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# Phytochemical and in vitro Antibacterial Evaluation of the Ethanolic Extract of the Stem Bark of *Entada africana* Guill. & Perr and *Sarcocephalus latifolus*

**J. C. Ifemeje[1*], C. Egbuna[1], S. C. Udedi[2] and H. I. Iheukwumere[3]**

*[1]Department of Biochemistry, Faculty of Science, Anambra State University, Uli, Nigeria.*
*[2]Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Nigeria.*
*[3]Department of Microbiology, Faculty of Science, Anambra State University, Uli, Nigeria.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Author JCI conceived, designed and carried out the phytochemical analysis. Author SCU wrote the protocol and first draft of the manuscript. Authors JCI and CE managed the literature searches, analysed and interpreted the results. Author HII performed the microbial studies. All authors read, corrected and approved the final manuscript.*

***Original Research Article***

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## ABSTRACT

**Aims:** The phytochemical composition and the *in vitro* antibacterial activity of the ethanolic extract of the stem bark of *Entada africana* Guill. & Perr. and *Sarcocephalus latifolus,* on *Staphylococcus aureus, Salmonella typhi, Escherichia coli* and *Bacillus subtilis* were investigated.
**Study design:** The plants stem bark samples were collected from Idu, Industrial Area of the Federal Capital Territory, Abuja, and were characterized by Mrs. Jamilat at the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The bacterial isolates were obtained from the Microbiology Laboratory, Anambra State University, Uli, Nigeria.
**Place and Duration of Study:** Analysis on the plant samples were done at the Department of Biochemistry and Department of Microbiology, Anambra State University, between January 2014 and February 2014.

**Corresponding author: Email: jcifemeje@yahoo.co.uk;*

**Methodology:** Phytochemical screening was done following standard methods. Agar well diffusion technique was used to screen the extracts for antibacterial activity. MIC and MBC for the various extracts were determined by the tube dilution technique. Ciprofloxacin, a standard antibiotic was used as control.
**Results:** The result of the phytochemical analysis revealed the presence of tannins, alkaloids, terpenes, resins, sterols, anthraquinone, phenols and saponins in *E. africana* while *S. latifolus* contained alkaloids, saponins, flavonoids, glycosides, anthraquinone, terpenes, phenols, resins and saponins. The *in vitro* antibacterial analysis revealed that the ethanolic extract of *E. africana* inhibited the growth of *S. typhi* and *B. subtilis* significantly while that of *S. latifolus* inhibited the growth of *E. coli* as well *B. subtilis* significantly with the zones of inhibition ranging from 12.00±0.02 to 0.80±0.01. The MIC and MBC for the organisms were at 5 mg/ml and 10 mg/ml.
**Conclusion:** The ethanolic extract of the stem bark of the plant samples exhibited antibacterial activity and thus could serve as a source for useful drugs.

*Keywords: Phytochemicals; Entada africana Guill. & Perr.; Sarcocephalus latifolus; bacteria.*

## 1. INTRODUCTION

All over the world, medicinal plants serves as a potent weapon in the diagnosis of diseases, making of traditional and modern medicines and in the treatment of various diseases which are caused by microbial agents such as bacteria, viruses and fungi [1,2]. These diseases if not treated appropriately using suitable herbs or drugs could degenerate into severe medical health complications. However, in Nigeria, as indeed most parts of Africa, indigenous knowledge of traditional medical practices is passed on orally and thus, memory plays a very prominent role in the training of a practitioner [3]. The therapeutic value of plants used in traditional medicine is derived from the presence of phytochemical principles [4], such as alkaloids, tannins and phenols which are found in parts of plants [5].

*Entada africana* Guill. & Perr. is a small tree of 4 to 10 meters in height and 90 cm in girth, with local names as 'Dorot' in Arabic and 'Ogurobe' in Yoruba, Nigeria [6]. It belongs to the phylum Magnoliophytas and family Fabaceae. The stem bark is said to have abortive effects, while a root decoction is a stimulating agent and tonic [7]. The plant is said to have antidote effects against various toxic agents because of its emetic properties [7]. Mbatchou et al. [6] stated that healing and fever reducing beverages are prepared from leaves, stem bark, roots, and shoots. Mbatchou et al*.,* [6] also stated that the ethanolic extract of the stem bark of *E. africana* inhibited the growth of some bacteria such as *S. typhi.* Nevertheless, the bark of the root and stem yields a long fiber used for cordage, commonly for roof binding and grass matting as well a low-quality gum [7]. In Northern Nigeria and Ghana, an infusion of the stem bark is taken as a tonic for stomach ache [7]. The leaves constitute a good wound dressing, preventing suppuration [7,8].

*Sarcocephalus latifolus* also called *Nauclea latifolia* is a straggling shrub or small tree of about 10 ft (3.05 m) high. It belongs to the family *Rubiaceae*, native to savannah forest and fringe tropical forests of West Africa [9]. It is known locally in Nigeria as 'Egbo egbesi' in Yoruba, 'Ubulu inu' in Igbo, and 'Tabasiya' in Hausa [10]. It is called 'African quinine' in Northern Nigeria in which a cold infusion of the stem bark is taken as a diuretic and anthelmintic [11]. The Fulanis in Nigeria uses the leaf extract to regularly deworm animals [12]. Other local uses of *Nauclea latifolia* include treatment of malaria, hypertension, diarrhea, tuberculosis, dysentery and also as a laxative [13]. It is also used in the treatment

of gastrointestinal tract disorders, sleeping sickness and prolongs menstrual flow [14,15]. The bark is said to be useful in the treatment of wounds, cough, and gonorrhea in Nigeria [16].

Bacteria are prokaryotic microorganisms numbered in quantum in which some are pathogenic and others beneficial to man. *Staphylococcus aureus* is a gram-positive coccal bacterium which is a successful pathogen due to a combination of nasal carriage and bacterial immuno-evasive strategies and causes a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, cellulitis folliculitis, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, and host of others [17,18]. *Salmonella typhi* is a gram-negative bacterium which causes typhoid fever and could be deadly [19]. It is a strong pathogen for humans due to its resistance to the innate immune response system [20]. *Escherichia coli* is a gram-negative bacterium with some virulent strains typically causing a bout of diarrhea that is unpleasant in healthy adults and is often lethal to children in the developing world [21]. *Bacillus subtilis* is a gram-positive bacterium which is known to cause disease in severely immunocompromised patients, and rarely causes food poisoning [22,23].

Therefore, the purpose of this study was to evaluate the phytochemical constituents and the possible antibacterial activities of *E. africana* Guill. & Perr. and *S. latifolus* so as to enhance assured traditional usage and hence give a spotlight for the usage in developing effective antibacterial drugs.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection and Preparation

The plants stem barks were collected from Idu, Industrial Area of the Federal Capital Territory, Abuja, and were characterized by Mrs. Jamilat at the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The plant materials were washed separately under running tap water, followed by distilled water. The washed plant leaves were air-dried for two weeks and later pulverized into fine powder and stored. Voucher specimens for *E. africana* (NIPRD/H/5743) and *S. latifolus* (NIPRD/H/5739) were deposited in the herbarium of NIPRD. The pathogenic bacteria isolates (*S. aureus* ATCC 25923*, S. typhi* ATCC 700931*, E. coli* ATCC 8739 and *B. subtilis* ATCC 6633) were obtained from the Microbiology Laboratory of the Department of Microbiology, Anambra State University. The bacteria isolates were recharacterized and reidendified using biochemical and microbiological procedures of Cowan and Steel [24].

### 2.2 Preparation of Ethanolic Extract

Ethanolic extract was prepared using 95% ethanol. In 500 ml 80 % ethanol, 50 g of sample was added. After constant shaking at 150 rpm for 72 hour at room temperature, the sample was filtered. Filterate was incubated at 40℃ till all the solvent was evaporated leaving behind the crude ethanolic extract.

### 2.3 Methods for Phytochemical Screening

Phytochemical screenings of the stem bark extract were carried out in order to determine the presence of selected phytochemicals following standard methods. Tannins, alkaloids, sterols

were determined by the method described by Sabri et al. [25]. Phenol (Ferric Chloride Test) and saponins were determined respectively by the methods of Prashant et al. [26] and Sofowora [27]. Flavonoids was determined by the method of Trease and Evans [28], glycosides were determined by the method of Mbatchou et al. [6], resins by the method of Bello et al. [29], anthraqinone (Borntrager's test) by the method of Onwukaeme et al. [30], terpenes (Salkowski test) by the method of Edeoga et al. [31].

## 2.4 Antibacterial Test

### 2.4.1 Preparation of concentrations of solvents soluble fractions of extracts

Stock solutions of 10 mg/ml concentrations of solvent soluble fractions of extracts of the plants were prepared by dissolving 5 mg of each fraction extract in 0.5 ml of dimethyl sulphoxide (DMSO). From the stock solution, concentrations of 2 mg/ml were made. Concentrations of Ciprofloxacin standard antibiotic were similarly prepared as those of the plant extracts. They were stored in sterilized vials and kept at the lower compartment of a refrigerator until required for use [6]. Mueller-Hinton agar was prepared and autoclaved at 121ºC for 15 minutes at 1atm as described by Sahoo et al. [32] and Uma et al. [33].

### 2.4.2 Antibacterial sensitivity test

The bacteria were cultured on nutrient agar and incubated at 37ºC for 24 hours. The bacteria were repeatedly cultured to obtain pure isolates. Morphological and biochemical reactions were carried out to ascertain proper identification. They were inoculated onto nutrient agar slant and stored at 4ºC using agar diffusion method [34]. Mueller-Hinton agar plates were inoculated with standard test inocula by direct streaking, and plates were properly labeled. A sterile cork borer (5 mm in diameter) was used to make wells in the plates for the extracts. An aliquot (0.1 ml) of the fractions was dispensed into each well. The plates were left to allow diffusion of extracts before being placed in the incubator at 37ºC for 24 hours. The relative susceptibility of the organism to the extracts was indicated by zones of inhibition produced after incubation.

### 2.4.3 Determination of minimum inhibitory concentration

Ten (10) mg/ml of extract solution was serially diluted in Muller-Hinton broth to give decreasing concentrations of 5 mg/ml, and 2.5 mg/ml. An aliquot (0.1 ml) of broth culture left overnight of test microorganism (concentration 1.5 x $10^8$ cfu/ml) in sterile normal saline was introduced into each extract dilution. The mixtures in sterile test tubes were incubated (37ºC, 24 hours) and observed for growth or inhibition. Ciprofloxacin (a synthetic broad spectrum antibacterial drug) was used as positive control. The minimum inhibitory concentration was the lowest concentration of the extract solution that inhibited microbial growth.

### 2.4.4 Minimum bactericidal concentration

A loopful of the test mixture was removed from each MIC tube that showed no growth, inoculated onto antibiotic-free Mueller-Hinton agar plate, incubated (37ºC, 24 hours) and inspected for presence of colonies indicating growth. The minimal bactericidal concentration is the lowest concentration of extract that showed no bacterial growth.

## 3. RESULTS AND DISCUSSION

The results of the phytochemical analysis presented in (Table 1) revealed that eight out of ten phytochemicals screened were present in the stem bark of *E. africana.* They included tannins, alkaloids, terpenes, and resins in high quantity. This was consistent to those reported by Mbatchou et al. [6]. Sterols, anthraquinone, phenols, and saponins were also present but in low amount. The antimicrobial property of *E. africana* as reported by Gislene et al. [35] is associated with the class of phytochemicals found present. However, the phytochemical constituent of the stem bark of *S. latifolus* (Table 1) include alkaloids and saponins in high amount, flavonoids, glycosides, anthraquinone, terpenes, phenols, resins and tannins in low amount.

**Table 1. Phytochemical screening of the ethanolic extract of the stem bark of *E. africana* and *S. latifolus***

| Phytochemicals | Stem bark | |
|---|---|---|
| | ***E. africana*** | ***S. latifolus*** |
| Tannins | ++ | + |
| Alkaloids | ++ | ++ |
| Flavonoids | - | + |
| Glycosides | - | + |
| Anthraquinone | + | + |
| Terpenes | ++ | + |
| Sterols | + | - |
| Phenols | + | + |
| Resins | ++ | + |
| Saponins | + | ++ |

**key: ++ = Highly present, + = Slightly present, - = Not detected*

Antibacterial activities were expressed as zones of inhibition in millimeters (Table 2). An observable zone of inhibition of growth of each microorganism served as a measure for concluding that a bacterium is sensitive or not. The result of the analysis however revealed that the ethanolic extract of the stem bark of *E. africana* exhibited anti-microbial activity on *S. typhi* (12.00±0.02) and *B. subtilis* (8.02±0.05). Antimicrobial activity of the ethanolic extract of the stem bark of *E. africana* was also observed on *S. aureus* (0.80±0.01) and *E.coli* (0.90±0.00) which could be of little or no importance. While the ethanolic extract of *S. latifolus* inhibited the growth of *E. coli* (11.12±0.01) and *B. subtilis* (9.01±0.01) with less activities on *S. aureus* (1.10±0.02) and *S. typhi* (2.20±0.00).

**Table 2. Zones of inhibition (mm) of extracts (10 mg/ml) against bacterial isolates**

| Test Bacteria | Ethanolic extract of the stem bark of the sample | | Ciprofloxacin |
|---|---|---|---|
| | ***E. africana*** | ***S. latifolus*** | |
| *S. aureus* | 0.80±0.01 | 1.10±0.02 | 7.03±0.01 |
| *S. typhi* | 12.00±0.02 | 2.20±0.00 | 15.00±0.00 |
| *E. coli* | 0.90±0.00 | 11.12±0.01 | 10.10±0.02 |
| *B. subtilis* | 8.02±0.05 | 9.01±0.01 | 12.00±0.01 |

** Values = mean ± SD of triplicate determination*

The MIC and MBC results are presented in (Table 3) and (Table 4) respectively. An aliquot of the ethanolic extract 10 mg/ml, 5 mg/ml and 2.5 mg/ml were used for all the bacterial

isolates. MBC tests were carried out to establish whether the extracts were bactericidal or bacteriostatic. MBC, in general was found to be 10 mg/ml for ethanolic extracts of both plants stem bark. The MIC and MBC for Ciprofloxacin was affective at 10 mg/ml and 5 mg/ml.

**Table 3. Minimum inhibitiory concentration (MIC) of the stem bark extracts on bacteria isolates**

| Test Bacteria | Ethanolic extract of the stem bark of *E. africana* | | | Ethanolic extract of the stem bark of *S. latifolus* | | | Ciprofloxacin | | |
|---|---|---|---|---|---|---|---|---|---|
| | 10 mg/ml | 5 mg/ml | 2.5 mg/ml | 10 mg/ml | 5 mg/ml | 2.5 mg/ml | 10 mg/ml | 5 mg/ml | 2.5 mg/ml |
| *S. aureus* | ++ | ++ | +++ | + | + | ++ | - | - | + |
| *S. typhi* | - | - | + | - | + | ++ | - | - | - |
| *E.coli* | + | ++ | ++ | - | - | + | - | - | - |
| *B. subtilis* | - | + | ++ | - | + | + | - | + | - |

**key: - = No colony growth, + = Scanty colony growth, ++ = Moderate colony growth, +++ = Heavy colony growth*

**Table 4. Minimum bactericidal concentration (MBC) of the stem bark extracts on bacterial isolates**

| Test Bacteria | Ethanolic extract of the stem bark of *E. africana* | | | Ethanolic extract of the stem bark of *S. latifolus* | | | Ciproflaxacin | | |
|---|---|---|---|---|---|---|---|---|---|
| | 10 mg/ml | 5 mg/ml | 2.5 mg/ml | 10 mg/ml | 5 mg/ml | 2.5 mg/ml | 10 mg/ml | 5 mg/ml | 2.5 mg/ml |
| *S. aureus* | ++ | +++ | ++++ | + | + | +++ | - | + | ++ |
| *S. typhi* | - | + | + | - | ++ | +++ | - | - | ++ |
| *E. coli* | + | ++ | +++ | - | + | ++ | - | + | + |
| *B. subtilis* | - | + | ++ | - | + | + | - | + | - |

**key: - = No colony growth, + = Scanty colony growth, ++ = Moderate colony growth, +++ = Heavy colony growth*

The phytochemical results revealed that the plants contained bioactive agents which are connected with antimicrobial properties in plants [36]. Flavonoids are reported to have antibacterial, anti-inflammatory, anticancer, antifungal, antiallergic, and diuretic properties [1]. Tannins are known to possess general antimicrobial and antioxidant activities [37]. Other compounds like saponins and anthraquinone have been implicated as bioactive antibacterial agents of plants [27,38,39]. Alkaloids are medicinal agents for their analgesic antispasmodic and antibacterial effects [1]. The high presence of resins in the stem bark sample of *E. africana* confirms the report of Orwa et al. [7] that it yields low-quality gum. Terpenes have also been reported to be active against bacteria [40].

However, it could be deduced that the ethanolic extract of *E. africana* inhibited the growth of *S. typhi* which was consistent as those reported by Mbatchou et al. [6]. *E. africana* also has anti-bacterial activity on *B. subtilis.* This could be attributed to the presence of tannins which have shown potential antiviral [41], antibacterial [42] and antiparasitic effects [43]. The ethanolic extract of *S. latifolus* inhibited the growth of *E. coli* and *B. subtilis* which was in conformity with what was demonstrated by Okiei et al. [44] that ethanolic extract of *S. latifolus* showed antibacterial property. Isah et al. [45] reported also that *S. latifolus* inhibited the growth of *S. typhi, B. subtilis, E. coli, and S. aureus.* From Tables 3 and 4, it was observed that the MIC and MBC for the organisms were at 5 mg/ml and 10 mg/ml.

## 4. CONCLUSION

The study revealed that the stem bark of *E. africana* and *S. latifolus* contained different phytochemicals. The stem bark of *E. africana* inhibited the growth of *S. typhi* and *B. subtilis* while *S. latifolus* inhibited the growth of *E.coli* and *B. subtilis*. This medicinal property of *E. africana and S. latifolus* could help in the treatment of bacterial infections and as well serve as resource for useful drugs.

## CONSENT

Not applicable.

## ETHICAL APPROVAL

Not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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