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Original Research Article

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

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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 Colum 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 Colum 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 embrocating 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

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 mil 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 mil fractions collected in 250 mil glass beakers. The collected fractions were concentrated to dryness using a rotary evaporator at 40^0C [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 160^0C for 2 hours. Serial dilution of the extract was made using 8 concentrations (20g/mil, 10g/mil, 5g/mil, 2.5g/mil, 1.25g/mil, 0.625g/mil, 0.3125g/mil and 0.15625g/mil) of the purified extract of *Bridelia ferruginea* were prepared. 1mil or gram per dry mass after calculations using the $C_1V_1 = C_2v_2$ 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 37^0C 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_2SO_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 2mi1 of $Fec1_3$,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 mil 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 10mil of benzene; the mixture was filtered and 5 mil 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 mil of the extract was mixed with 2 mil of glacial acetic acid containing one drop ferric chloride solution. To this, 1 mil 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 mil 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 mil of $Fecl_3$ reagent was added and 1 mil 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].

Quantitative Method of Analyses of *Bridelia ferruginea*

(i) **Saponins**

The grinded plant samples (20 g) were extracted with 20% aqueous ethanol by using a water bath maintained at 55°C, for 4 hour with stirring. After filtration the residue was re-extracted with 200 ml of 20% ethanol. The combined extracts were reduced to 40

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 mil of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50 mil volumetric flask and made up to the marked level. Then, 5 mil of the filtrate was transferred into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 M Hcl and 0.008 M potassium ferrocyanide. 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 mil beaker and 200mil 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 HNO 3 /H 2O2 (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-Kjeldahl (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% H_2SO_4 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/mil, 10mg/mil, and 5mg/mil 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/mil, *Klebsiella pneumonia* and *Candida albican*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/milof 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 using ethyl acetate as the eluting solvent. At concentrations of 20mg/mil 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 albican*at 6.0mm in the same concentration. At concentrations 10mg/mil, all microorganisms tested has the same zones of inhibition at 5.0mm respectively. At concentration 5.0, 2.5 and 1.25mg/mil, *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/mil, *Klebsiella pneumonia* has the highest zones of inhibition of 2.0mm while *Candida albican, Staphylococcus aureus*and *Escherichia coli* has the lowest value of 1.0mm respectively. At 0.3125mg/mil, all the isolates have the same value of zone of inhibition of 1.0mm. At concentration of 0.1562mg/mil *Candida albican*has the highest zones of inhibition of 1.0mm while *Klebsiella pneumonia* has the same lowest value of 0.5mm.

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/mil and 10mg/mil of purified ethyl acetate leaf extracts, followed by *Candida albican* with 5.0mm and 3.0mm, then *Staphylococus aureus* with 5.0mm and 2.0mm while *Klebsiella pneumonia* has the least value of 2.0mm at that concentrations. At concentration 5mg/mil of purified ethyl acetate leaf extracts of *Bridelia ferruginea*. At concentration 1.25mg/mil, *Candida albican* and *Escherichia coli* have the same highest value of 1.0mm while *Klebsiella pneumonia* and *Staphylococus aureus* is the lowest with 0.0mm. At concentration 0.625mg/ml only *Candida albican* 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 albican* 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 albican, 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/mil 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 vale of 3.0mm respectively. At concentrations of 2.5mg/ml *Candida albican*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 albican*. have the highest value of 3.0mm, *Klebsiella pneumonia* 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 albican* 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. At concentration 0.1563 mg/ml *Klebsiella pneumonia and Candida albican* have the highest 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/mil 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 albican* and *Escherichia coli* while has the least value of 6.0mm. At concentration 10mg/mil, *Staphylococcus aureus* has the highest zone of inhibition of 6.0mm, *Klebsiella pneumonia* has the value of 5.0mm while *Candida albican* and *Escherichia coli* while has the least value of 4.0mm.At concentration 5mg/mil *Candida albican, 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/mil, *Klebsiella pneumonia* has the highest value while *Staphylococcus aureus, Candida albican* and *Escherichia coli* have the lowest zone of inhibition. At concentrations 1.25mg/mil, *Klebsiella pneumonia* have the highest value of 3.0mm, *Staphylococcus aureus* and *Candida albican*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/mil of purified ethanol bark extracts. *Staphylococcus aureus*has the value of zone of inhibition of 2.0,1.0 0.0mm,*Candida albican* and *Escherichia coli* have the value of 1.0mm in at all the three concentrations while *Klebsiella pneumonia* have the lowest value of 0.0mm respectively.

Table 7 shows the phytochemical screening of *Brideliaf erruginea*, 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, , phenoland 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 the *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 29.34grams in bark extracts of *Bridelia ferruginea* and 32.98grams in leaf extracts of the same plant extract. while Copper has the least value of 2.16grams in the bark extracts and 0.02grams 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 alkaloid. 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 60.90grams in bark and 60.55grams in leaf. The least proximate nutrient is moisture content has 3.79grams in barks and 3.74grams 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

Conc. mg/ml	*Candida lbican* ATCC 90029	*Klebsiella pneumonia* ATCC 35659	*Staphylococcus aureus* ATCC 29213	*Escherichia coli* ATCC 25922
20	5.0	6.0	6.0	4.0
10	4.0	4.0	4.0	2.0
5	2.0	3.0	3.0	2.0
2.5	2.0	2.0	2.0	2.0
1.25	1.0	1.0	0.0	0.0
0.625	1.0	1.0	0.0	0.0
0.3125	1.0	1.0	0.0	0.0
0.1563	0.0	2.0	0.0	0.0

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

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

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

Table 3: Fraction three antimicrobial activity of the purified ethyl acetate leaf extract of brideliaferruginea, using n-hexane as the eluting solvent

Conc. mg/ml	*Candida albican* ATCC 90029	*Klebsiella pneumonia* ATCC 35659	*Staphylococcus aureus* ATCC 29213	*Escherichia coli* ATCC 25922
20	5.0	2.0	5.0	7.0
10	3.0	2.0	2.0	5.0
5	3.0	2.0	2.0	2.0
2.5	2.0	0.0	1.0	2.0
1.25	1.0	0.0	0.0	1.0
0.625	1.0	0.0	0.0	0.0
0.3125	0.0	0.0	0.0	0.0
0.1563	0.0	0.0	0.0	0.0

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

Conc. mg/ml	*Candida albican* ATCC 90029	*Klebsiella pneumonia* ATCC 35659	*Staphylococcus aureus* ATCC 29213	*Escherichia coli* ATCC 25922
20	2.0	3.0	0.0	3.0
10	0.0	2.0	0.0	2.0
5	0.0	1.0	0.0	2.0
2.5	0.0	0.0	0.0	1.0
1.25	0.0	0.0	0.0	0.0
0.625	0.0	0.0	0.0	0.0
0.3125	0.0	0.0	0.0	0.0
0.1563	0.0	0.0	0.0	0.0

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

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

Conc. mg/ml	*Candida albican* ATCC 90029	*Klebsiella pneumonia* ATCC 35659	*Staphylococcus aureus* ATCC 29213	*Escherichia coli* ATCC 25922
20	5.0	6.0	4.0	4.0
10	5.0	5.0	4.0	3.0
5	4.0	3.0	3.0	3.0
2.5	3.0	2.0	2.0	3.0
1.25	3.0	2.0	0.0	0.0
0.625	2.0	1.0	0.0	0.0
0.3125	2.0	1.0	0.0	0.0
0.1563	1.0	1.0	0.0	0.0

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

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

Conc. mg/ml	*Candida albican* ATCC 90029	*Klebsiella pneumonia* ATCC 35659	*Staphylococcus aureus* ATCC 29213	*Escherichia coli* ATCC 25922
20	6.0	7.0	8.0	6.0
10	4.0	5.0	6.0	4.0
5	4.0	4.0	4.0	3.0
2.5	3.0	4.0	3.0	3.0
1.25	2.0	1.0	2.0	3.0
0.625	1.0	0.0	2.0	1.0
0.3125	1.0	0.0	1.0	1.0
0.1563	1.0	0.0	0.0	1.0

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

Table 7: Qualitative Analysis of the Phytochemical Screening ofbrideliaferruginea

SAMPLE	Alkaloid	Cardiac Glycoside	Steroid	Anthraquinone	Phenol	Tannins	Saponin	Flavonoids
Bridelia ferruginea leaf	- ve	+ ve	+ ve	+ ve	+ ve	+ ve	- ve	+ ve
Bridelia ferruginea bark	-ve	+ ve	+ ve	- ve	+ ve	+ ve	- ve	- ve

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

Plant sample used	Na	K	Ca	Mg	Zn	Fe	Pb	Cu	Mn
Bridelia ferruginea leaf	19.00	23.98	22.12	23.34	16.89	22.12	3.56	0.00	5.79
Bridelia ferruginea bark	19.08	24.98	25.12	29.34	16.	22.0	7.56	2.16	9.79

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

Parameters	*Bridelia ferruginea* leaf	*Bridelia ferruginea* bark
Tannin	2.32	2.37
Phenol	2.56	2.49
Phylate	15.68	15.79
Oxalate	6.57	6.51
Saponin	9.78	9.70
Flavonoid	6.43	6.56
Alkaloids	4.25	4.31

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

S/N	% Ash	% MC	% CP	% Fat	% Fibre	%CHO
Bridelia ferruginea leaf	9.37	3.74	14.68	7.22	4.33	60.55
Bridelia ferruginea bark	9.39	3.79	14.72	6.82	4.46	60.90

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 m any 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 deleterous 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 tritrpenoid 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), Potasium (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.

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