</td>
<td>4.0</td>
<td>4.0</td>
<td>3.0</td>
<td>4.0</td>
</tr>
<tr>
<td>2.5</td>
<td>3.0</td>
<td>3.0</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>1.25</td>
<td>2.0</td>
<td>2.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>0.625</td>
<td>1.0</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>0.3125</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>0.1563</td>
<td>1.0</td>
<td>0.5</td>
<td>0.6</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Antimicrobial with interpretative criteria using clinical laboratory international standard (CLSI). Unit of inhibition –mm, susceptible ≤ 4µg/ml or 20mm
Table 3: Fraction three antimicrobial activity of the purified ethyl acetate leaf extract of *Bridelia ferruginea*, using *n*-hexane as the eluting solvent

<table>
<thead>
<tr>
<th>Conc. mg/ml</th>
<th>Candida albicans ATCC 90029</th>
<th>Klebsiella pneumonia ATCC 35659</th>
<th>Staphylococcus aureus ATCC 29213</th>
<th>Escherichia coli ATCC 25922</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>5.0</td>
<td>2.0</td>
<td>5.0</td>
<td>7.0</td>
</tr>
<tr>
<td>10</td>
<td>3.0</td>
<td>2.0</td>
<td>2.0</td>
<td>5.0</td>
</tr>
<tr>
<td>5</td>
<td>3.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>2.5</td>
<td>2.0</td>
<td>0.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>1.25</td>
<td>1.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>0.625</td>
<td>1.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.3125</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.1563</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Antimicrobial with interpretative criteria using clinical laboratory international standard (CLSI). Unit of inhibition –mm, susceptible ≤ 4µg/ml or 20mm

**ETHYL ACETATE BARK EXTRACTS OF *Bridelia Ferruginea***

Table 4: Fraction four of antimicrobial activity of the purified ethyl acetate bark extracts of *Bridelia ferruginea*, using ethanol as the eluting solvent

<table>
<thead>
<tr>
<th>Conc. mg/ml</th>
<th>Candida albicans ATCC 90029</th>
<th>Klebsiella pneumonia ATCC 35659</th>
<th>Staphylococcus aureus ATCC 29213</th>
<th>Escherichia coli ATCC 25922</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>2.0</td>
<td>3.0</td>
<td>0.0</td>
<td>3.0</td>
</tr>
<tr>
<td>10</td>
<td>0.0</td>
<td>2.0</td>
<td>0.0</td>
<td>2.0</td>
</tr>
<tr>
<td>5</td>
<td>0.0</td>
<td>1.0</td>
<td>0.0</td>
<td>2.0</td>
</tr>
<tr>
<td>2.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>1.25</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.625</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.3125</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.1563</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Antimicrobial with interpretative criteria using clinical laboratory international standard (CLSI). Unit of inhibition –mm, susceptible ≤ 4µg/ml or 20mm

Table 5: Fraction five of antimicrobial activity of the purified ethyl acetate bark extracts of *Bridelia ferruginea*, using ethyl acetate as the eluting solvent

<table>
<thead>
<tr>
<th>Conc. mg/ml</th>
<th>Candida albicans ATCC 90029</th>
<th>Klebsiella pneumonia ATCC 35659</th>
<th>Staphylococcus aureus ATCC 29213</th>
<th>Escherichia coli ATCC 25922</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>5.0</td>
<td>6.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>10</td>
<td>5.0</td>
<td>5.0</td>
<td>4.0</td>
<td>3.0</td>
</tr>
<tr>
<td>5</td>
<td>4.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>2.5</td>
<td>3.0</td>
<td>2.0</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>1.25</td>
<td>3.0</td>
<td>2.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.625</td>
<td>2.0</td>
<td>1.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.3125</td>
<td>2.0</td>
<td>1.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.1563</td>
<td>1.0</td>
<td>1.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Antimicrobial with interpretative criteria using clinical laboratory international standard (CLSI). Unit of inhibition –mm, susceptible ≤ 4µg/ml or 20mm

Table 6: Fraction six antimicrobial activity of the purified ethanol bark extracts of *Bridelia ferruginea* using *n*-hexane as the eluting solvent

<table>
<thead>
<tr>
<th>Conc. mg/ml</th>
<th>Candida albicans ATCC 90029</th>
<th>Klebsiella pneumonia ATCC 35659</th>
<th>Staphylococcus aureus ATCC 29213</th>
<th>Escherichia coli ATCC 25922</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>6.0</td>
<td>7.0</td>
<td>8.0</td>
<td>6.0</td>
</tr>
<tr>
<td>10</td>
<td>4.0</td>
<td>5.0</td>
<td>6.0</td>
<td>4.0</td>
</tr>
<tr>
<td>5</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>3.0</td>
</tr>
<tr>
<td>2.5</td>
<td>3.0</td>
<td>4.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>1.25</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>0.625</td>
<td>1.0</td>
<td>0.0</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>0.3125</td>
<td>1.0</td>
<td>0.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>0.1563</td>
<td>1.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Antimicrobial with interpretative criteria using clinical laboratory international standard (CLSI). Unit of inhibition –mm, susceptible ≤ 4µg/ml or 20mm.

Available Online: [http://scholarsmepub.com/sjpm/](http://scholarsmepub.com/sjpm/)
Table 7: Qualitative Analysis of the Phytochemical Screening of Bridelia ferruginea

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>Alkaloid</th>
<th>Cardiac Glycoside</th>
<th>Steroid</th>
<th>Anthraquinone</th>
<th>Phenol</th>
<th>Tannins</th>
<th>Saponin</th>
<th>Flavonoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bridelia ferruginea leaf</td>
<td>- ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>- ve</td>
<td>+ ve</td>
<td></td>
</tr>
<tr>
<td>Bridelia ferruginea bark</td>
<td>- ve</td>
<td>+ ve</td>
<td>+ ve</td>
<td>- ve</td>
<td>+ ve</td>
<td>- ve</td>
<td>- ve</td>
<td></td>
</tr>
</tbody>
</table>

Table 8: Quantitative Analyses of Elemental composition Present in Bridelia ferruginea Extract (mg/100g)

<table>
<thead>
<tr>
<th>Plant sample used</th>
<th>Na</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Zn</th>
<th>Fe</th>
<th>Pb</th>
<th>Cu</th>
<th>Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bridelia ferruginea leaf</td>
<td>19.00</td>
<td>23.98</td>
<td>22.12</td>
<td>23.34</td>
<td>16.89</td>
<td>22.12</td>
<td>3.56</td>
<td>0.00</td>
<td>5.79</td>
</tr>
<tr>
<td>Bridelia ferruginea bark</td>
<td>19.08</td>
<td>24.98</td>
<td>25.12</td>
<td>29.34</td>
<td>16.00</td>
<td>22.0</td>
<td>7.56</td>
<td>2.16</td>
<td>9.79</td>
</tr>
</tbody>
</table>

Table 9: Quantitative Analyses Of Anti–nutrients Present in Bridelia ferruginea Extracts Result in Percentage (%)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Bridelia ferruginea leaf</th>
<th>Bridelia ferruginea bark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannin</td>
<td>2.32</td>
<td>2.37</td>
</tr>
<tr>
<td>Phenol</td>
<td>2.56</td>
<td>2.49</td>
</tr>
<tr>
<td>Phylate</td>
<td>15.68</td>
<td>15.79</td>
</tr>
<tr>
<td>Oxalate</td>
<td>6.57</td>
<td>6.51</td>
</tr>
<tr>
<td>Saponin</td>
<td>9.78</td>
<td>9.70</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>6.43</td>
<td>6.56</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>4.25</td>
<td>4.31</td>
</tr>
</tbody>
</table>

Table 10: Quantitative analyses Of Proximate Nutrient Composition of Bridelia ferruginea Extracts

<table>
<thead>
<tr>
<th>S/N</th>
<th>% Ash</th>
<th>% MC</th>
<th>% CP</th>
<th>% Fat</th>
<th>% Fibre</th>
<th>%CHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bridelia ferruginea leaf</td>
<td>9.37</td>
<td>3.74</td>
<td>14.68</td>
<td>7.22</td>
<td>4.33</td>
<td>60.55</td>
</tr>
<tr>
<td>Bridelia ferruginea bark</td>
<td>9.39</td>
<td>3.79</td>
<td>14.72</td>
<td>6.82</td>
<td>4.46</td>
<td>60.90</td>
</tr>
</tbody>
</table>

DISCUSSION AND CONCLUSION

Plants are important source of potential antimicrobial useful structures for the development of new chemotherapeutic agents. The first step in towards this goals is the in-vitro antimicrobial assay [4]. The need to develop new antimicrobial agent and antibiotics from the leaf and bark of medicinal plant from the fact that microorganisms are developing resistance to many drug and the death rate from infectious diseases such as sexually transmitted infections, skin infection and many others. The medicinal value of this plant lies in some chemical substances that produce a definite physiological action on the human body [12].

Some bioactive components of the plants are used to control the effect of deleterious organism like clinical species of organism which is the cause of infection, the subject matter of this research work. This can be used to prove the high antibacterial activities exhibited by the leaf extracts of Bridelia ferruginea in all the solvent used for extraction. This is to say that medicinal plants is a very important tool to human wellbeing and it can be used to combat infectious diseases [13].

The result of the study revealed that ethyl acetate extract of Bridelia ferruginea leaf and bark eluted with N-hexane, ethyl acetate and ethanol have antimicrobial effect on the clinical strain of the microorganisms used for this study. The ethyl acetate leaf and bark were effective in various eluting solvent which are; (N-hexane, ethyl acetate and ethanol) at different concentration. The leaf and bark extract of the ethyl acetate which eluted by N hexane (Table 3and 6), ethyl acetate (Table 2 and 5) and ethanol (Table 1 and 4) against the tested microorganisms. These results support the potential for developing antimicrobials from higher plants and it will lead to the development of phytomedicine to act against microbes.

Extracts from Bridelia ferruginea leaf and bark have been used for centuries in folk medicine for the treatment of liver disorders. Bridelia ferruginea leaf and bark are the main flavolignan occurring in the flavonoids mixture silymarin of this plant had shown positive effect on liver. Besides being hepatoprotective, Bridelia ferruginea leaf has been extensively evidenced to induce apoptosis, reduce and/or inhibit cell
proliferation and tumor angiogenesis in human lung, bladder and prostate cancer models.

This result reveals that phytochemical are very useful bioactive component to human being. The presence of cardiac glycosides is an indication of medical significant of the leaf and the bark of Bridelia ferruginea. Cardiac glycoside are cardioactive compound belonging to triterpenoid class of compound as reported by [21]. The phytochemical constituents of Bridelia ferruginea are summaries in table 7. The bioactive compounds are known to act by different mechanisms and exact antimicrobial action. Steroid has been reported to have antibacterial properties.

The activity due to different minerals identified in Bridelia ferruginea leaf and bark also plays a major role in the prevention of infectious and communicable diseases. This is an important attribute of this medicinal plant. Minerals like Sodium (Na), Calcium (Ca), Magnesium (Mg), Zinc (Zn), Iron (Fe), Lead (Pb), Copper(Cu), Manganese (Mn), Potassium (K) and Phosphorus (P) plays a major role in infectious diseases, the chemical balance of the human system and food preservation [20]. The toxic elements like Pb were absent in all parts of Bridelia ferruginea leaf and bark under investigation.

The correlation between membrane lipid and sensitivity for steroidal compound indicate the mechanism in which steroids specifically associate with membrane lipid and exact action by causing leakages from liposome as reported by [23]. Tanin binds to proline synthesis [22].

CONCLUSION

The results confirm the validity of the use of Bridelia ferruginea plant in traditional medicine and it possess compound with antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. Therefore, it is quite easy to use as an herbal medicine as compare to chemically synthesized drug.

RECOMMENDATION

It is thereby recommended to explore and purify more of medicinal plants such as the one studied; Bridelia ferruginea to fight against public health problems. Also, more solvents should be used to extract the plant in order to determine other compositions of elemental components of the plant for antimicrobial properties against some tested pathogenic microorganisms.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to all the technical staffs of the laboratory unit of Both the Department of Microbiology, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria and Obafemi Awolowo University, Ile Ife, Osun State, Nigeria for their support and all the technical assistance rendered during the course of this research work.

REFERENCES


