

Full Length Research Paper

Antimicrobial and phytochemical analysis of leaves and bark extracts from *Bridelia ferruginea*

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Five bacteria and three fungi were grown and tested against the effects of crude ethanolic, methanolic and water extracts of *Bridelia ferruginea* leaves and bark. The leaf water extract had zero antimicrobial activity. The overall antimicrobial activity recorded zones of inhibition ranging between 9 and 20 mm. The crude extracts were not active against the fungi tested. The minimum inhibitory concentration (MIC) of the active extracts was determined. Half strength (10 g/ml) concentrations of the bark ethanol and methanol extracts were the MICs against *Citrobacter* sp. and *Bacillus subtilis*. While quarter strength (5 g/ml) concentrations of the bark methanol and ethanol extracts were the MICs against *Staphylococcus aureus* and *Micrococcus luteus*. The phytochemical analysis carried out on *B. ferruginea* leaves and bark detected the presence of alkaloids, flavonoids, tannin, cardiac glycosides, anthraquinone, phlobatinnin and saponins and was negative for anthocyanin.

Key words: *Bridelia ferruginea*, phytochemical analysis, antimicrobial activity, zone of inhibition.

INTRODUCTION

All over the world, several hundreds of plants are good sources of medicinal agents and are used in traditional medicine for many different purposes, including bacterial and fungal infections (Obafemi et al., 2006). Traditionally, usage of plants in curing illnesses has deep roots in human history (Grabley and Thiericke, 1999). Ethnopharmacological uses of plants prevail among the Nigerian people. It has been pointed out by Baker et al. (1995) that plants continue to play a prominent role in primary health care of about 80% of the worlds' population. Over the years there have been alarming reports of multiple drug resistance in medically important strains of bacteria and fungi (Ozumba, 2003; Aibinu et al., 2004). The persistent increase in antibiotic resistant strains of organisms have led to the development of more potent antibiotics such as 3rd and 4th generation of Cephalosporin by pharmaceutical companies (Odugbemi, 2006). Many published reports have shown the effectiveness of traditional herbs against microorganisms. As a result, plants are one of the bedrocks for modern medicines to attain new principles (Evans et al., 2002).

Bridelia ferruginea is a shrub of 15 m high with crooked

bole up to 1.8 m in girth. It is the commonest *Bridelia* sp of the savanna woodland (Irobi et al., 1994). The bark, leaves and roots are ingredients of Yoruba (in Nigeria) infusions chiefly administered to children (Burkil, 1994). Similar use is made of root-decoction in Ivory Coast. The bark and the bright red infusion from it are commonly sold in Nigerian markets and shops for use as a mouth-wash and remedy for thrush in children. In Congo, a bark decoction is used for toothache and in Ivory Coast for dysentery and diarrhea or as a laxative (Gill, 1992). The bark is used as antidote against poison and arrow poison (Burkil, 1994). A leaf extract in saline solution is reported to produce a marked reduction of blood-sugar in laboratory rats and clinical trials have given a drop from 250 mg % to the normal less than 120 mg % after eight weeks of daily treatment (Iwu, 1980). A bark preparation is used for immunity against arrow poison and syphilis. Extract from the bark is mixed with the stem of *Costus afer* for the treatment of minor epilepsy (Akubue and Mittal, 1982). Root and stem barks are used for skin disease and eruption, the stem and root are rich in tannin and are used as chewing sticks/mouth washes (Burkil, 1994). Boiled water extract in Nigerian material has given positive action on Gram positive bacteria *Sarcina lutea* and *Staphylococcus aureus* (Malcolm and Sofowora, 1969).

Alkaloids are a group of mildly alkaline compounds

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mostly of plant origin. Some 30 of the known alkaloids are used in medicine. Tannic acid is valuable as an external medicine because it is astringent and styptic. Cardiac glycosides are used to treat heart problems. Saponins occur widely in varieties of plants. They are used as cleaning agents and as foam producers. When ingested orally, they have a bitter taste and are practically non-poisonous to warm-blooded animals. Some of the saponins are useful as raw materials for synthesis of steroid hormones (Encarta, 2007).

The aims and objectives of this study are to extract antimicrobial substances from *B. ferruginea* leaves and bark using distilled water, ethanol and methanol, test the effects of these crude agents on some pathogenic bacteria and fungi, and carry out phytochemical screening of the extracts.

MATERIALS AND METHODS

Collection of plant materials

Fresh leaves and bark of *B. ferruginea* were collected from the permanent site of Bowen University, Iwo and identified in the department of Biological sciences, Bowen University, Iwo, Nigeria.

Preparation of extracts

The samples were air dried at room temperature for three days, then oven-dried at 50°C for 5 - 7 days to remove the residual moisture. The dried leaves and bark were pulverized and stored in an air-tight container for further use. Three different solvents were used for the antimicrobial studies; distilled water, ethanol and methanol. Equivalent amounts of crushed samples (20 g) of the leaves and bark were soaked in 120 ml of each solvent and sealed properly to prevent evaporation. The suspended solutions were left to stand for 7 days and then filtered. The six filtrates were stored at room temperature (Obafemi et al., 2006).

Test organisms

Eight microorganisms were used in this study. They were obtained from the laboratory stock of the Department of Biological Sciences, Bowen University Iwo, Osun state. The organisms included five bacteria, *Escherichia coli*, *Citrobacter* spp, *S. aureus*, *Micrococcus luteus* and *Bacillus subtilis* and three fungi, *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans*.

Antibacterial activity

The antibacterial sensitivity testing of the plant was determined using the agar-well diffusion method (Irobi et al., 1994). The bacterial isolates were grown in nutrient broth (lab M) for 18 h before use and standardized to 0.5 McFarland standards (10^6 cfu ml⁻¹). The inoculum suspensions were tested against the effect of the extracts at a concentration of 20 g/ml. Two hundred microliter of the standardized cell suspensions were spread on a Mueller-Hinton agar. Wells were then bored into the agar using a sterile 5 mm diameter cork borer. Approximately 100 µl of the crude extracts were dispensed into the wells and properly labeled. The preparations were left to diffuse before incubation (Owoseni and Ogunnusi, 2006). The preparations were left to diffuse before

incubation. The inoculated plates were incubated at 37°C for 24 h. The plates were then observed for clearing around the wells, that is, zones of inhibition.

Antifungal activity

The fungal isolates were allowed to grow on a Potato Dextrose Agar (PDA) (Oxoid) at 25°C until they sporulated. The fungal spores were harvested after sporulation by pouring a mixture of sterile glycerol and distilled water to the surface of the plate and later scraped the spores with a sterile glass rod. The harvested fungal spores and bacterial isolates were standardized to an OD_{600nm} of 0.1 before use. One hundred microliter of the standardized fungal spore suspension was evenly spread on the SDA (Oxoid) using a glass spreader. Wells were then bored into the agar media using a sterile 5 mm cork borer and the wells filled with the solution of the extract taking care not to allow spillage of the solution to the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 h to allow for proper diffusion of the extract into the media. Plates were incubated at 25°C for 96 h and later observed for zones of inhibition (Igbinosa et al., 2009).

Antibiotic susceptibility test

Standard antibiotic discs were used in carrying out the susceptibility tests for the bacterial isolates. Different antibiotic discs were used for the Gram positive and Gram negative bacteria respectively. The agar was allowed to solidify in the Petri plates while sterile forceps was used to aseptically place the discs of known antibiotics on already inoculated Mueller-Hinton agar plates. The antibiotics used were Chloramphenicol (30 µg), Ceftriazone (30 µg), Nitrofurantoin (200 µg), Cotrimozazole (25 µg), Tetracycline (25 µg), Cephalexin (15 µg), Ofloxacin (10 µg), Ciprofloxacin (10 µg), Gentamycin (10 µg), Ampicillin (25 µg), Augmentin (30 µg), Ampiclox (30 µg), Linomycin (30 µg), Streptomycin (10 µg) and Erythromycin (5 µg). The plates were incubated for 24 h at 37°C. The zones of inhibition indicating the susceptibility of the organisms to the antibiotics were observed and recorded (Olajuyigbe and Awoniyi, 2005; Si et al., 2006).

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the extract inhibiting the visible growth of any microorganism. The MIC of different concentrations of the extracts was determined using two-fold dilutions method (Russel and Furr, 1977), that is, half strength (10 g/ml) and quarter strength (5 g/ml) of extracts; in addition to the normal strength (20 g/ml) while ethanol and methanol were used as control. The 5 mm cork borer was used to bore four wells in the already solidified media inoculated with the microorganisms. The different extract concentrations were dispensed into each well and properly labeled. The preparation was left to diffuse before incubating at 37°C for 24 h for the bacterial strains. The lowest concentration of antimicrobial agent that completely prevented the growth of the microorganisms was taken as the minimum inhibitory concentration (MIC) of the extract. The zone of inhibition was observed and recorded (Osole et al., 2005).

Phytochemical analysis

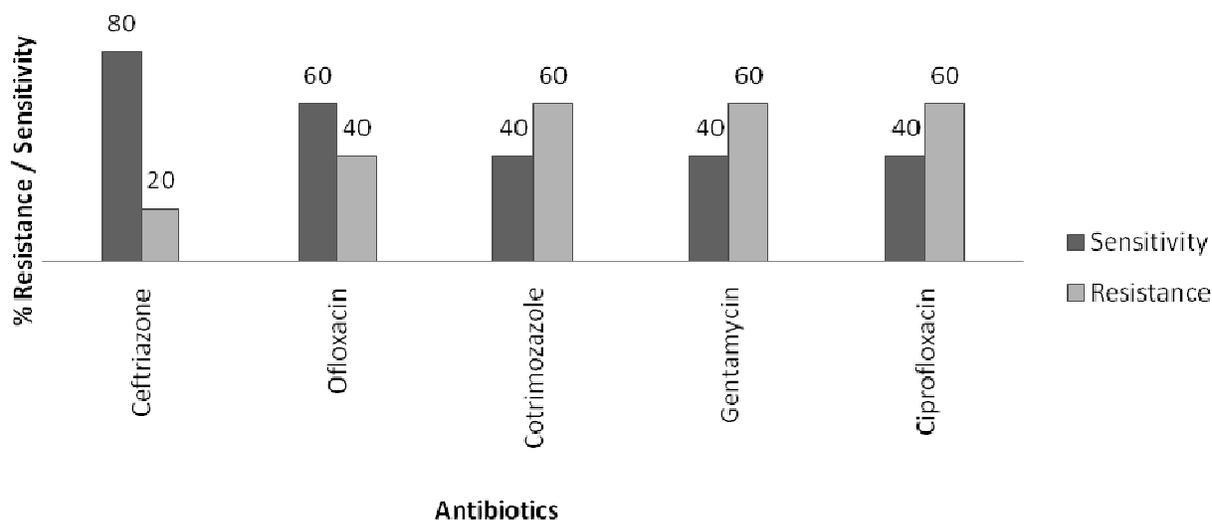
The ground pulverized leaves and barks were transferred into a soxhlet extractor and mounted using methanol. After concentrating the extracts, a paste was obtained and kept in the refrigerator for

Table 1. Antimicrobial activities of solvent extracts of *B. ferruginea* leaves and bark.

Microorganism	Gram	Zone of Inhibition (mm) ^a							
		*LW	LE	LM	BW	BE	BM	M	E
Bacteria									
<i>E. coli</i>	-	-	-	-	-	-	-	-	-
<i>Citrobacter</i>	-	-	20	-	9	9	13	-	-
<i>S. aureus</i>	+	-	20	14	10	14	15	-	-
<i>M. luteus</i>	+	-	-	-	-	17.5	15	-	-
<i>B. subtilis</i>	+	-	15	-	-	10	-	-	-
Fungi									
<i>A. flavus</i>	NA	-	-	-	-	-	-	-	-
<i>A. niger</i>	NA	-	-	-	-	-	-	-	-
<i>C. albicans</i>	NA	-	-	-	-	-	-	-	-

^a at a concentration of 20g/ml, (-) no inhibition, NA- not applicable

*LW: Leaf- water extract; LE: Leaf-ethanolic extract; LM: Leaf-methanolic extract; BW: Bark-water extract; BE: Bark-ethanolic extract; BM: Bark-methanolic extract; M: methanol; E: ethanol.

**Figure 1.** Percentage resistance and sensitivity patterns of isolates.

further analysis. Phytochemical screening was carried out to determine the presence of active secondary metabolites present in the plant extracts. The phytochemical assays for alkaloids, flavonoids, cardiac glycosides, tannins, phlobatannins, saponins and anthraquinones were carried out according to established procedures (Rai and Obayemi, 1973; Rai and Abdulahi, 1978; Aliyu and Nwude, 1982; Sofowora, 1986; Elujoba et al., 1989; Trease and Evans, 1989; Harborne, 1998).

RESULTS

Eight microorganisms, five bacteria and three fungi were tested against the effect of the crude extracts. Of the six prepared plant extracts, only five had effect on the growth inhibition of the organisms, but only two of the plant extracts had maximal efficacy. The bark-ethanolic extract

and the bark-methanolic extract were the most effective (Table 1). None of the extracts had effect on the fungal isolates.

Figure 1 shows the percentage resistance and sensitivity patterns of the antibiotics on the test organisms. Cotrimozazole, Gentamycin and Ciprofloxacin had the same sensitivity of 40% on the organisms while Ceftriazone had the highest percentage sensitivity (80%) on the organisms. Figures 2 and 3 show the minimum inhibitory concentration of the bark methanolic and ethanolic extracts on four of the test organisms. The results show that the diluted extracts also have antimicrobial activities. The minimum inhibitory concentration for *S. aureus* would be less than 5 mg/ml while the MIC for *Citrobacter* spp. was 5 mg/ml using the bark-methanol extract (Figure 2). The MIC of the bark-ethanol extract was 5 mg/ml for *B.*

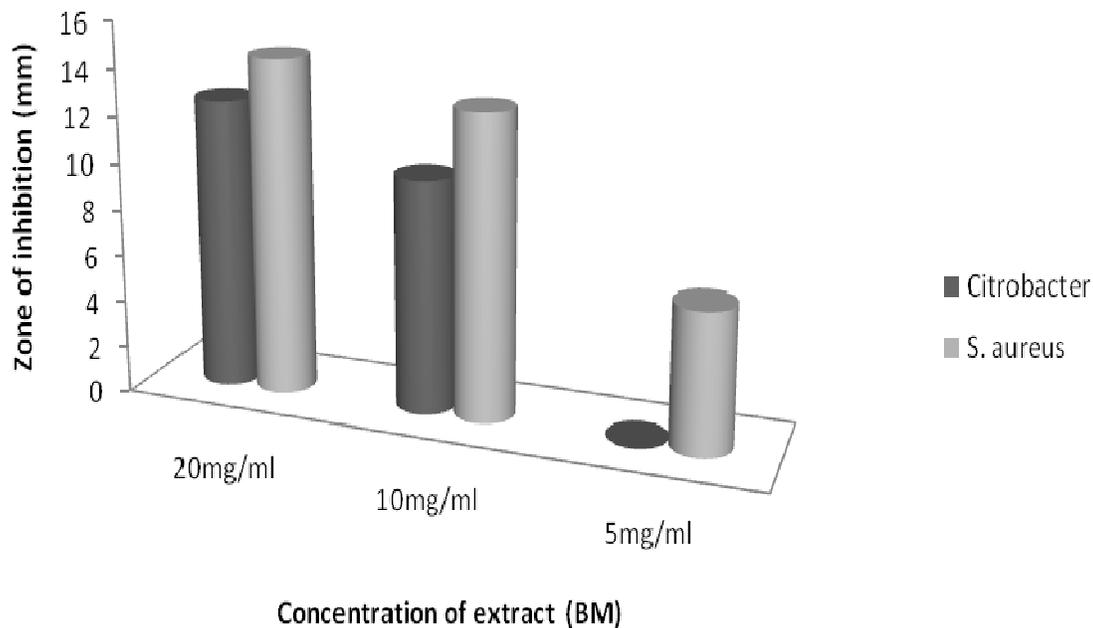


Figure 2. Minimum inhibitory concentration of bark-methanolic extract (BM) on *Citrobacter* and *S. aureus*.

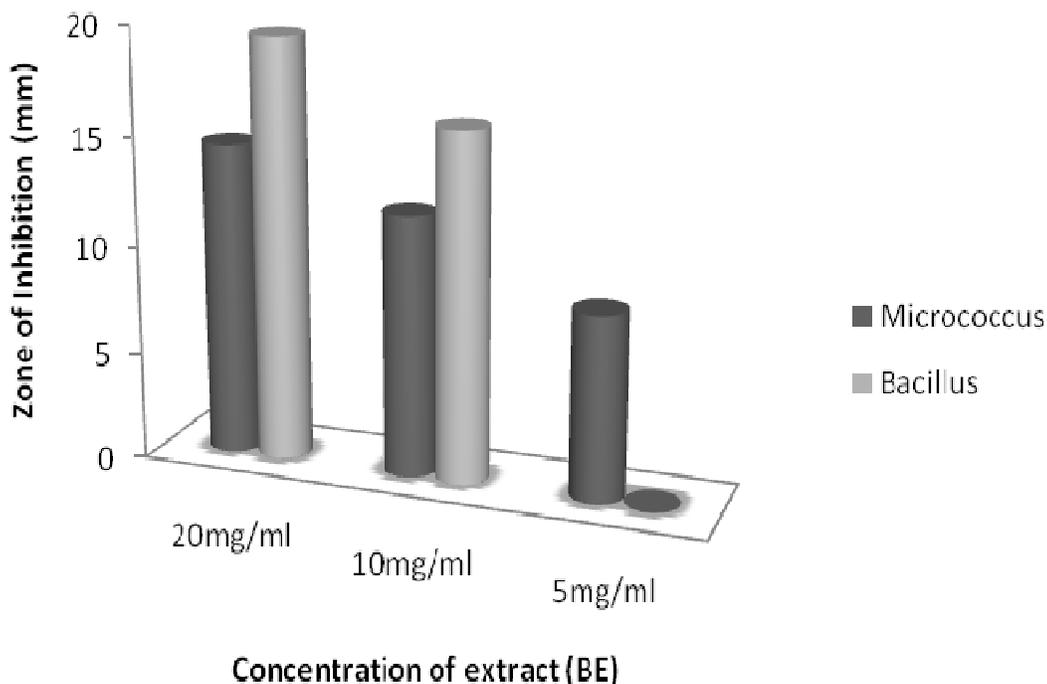


Figure 3. Minimum inhibitory concentration of Bark-ethanolic extract (BE) on *M. luteus* and *B. subtilis*.

subtilis and was less than 5 mg/ml for *Micrococcus luteus*.

Table 2 shows the results of the phytochemical analyses of *B. ferruginea* leaf and bark. Out of the phytochemicals tested, only anthocyanin a cyanogenic glycoside was not present in the plant parts tested.

DISCUSSION

The bark extracts of *B. ferruginea* using methanol and ethanol as extracting solvents presented a better inhibitory effect on the test organisms than extracts obtained from the leaves. This could be attributed to the concentration

Table 2. Phytochemical analyses of *B. ferruginea* leaf and bark.

Test	Bridelia Leaf	Bridelia Bark
Alkaloids	Present	Present
Flavonoids	Flavonoid present Catechol tannin suspected	Flavonoid present Catechol tannin suspected
Tannin	Tannin confirmed Taniferous confirmed	Tannin confirmed Taniferous confirmed
Cardiac glycosides	Deoxysugar present Steroidal ring present Steroidal nucleus present Cardenoloids present	Deoxysugar present Steroidal ring present Steroidal nucleus present Cardenoloids present
Anthraquinone	Free anthraquinone present Bound anthraquinone present	Free anthraquinone present Bound anthraquinone present
Phlobatinnins	Present	Present
Saponin	Saponin confirmed	Saponin confirmed
Cyanogenic glycosides	Anthocyanin absent/negative	Anthocyanin absent/negative

of the active substance causing the inhibitory effect which could have been higher in the bark and the metabolites of the barks are probably different from those of the leaves. The antimicrobial activity was more pronounced against bacteria than fungi. The use of methanol and ethanol as extracting solvents proved to be more efficient in extracting the active compounds than using water as solvent. This could be ascribed to the alcoholic aqueous environment which promotes easy extraction as reported by Nostro et al. (2000). The non-activity of the water extract against most bacterial strains investigated in this study is in agreement with previous works which show that aqueous extracts of plants generally showed little or no antibacterial activities (Aiyegoro et al., 2008; Igbinsola et al., 2009). This finding agrees with Gunnar et al. (1991), who reported that different extracts of plants show different antimicrobial activities on an organism.

The MIC illustrates a decreasing inhibitory effect of the bark extracts as the concentration decreases. This implies that antimicrobial activity of a substance is concentration dependent, which is in concordance with the report of Oboh and Abulu (1997), that antimicrobial activity is a function of the active ingredient reaching an organism. From the results, the extracts seem to be more active against Gram-positive bacteria than Gram-negative bacteria. As reported by Nostro et al. (2000), the reason for the different sensitivity between Gram negative and Gram positive could be ascribed to the morphological differences between these microorganisms. Gram-negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components, which makes the cell wall partially impermeable. The Gram-positive bacteria are thus, more susceptible, having only an outer peptidoglycan layer which is not an effective permeability barrier (Nikaido and Vaara, 1985).

Previous studies demonstrated the presence of Flavonoid

components (Maffaei-Facino et al., 1970) in *Helichrysum italicum*. Other authors showed the presence of terpenes and isomers of nepetalactone in *Nepeta cataria* and their bacteriostatic and fungistatic properties (Bourrel et al., 1993). However, it is difficult to compare the data of this study with literature because several variables influence the results, such as the type of plant used, the climatic and environmental factors of the plant, the method of extraction and the use of appropriate solvents.

The phytochemical analysis of ethanol extracts of *B. ferruginea* leaves and bark tested positive to seven constituents except the cyanogenic glycoside-anthocyanin which was negative. These compounds are known to be biologically active and therefore aid antimicrobial activities of the plant. This result is in line with the work of Kolawole et al. (2006) who recorded that antidiabetic activities of these plant extracts could be due to the presence of tannins, polyphenols, steroids, triterpenes and alkaloids as revealed in the phytochemical screening. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003). The plant can also be used in the brewing industries in clarifying beer and wine because of the presence of tannins.

Similar results were obtained by Ogunkunle and Ladejobi (2006) who worked on extracts of *Senna* sp. The presence of these chemical constituents underscores the importance of these plants in medicine. According to Microsoft Encarta (2007), all alkaloids contain quinine, morphine and reserpine which are used for malaria, pain relief and valuable tranquilizers respectively. Alkaloids are one of the largest groups of phytochemicals in plants, have amazing effects on humans and has led to the development of powerful pain killer medications (Kam and Liew, 2002). This suggests that *B. ferruginea* can be a useful source for malaria drug, pain

relief and tranquiliser. The leaf and bark extracts have also been reported to reduce plasma glucose levels in diabetic patients (Iwu, 1980; Kolawole et al., 2006).

However, further experimental and research efforts on the plants and their extracts are needed to be able to specify the pharmacological implication. Other details needed will include tests using other solvents, infrared spectrometry, MS and NMR of the constituents of the extracts.

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