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# *In vitro* Antioxidant and Antibacterial activity of *Bridelia atroviridis* (Arasado)

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author OOS prepared the experiment. Author OOE helped in the preparation of the extract and author MOA did the microbial aspect of the work. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** *Bridelia atroviridis* methanolic leaf extracts was assessed for antioxidant and antibacterial activity.
**Place and Duration of Study:** The study was carried out in the Microbiology Laboratory, Department of Science Laboratory Technology, School of Pure and Applied Science, Lagos State Polytechnic, Ikorodu, Lagos- Nigeria for the period of three months between September and November 2015.
**Methodology:** Antioxidant compounds lycophene, β-carotene, total phenolic and total flavonoid content were evaluated using the method described by Nagata and Yamashita, aluminum chloride colorimetric assay and Folin-Ciocalteau assay respectively. DPPH radical scavenging activity method was utilized in assessing the antioxidant capacity of the plant extract while the antimicrobial activity was evaluated by the agar diffusion technique.

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**Results:** The *Bridelia atroviridis* methanolic leaf extracts assessed in this study possessed significant amount of antioxidant compounds lycophene, β-carotene, total phenol and flavonoids. The extract exhibited antioxidant activity by scavenging DPPH radicals in a dose dependent pattern with $IC_{50}$ of 51.24 µg/mL compared to vitamin C with $IC_{50}$ of 41.67 µg/mL.
**Conclusion:** *Bridelia atroviridis* methanolic leaf extracts is a potential source of drugs with antioxidant and antimicrobial activity.

*Keywords: Antioxidant; antibacterial; Bridelia atroviridis; methanolic extract.*

## 1. INTRODUCTION

Infectious diseases caused by fungi, bacteria, viruses and parasites are the leading cause of death worldwide and according to WHO estimations [1], it is responsible for 76% of deaths in Nigeria alongside maternal, perinatal and nutritional condition. The only solution to this public health problem is use of antibiotics. However, the emergence of new infections and antibiotic resistant microbial strains arising from random use of antibiotics has threatened the effectiveness of numerous antibiotics [2,3]. This has forced the search for new antimicrobial substances from various sources [4] including plants -nature's drug store to curb this menace. Various plants and their parts have been used in different recipes for the treatment of many infectious diseases throughout the memoire of mankind. Many scientific validations of the antimicrobial activities of plant extracts exist, however, there is a constant and dire need to find and develop new lead antimicrobial compounds with concordant antioxidant activity. Therefore, researchers are progressively focusing on medicinal plants for new leads to develop better drugs against microbial infections.

*Bridelia atroviridis* is an ethno botanical commonly used in the treatment of various diseases and ailments by traditional medical practitioners in Nigeria. Due to its purported antibacterial and analgesic properties, the bark is used for curing rheumatic troubles, genitourinary, purgative, diuretic, and aphrodisiac and management of toothache, gonorrhoea and other venereal diseases [5,6]. However, despite the numerous medicinal benefits attributed to this plant, scarce are the scientific reports validating these claims, thus, this study is aimed at investigating the antioxidant and antimicrobial activity of *Bridelia atroviridis* against selected test organisms.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Identification of Plant Material

Fresh leaves of *Bridelia atroviridis* were obtained from Mushin Herbal Market of Lagos State and identified at the Botany unit, Science Laboratory Department, Lagos State Polytechnic. The leaves were dried at room temperature away from direct sunlight and were later reduced to powder form using an electric blender. The powder plant materials were stored in air tight containers until required.

### 2.2 Preparation of Plant Extracts Extraction

Active components of powdered *Bridelia atroviridis* leaves were extracted by soaking 100 g in 500 mL of methanol for 24 hours. The extract was filtered and concentrated under reduced pressure in a rotary evaporator giving a percentage yield of *6.12%.*

### 2.3 Total Phenolic Content

The total phenolic content of the extract was determined spectrometrically using the method of Chun et al. [7]. Folin-Ciocalteu's reagent (1mL) was added to sample (1 mL, 1.0 mg/mL) and mixed thoroughly. To this mixture, 4 ml of sodium carbonate (75 g/L) and 10 ml of distilled water were added and thoroughly mixed. The mixture was allowed to stand for 90minutes at room temperature. The absorbance of the reaction mixture was taken at 550 nm and total phenolic content was deduced using a calibration curve for gallic acid. The results were expressed as the gallic acid equivalent per gram of dry weight of extract (mg of GAE/g of extract). All samples were analyzed in triplicate and values expressed as mean ± SD.

## 2.4 Total Flavonoid Assay

The aluminum chloride colorimetric assay described by Chang et al. [8] was used to determine the total flavonoid content of the plant extract. The plant extract (0.5 mL) was separately mixed with methanol (1.5 mL), $AlCl_3$ (0.1 mL, 10%), sodium acetate (0.1 mL, 1 M) and distilled water (2.8 mL). The reaction mixture was left for 30 minutes at room temperature and the absorbance was read at 415 nm. The total flavonoid content was calculated using a calibration curve for quercitin and results were expressed as the gallic acid equivalent per gram of dry weight of extract (mg of GAE/g of extract). All samples were analyzed in triplicate and values expressed as mean ± SD.

## 2.5 Determination of β-Carotene and Lycopene Content of the Extracts

Using the method of Nagata and Yamashita [9], the methanolic leaf extract of *Bridelia atroviridis* (0.1 g) was weighed into a beaker and 10 mL of acetone: hexane mixture (4:6) was added, vigorously shaken for 5 minutes and filtered through a filter. Absorbance of the filtrate was measured at 453, 505, and 663 nm and the concentration of β-Carotene and lycopene in the plants were calculated according to the following equations:

**β- Carotene** (mg/100 mL) = 0.216 (A663) + 0.304 (A505) + 0.452 (A453)

**Lycopene** (mg/100 mL) = 0.0458 (A663) + 0.372 (A505) 0.0806 (A453)

The assay was carried out in triplicates and expressed as mg of carotenoid/g of the extract.

## 2.6 DPPH free Radical Scavenging Activity

DPPH stock solution prepared in methanol was added to 1 ml of extract solution and vitamin C at different concentrations (25, 50, 75 and 100 µg/ml). After 30 min, absorbance was measured at 517 nm and scavenging activity was expressed as the percentage inhibition calculated using the formula:

% Anti-radical activity = (Control Absorbance - Sample Absorbance × 100)/ Control Absorbance

Where: Control Absorbance is absorbance of DPPH solution

## 2.7 Test Organisms

The bacterial strains *Staphylococcus aureus* (Gram-positive), *Escherichia coli* (Gram-negative) and fungal strain, *Candida albican* used in this investigation were obtained from Microbiology Department, University of Lagos, Nigeria. The microorganisms were maintained at 4℃ on Nutrient Agar slant and fresh subcultures were made before use.

## 2.8 Antibacterial Test Using the Agar Diffusion Method

The antibacterial activity of the methanolic leaf extract of *Bridelia atroviridis w*as determined using the agar diffusion method. Briefly, all the extracts were dissolved in dimethyl sulfoxide (DMSO, 5%) in order to obtain concentrations of 50 mg/mL. Inoculum of the bacterial strains ($10^8$ CFU/mL) was then plated using sterile swabs into sterilized Petri dishes containing 20 mL of Nutrient agar. Wells with diameter of 6 mm wells were cut and filled with 100 µL of extract and ampicillin (100 µL, 50 mg/mL) into respective plates while DMSO was used as negative control. The Petri dishes were pre-incubated at room temperature for 3 hours in order to allow complete diffusion of the extracts before incubating at 37℃ for 24 hours.

## 3. RESULTS

Antioxidant compounds total phenolics, flavonoids, β-carotene and lycophene present in the plant extract was shown in Fig 1.

The free radical scavenging activity of *Bridelia atroviridis* evaluated using DPPH radical scavenging activity is shown above in Fig. 2. Ascorbic acid used as reference standard. The result showed that the plant extract was able to scavenge the DPPH radicals, obvious in the % increase observed with the concentration increase and were superb in comparison to standard ascorbic acid used in this study. However, considering the $IC_{50}$ value for *Bridelia atroviridis* and ascorbic acid are 51.24 µg/mL and 41.67 µg/mL respectively, vitamin C is a more effective antioxidant.

The result of antimicrobial activity of *Bridelia atroviridis methanolic* leaf extract is shown in Fig. 3. All the organisms were susceptible to the plant extracts and standard drug ampicillin though *Bridelia atroviridis* produced a wider zone of inhibition.

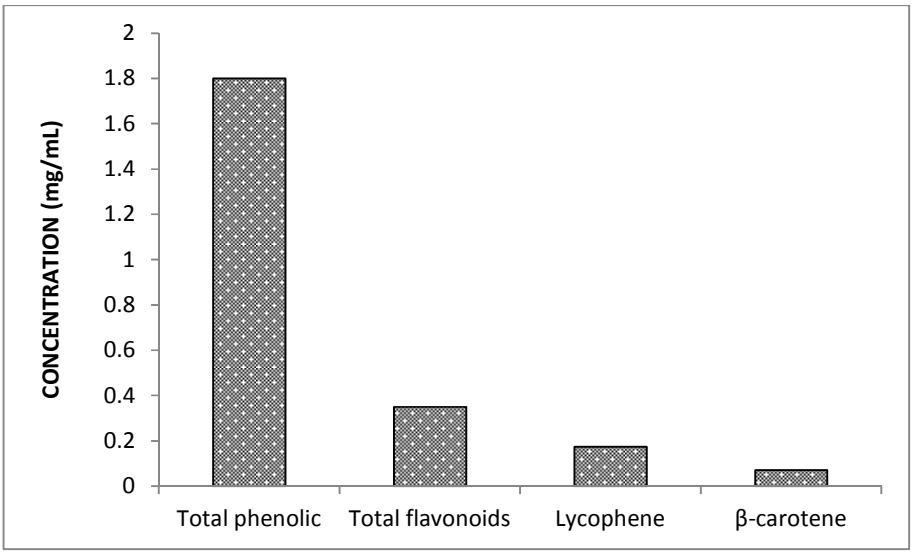


**Fig. 1. Concentration of antioxidant compounds in the plant extract**
*Values are expressed as mean values of three determinants*

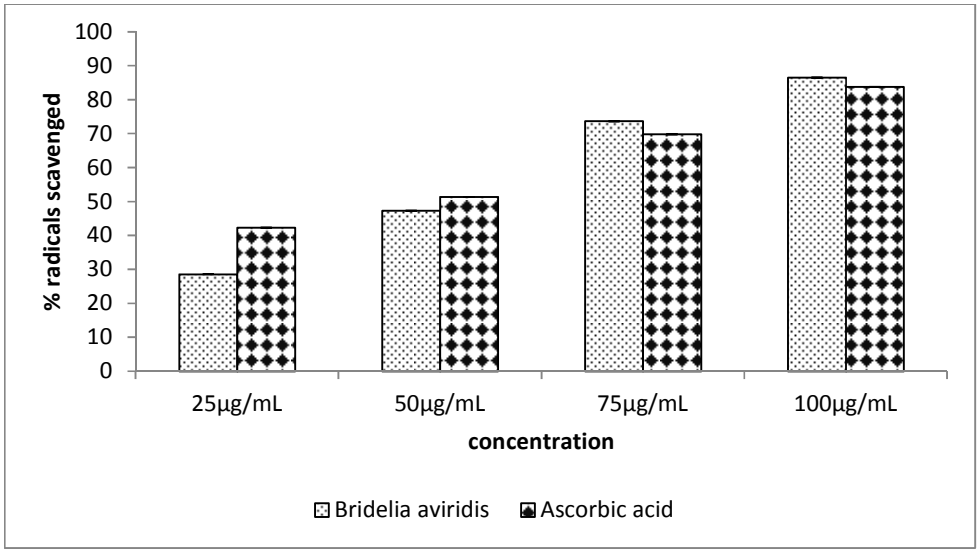


**Fig. 2. Percentage DPPH radical scavenging activity of *Bridelia atroviridis* methanolic leaf extract**
*Values are expressed as mean ± SD values of three determinants*

## 4. DISCUSSION

Antioxidants are compounds that defuse free radical attack on biological molecules by donating electrons to the highly reactive species. Lycophene, β-carotene, phenolics and flavonoids are phytochemicals with potent antioxidant activity as reported by various researchers [10, 11]. From the result obtained, phenolic compounds were the main antioxidant constituents found in methanolic leaf extract of *Bridelia atroviridis* while β-carotene and lycophene showed minimal concentration. This is in agreement with reports of other authors [12,13]. Flavonoids and phenolic compounds are widely distributed in nature [14] and have been extensively studied because of their physiological roles such as antioxidant, antitumor and antimutagenic activities [15-17].

The presence of these compounds in the methanolic extract of *Bridelia atroviridis* leaves confers the extract with antioxidant property, since various reports associates the antioxidant activity of plant materials with the content of phenolic compounds. This is evident in the result obtained from the DPPH radical scavenging activity, a method vastly employed in antioxidant activity analysis which presents a prompt means for screening the extracts [18,19,17].

The antimicrobial activity of BA was performed using the agar diffusion method by employing a Gram positive bacterium (*S. aureus),* Gram negative bacteria (*E. coli*) *and* a fungus (*C. albican*) as test organisms in order to establish its effectiveness against a wide range of organisms. The zone of inhibition obtained from this study was prominent in all the test organisms, though; *S. aureus* was most susceptible to the plant extract, followed by *C. albicans* while the least susceptible was *E. coli* producing a zone inhibition of 30.66±1.15, 30.33±1.53 and 26.00±1.00 mm respectively. The activities of BA methanolic leaf extracts were compared with standard antibiotics, ampicillin as illustrated in Fig. 3.

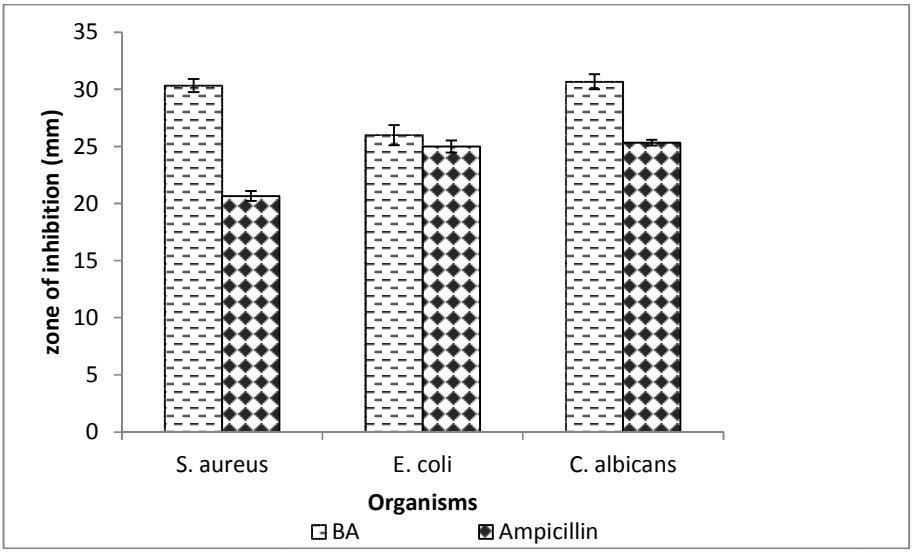


**Fig. 3. Zone of inhibition of plant extract and standard drug against test organisms at a concentration of 50 mg/mL**

*Values are expressed as mean ± SD of three determinants*

**Table 1. Concentration of antioxidant compounds in the plant extract**

| Total phenolic | Total flavonoids | Lycophene | β-carotene |
|---|---|---|---|
| 1.8 | 0.35 | 0.174 | 0.071 |

**Table 2. Percentage DPPH radical scavenging activity of *Bridelia atroviridis* methanolic leaf extract**

| | 25 µg/mL | 50 µg/Ml | 75 µg/mL | 100 µg/Ml |
|---|---|---|---|---|
| *Bridelia aviridis* | 28.6±0.02 | 47.28±0.02 | 73.67±0.04 | 86.51±0.09 |
| Ascorbic acid | 42.3±0.01 | 51.36±0.02 | 69.83±0.04 | 83.78±0.01 |

**Table 3. Zone of inhibition of plant extract and standard drug against test organisms at a concentration of 50 mg/mL**

| | *S. aureus* | *E. coli* | *C. albicans* |
|---|---|---|---|
| BA | 30.33±1.0 | 26±1.53 | 30.67±1.15 |
| Ampicillin | 20.67±0.44 | 25±0.53 | 25.33±0.25 |

Agyare et al. [20] have reported the antimicrobial activity of BA leaf extracts; however, the results obtained in this study were higher than that observed in their study. Antimicrobial activity might be due to the active components present in the extracts plant extract which is dependent on mode of extraction of active principles, distribution of antimicrobial substances, which varied from species to species [21] and location of plant, the microbial strains used and the concentration of plant extracts. The antimicrobial activity of plant extracts may be attributed to the presence of phytochemicals like saponin [22], alkaloids [23], tannins [24,25] present in the extracts. These findings have great practical applications in the recent times as infectious diseases are leading cause of death worldwide.

## 5. CONCLUSION

From the results obtained in this study, the methanol leaf extract of *Bridelia atroviridis* possessed both antioxidant and antibacterial activity evident in their ability to scavenge DPPH radicals and inhibiting the growth of the test organisms. Thus, *Bridelia atroviridis* is a potential source of natural antibiotics with broad spectrum activity complemented by its antioxidant property which is useful in the prevention and management of various diseases associated with oxidative stress.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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