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# PRELIMINARY PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF THE STEM BARK EXTRACTS OF *BAUHINIA RUFESCENS* LAM USING SOME SELECTED PATHOGENS

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***ABSTRACT***
***Bauhinia rufescens Lam (Leguminosea-Caesalpinoideae) stem bark was extracted using methanol and fractionated using ethylacetate, butanol and water. The extract and fractions were subjected to preliminary phytochemical screening using standard procedures followed by antimicrobial screening using disc diffusion and broth dilution techniques. The extract and fractions showed the presence of carbohydrate, tannins, flavonoids, saponins, terpenes and steroids. The antimicrobial screening of the extract and fractions against clinical isolates of Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli and Candida albicans, using disc diffusion method at disc potency of 100µg/disc showed inhibitory activity on the test isolates with zone of inhibition ranging from 16-37mm. The methanolic and ethylacetate extracts showed the least MIC of 1.25mg/mL. The result of the study confirms the traditional use of the stem bark of B. rufescens in the treatment of infections caused by susceptible microorganisms.***

***Keywords: Bauhinia rufescens, Stem bark, Phytochemical screening, Antimicrobial activity***

## INTRODUCTION

Infectious diseases are the number one cause of death worldwide and they account for approximately 50% of deaths in tropical countries (Iwu *et al*., 1999). In spite of the fact that there is a wide range of antibiotics currently available for treatment of infectious diseases, there are still a lot of problems associated with chemotherapy such as the development of resistance to chemotherapeutic agents as a result of over usage (Reuter, 2005). Among the set back includes the high cost of chemotherapeutic agents which necessitates the study of local medicinal plants used in the treatment of infectious diseases. Traditionally, plants are used for treatment of diseases in different parts of the world since time immemorial (Hostettmann *et al*., 2000).

*Bauhinia rufescens* Lam belonging to the family Leguminosea-Caesalpinoideae is a small tree that grows up to 8m high, found in the dry savannah area of West Africa extending to Sudan and often grown as an ornamental plant in villages. It is referred to as "Jirga" in Hausa. The stem bark is astringent and used in dressing wound. It is also used to treat diarrhoea, dysentery, jaundice, syphilis, leprosy and smallpox. The root contains high concentration of tannins and thus is used in tanning leather in Northern Nigeria (Burkill, 1995). Flavonoids such as quercetin and kaempferitrine (lespedin) have been isolated from the leaves of a Brazilian species of Bauhinia-*B. forticata* (Silva *et al*., 2000). The screening for phytochemical and antimicrobial activity of the stem bark has not been carried out to our knowledge. This study therefore aims at investigating the phytochemical constituents and antimicrobial activity of the stem bark extracts of *B. rufescens* .

## MATERIALS AND METHODS

### Plant Material

The stem bark of *B. rufescens* was collected in a village near Samaru, Zaria-Nigeria in October, 2008 after the plant was identified and authenticated in the Herbarium of the Department of Biological sciences, Ahmadu Bello University, Zaria, Nigeria, where a voucher specimen (No 427) has been deposited. The stem barks were air-dried and powdered using mortar and pestle.

### Extraction Procedure

Two hundred grams of the powdered stem bark materials were extracted with methanol after defatting with petroleum ether in a soxhlet extraction apparatus for 24 hours. The methanolic extract (ME) was evaporated using a rotary evaporator to a dry residue. ME was suspended in 200 ml of distilled water, filtered and partitioned exhaustively first with ethyl acetate to give ethyl acetate fraction (EAF), and then with n-butanol to give the butanol fraction (BF) and the residual aqueous fraction (AF).

### Phytochemical Screening

Various phytochemical tests were carried out on the extracts (ME, EAF, BF and AF) to detect the presence of bioactive constituents of the plant material (Treese and Evans, 1989; Sofowora, 1993).

**Test for carbohydrates**
Molish's test: A few drops of Molish's reagent was added to each of the extract dissolved in 2ml of water, followed by the addition of 1ml concentrated sulphuric acid down the side of the test tube to form a lower layer. A purple colour at the interface of the two liquids indicates the presence of carbohydrates.

**Test for flavonoids**
Shinoda test: To an alcoholic solution of each of the extract, three pieces of magnesium chips were added followed by a few drops of concentrated hydrochloric acid. Appearance of an orange, pink or red to purple colour indicates the presence of flavonoids.

**Test for saponins**
Froth test: About 0.5 g of the extract was shaken with water in a test tube. Frothing which persisted for 15 min indicates the presence of saponins.

**Test for tannins**
Ferric chloride test: A small quantity of the extract was boiled with water and filtered. Two drops of ferric chloride was added to the filtrate, formation of a blue-black, or green precipitate was taken as evidence for the presence of tannins.

**Test for sterols/terpenes**
Liebermann-Burchard test: 1 ml of anhydrous acetic acid was added to 1 ml chloroform and cooled to 0°C then one drop of concentrated sulphuric acid was added to the cooled mixture
followed by the extract. The solution was observed for blue, green, red or orange colour that changes with time.

**Antimicrobial Studies**
**Test Isolates:** The test isolates were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. The bacterial cultures were checked for purity and maintained in blood agar slant while the fungus was incubated in sabouraud's dextrose agar slant. The microorganisms include; *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans.*

**Disc Preparation:** One hundred improvised Whatman No. 1 filter paper discs (6mm in diameter) were placed in 10mg/mL of the extract in DMSO to form discs of 100µg potency.

**Inoculum Standardisation:** The isolates were dispensed in normal saline and the suspension made to match 0.5 MacFarland standards.

**Susceptibility Testing:** The disc diffusion method described by Bauer *et al*., (1966) was employed in the study. Discs prepared from the extracts were placed on blood agar plates which have been inoculated with test isolatess according to standard protocol described by National Committee of Clinical Laboratory Standard, (1993). The plates were incubated at 37°C for 24 hours for bacteria and at 25°C for 48 hours for fungus. The zone of inhibition was measured using transparent ruler across center of the discs and recorded in millimeters. Filter paper discs containing the solvents without the extracts served as negative control while standard Streptomycin 40µg/disc was the standard antibacterial drug used as positive control.

**Determination of minimum inhibitory concentration (MIC):** This was carried out on extracts that showed inhibitory effects on the test isolates. Broth dilution method was used for the determination (Collins *et al*., 1995). Nutrient broth was prepared according to the manufacturer's instructions. 10mls each of the broth was introduced into 5 screw-cap test tubes and sterilized at 121°C for 15 minutes and then allowed to cool.
Two-fold serial dilution of the extract with nutrient broth was carried out to give concentrations of 20, 10, 5, 2.5 and 1.25mg/mL. 0.1mL of the microorganism each were inoculated into the dilutions and incubated at 37°C for 24 hours. The lowest concentrations of the extracts which show no turbidity represent the MIC.

**Determination of minimum bactericidal concentration (MBC):** This was carried out to determine weather the isolates were killed or only their growths were inhibited. Blood agar plates were prepared according to the manufacturer's instructions, sterilized and poured into sterilized Petri dishes. The content of the MIC tubes and tubes with concentration greater than MIC were inoculated on separate plates by dipping a sterile wire loop into each test tube and streaking on the surface of the plates. The plates were then incubated at 37°C for 24 hours after which they were observed for growth. The MBC was the plate with the lowest concentration of the extract in which there occurs no growth.

**RESULTS**
The preliminary phytochemical screening of the stem bark extracts (ME, EAF, BF and AF) showed the presence of carbohydrates, flavonoids, saponins, tannins and sterol/terpenes (Table 1). The antimicrobial activity test showed that the test isolates were susceptible to the extracts at concentration of 100µg/disc with exception of *B. subtilis, E. coli* and *C. albicans* that were resistant to BF. The extracts exhibited zones of inhibition ranging from 6-28mm which were comparable to those produced by streptomycin 40µg/disc as shown in Table 2. The EAF showed a uniform MIC and MBC of 1.25 and 2.5mg/mL respectively against all the test microorganisms as shown in Table 3.

**Table 1: Phytochemical constituents of the stem bark extracts of *B. rufescens***

| Constituents | ME | EAF | BF | AF |
|---|---|---|---|---|
| Carbohydrates | + | - | + | + |
| Flavonoids | ++ | ++ | - | + |
| Saponins | + | - | ++ | - |
| Tannins | ++ | + | + | + |
| Sterol/terpenes | + | + | + | - |

NOTE: ++ = Highly present; + = Present; - = Absent

**Table 2: Susceptibility test of the isolates to the various extracts**

| | Concentration (100µg/disc)/ Zones of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| **Test isolates** | **ME** | **EAF** | **BF** | **AF** | **Streptomycin 40µg/disc** |
| *S. aureus* | 22 | 28 | 11 | 06 | 25 |
| *S. pyogenes* | 14 | 23 | 10 | 16 | 27 |
| *B. subtilis* | I9 | 22 | - | 09 | 20 |
| *E. coli* | 27 | 26 | - | 09 | 26 |
| *P. aeruginosa* | 24 | 27 | 12 | 11 | 30 |
| *C. albicans* | 20 | 20 | - | 12 | 26 |

**Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in mg/mL of the various extracts on the test microorganisms**

| Test isolates | ME | EAF | BF | AF |
|---|---|---|---|---|
| *S. aureus* | 1.25 (2.5) | 1.25 (2.5) | 2.5 (5) | 5 (10) |
| *S. pyogenes* | 2.5 (5) | 1.25 (2.5) | 2.5 (5) | 2.5 (5) |
| *B. subtilis* | 2.5 (5) | 1.25 (2.5) | - | 5 (10) |
| *E. coli* | 1.25 (2.5) | 1.25 (2.5) | - | 2.5 (5) |
| *P. aeruginosa* | 1.25 (2.5) | 1.25 (2.5) | 2.5 (5) | 5 (10) |
| *C. albicans* | 1.25 (2.5) | 1.25 (2.5) | - | 5 (10) |

NOTE: Values in parentheses represent the MBC

## DISCUSSION

The constituents screened for were found to be present in the methanolic extract while some of them were absent in the fractions. The methanolic extract and ethylacetate fractions showed the highest zones of inhibition especially against *S. aureus, E. coli* and *P. aeruginosa.* The antimicrobial activity of medicinal plants extracts have been attributed to the presence of phytochemical compounds like saponins, tannins and flavonoids contained in them (De and James, 2002). The presence of these compounds usually justifies the use of the plants for treatment of infections caused by susceptible pathogens. The extracts have shown significant antimicrobial activities against the isolates tested. Methanolic extract and ethylacetate fraction tested positive to flavonoids which are known to possess significant antibacterial and antimicrobial properties (Harborne and Williams, 2000). Phenolic compounds such as flavonoids and tannins are known to exhibit a wide range of interesting biological activities like antimicrobial, antiviral, antioxidant, hepatoprotective, cardioprotective, neuroprotective, cytotoxic, anticancer properties (Havsteen, 2002). These biological and pharmacological properties are usually attributed to their free radical scavenging efficacies, metal complexion capabilities and their ability to bind to proteins with a high degree of specificity (Fotie, 2008).

The extracts displayed appreciable antimicrobial activities against *S. aureus* and *E. coli* that are known to show above average resistance to most synthetic antimicrobial agents. These pathogens are known to cause majority of community and hospital acquired infections (Indrayan *et al*., 2002). The extracts, especially methanolic and ethylacetae extracts showed broad spectrum of activities against Gram positive and Gram negative bacteria as well as fungus. Bioactive compounds having antimicrobial activity may act via the inhibition of microbial growth and thereby offering protection to the plant against microbial infections (Ogunlana and Ramstad, 1975). The stem bark of *B. rufescens* may be said to possess bioactive compounds that have antimicrobial properties and thus justifies the use of this plant in traditional medicine for the treatment of infections.

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