

Phytochemical Screening and In Vitro Anti-Bacterial Studies of the Ethanolic Extract of the Stem bark of *Entada africana* Guill. & Perr. and *Sarcocephalus latifolus* on some selected Strains of Bacteria

ABSTRACT

Aims: The phytochemical composition and the in vitro anti-bacterial activity of the ethanolic extract of the stem bark of *Entada africana* Guill. & Perr. and *Sarcocephalus latifolus* on four strains of bacteria: *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Bacillus subtilis* was investigated.

Study design: The plants stem bark samples were collected from Idu, Industrial Area of the Federal Capital Territory, Abuja, and were characterized by a Botanist at the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria.

Place and Duration of Study: Analysis on the plant samples was done at the Department of Biochemistry and Department of Microbiology, Anambra State University, between January 2014 and February 2014.

Methodology: phytochemical screening on the ethanolic extracts of the plants stem barks was done using standard methods and equipment.

Results: The result of the phytochemical analysis revealed the presence of tannins, alkaloids, terpenes and resins at high concentration in the stem bark of *E. africana* with the little presence of sterols, anthraquinone, phenols and saponins. However, the phytochemical analysis of *S. latifolus* showed the presence of alkaloids and saponins in high concentration whereas flavonoids, glycosides, anthraquinone, terpenes, phenols, resins and saponins were present in low amount. The (%) moisture content of the stem bark of *E. africana* and *S. latifolus* were found in the order of 9.47 and 10.72 respectively while their (%) ash content were 5.67 and 6.21 respectively. The in vitro-bacteria analysis revealed that the ethanolic extract of *E. africana* inhibited the growth of *S. typhi* and *B. subtilis* while that of *S. latifolus* inhibited the growth of *E. coli* as well *B. subtilis*.

Conclusion: The ethanolic extract of the stem bark of the plant samples exhibited antimicrobial activity and thus could serve as a source for useful drugs.

Comment [C1]: check

Keywords: Phytochemicals, *Entada africana* Guill. & Perr., *Sarcocephalus latifolus*, Bacteria, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Bacillus subtilis*.

1. INTRODUCTION

All over the world, medicinal plants serves as a potent weapon in the diagnosis and treatment of various diseases which are specially caused by microbial agents such as bacteria, viruses, fungi etc. These diseases if not treated appropriately using suitable herb or drug 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 [1]. The therapeutic value of plants used in trado-medicine are derived from the presence of

45 phytochemical principles [2], which are found in parts of the plants such as alkaloids, tannins and phenols
46 etc [3].

47 *Entada africana* Guill. & Perr., is a small tree of 4 to 10 meters in height and 90 cm in girth, with local
48 names as Dorot in Arabic and Ogurobe in Yoruba, Nigeria [4]. It belongs to the phylum: Magnoliophytas
49 and family: Fabaceae. The stem-bark is said to have abortive effects, while a root decoction is a
50 stimulating agent and tonic. The plant is said to have antidote effects against various toxic agents
51 because of its emetic properties. Mbatchou *et al.*, [4] stated that healing and fever reducing beverages
52 are prepared from leaves, stembark, roots and shoots. The bark of the root and stem yields a long fiber
53 used for cordage, commonly for roof binding and grass matting as well a low-quality gum. In Northern
54 Nigeria and Ghana, an infusion of the leaves or of the stembark is taken as a tonic and for stomach ache.
55 The leaves also constitute a good wound dressing, preventing suppuration [5].

56 *Sarcocephalus latifolus* also called *Nauclea latifolia* is a straggling shrub or small tree of about 10ft high. It
57 belongs to the family, Rubiaceae, native to savannah forest and fringe tropical forests of West Africa [6]. It
58 is known locally in Nigeria as Egbo egbesi in Yoruba, Ubulu in Igbo, and Tabasiya in Hausa [7]. It is
59 called "African quinine" in Northern Nigeria, a cold infusion of the bark is taken as a diuretic and
60 anthelmintic [8]. The Fulanis in Nigeria uses the leaf extract to regularly deworm animals [9]. Other local
61 uses of *Nauclea latifolia* includes in the treatment of malaria, hypertension, diarrhea, tuberculosis,
62 dysentery and also a laxative [10].

63 The purpose of this study was to evaluate the phytochemical constituents and the anti-bacteria activities
64 of *Entada africana* Guill. & Perr., and *Sarcocephalus latifolus* on some selected strains of bacteria.

65 2. MATERIALS AND METHODS

66 **2.1 Sample Collection:** The plants stem barks were collected from Idu, Industrial Area of the Federal
67 Capital Territory, Abuja, and were characterized by a Botanist at the National Institute for Pharmaceutical
68 Research and Development (NIPRD), Abuja, Nigeria.

69 **2.2 Sample Preparation:** Plant materials were washed separately under running tap water, followed by
70 distilled water. The washed plant leaves were air-dried for two weeks and later pulverized into fine
71 powder and stored.

72 2.3 Methods for Phytochemical Screening

73 **2.3.1 Detection of Tannins:** Tannin was detected using the method of [11]. Two (2) mls of water was
74 introduced in test tube containing 2 mls of the aqueous extract of the samples and 1 to 2 drops of diluted
75 ferric chloride solution was added. A dark green or blue green coloration was observed indicating the
76 presence of tannins.

77 **2.3.2 Detection of Alkaloids:** Two (2) mls of 10% NH₄OH (pH=7) was added to 15 mls aqueous extract
78 of the samples. The alkaloid was extracted using 10mls of chloroform and 2mls of 10% HCl. This was
79 divided into two portions. Mayer's reagent was added to one portion and Wagner's reagent to the other.
80 The formation of a brown or white precipitate was regarded as positive for the presence of alkaloids [11].

81 **2.3.3 Determination of Phenols (Ferric Chloride Test):** Extracts were treated with 3-4 drops of ferric
82 chloride solution. Formation of bluish black colour indicated the presence of phenols [12].

83 **2.3.4 Determination of Anthraquinone:** Half a gram (0.5g) of each of the powdered samples were
84 shaken in different test tube with 10mls of chloroform for 15mins and then filtered. Equal volumes of
85 ammonia were added to the filtrates and shaken. A bright pink colour at the upper aqueous layer
86 indicated the presence of free anthraquinone.

87 **2.3.5 Determination of Glycosides:** Five (5) mls of the water extracts of the powdered leaves and stem
88 bark were obtained in different test tubes and boiled with 2.5 mls of dilute H_2SO_4 for 15 mins each. This
89 was later neutralized with 10% sodium hydroxide (NaOH), after which 5mls of Fehlings solution A and B
90 were added to both test tubes. The appearance of a brick red precipitate indicated the presence of
91 glycoside.

92 **2.3.6 Determination of Saponins:** To a small quantity of each of the powdered samples was added 95%
93 ethanol and boiled. The mixture was filtered and 2.5 mls of it added to 10mls of distilled water in a test
94 tube. The test tube was stoppered and shaken vigorously for about 30 seconds. It was allowed to stand
95 for 30 minutes. Honey-comb is indicative of the presence of saponins [13].

96 **2.3.7 Determination of Resins:** Fifteen (15) mls of petroleum ether was added to 0.1g of each of the
97 powdered samples and filtered into two test tubes. An equal volume of copper acetate solution was
98 added and shaken vigorously and allowed to separate. A green colour indicated the presence of resins.

99 **2.3.8 Determination of flavonoids:** Five grams (5g) of each of the powdered samples were completely
100 detanned with acetone. The residue was extracted in warm water after evaporating the acetone. Two
101 drops of 10% NaOH was added separately to 1ml of the detanned water extract. A yellow solution
102 indicated the presence of flavonoids.

103 **2.3.9 Determination of sterols:** Sterols were determined by the reaction of Liebermann. Ten (10) mls of
104 ethanolic extract was evaporated using rotary evaporator. The residue was dissolved in 0.5 ml hot acetic
105 anhydride; then chloroform treated with the reagent of Liebermann Burchardt was added to 0.5mls of the
106 filtrate. The appearance, at the interphase, a ring of blue-green, showed a positive reaction [11].
107

108 **2.3.10 Determination of Terpenes:** Five (5g) of each of the sample was extracted by maceration with
109 50mls of 95% ethyl acetate, filtered and the filtrate evaporated to dryness. The residues were dissolved in
110 10mls of anhydrous chloroform and filtered. The filtrate was mixed with 1ml of acetic anhydride followed
111 by addition of 1ml Conc. H_2SO_4 down the wall of the test tube to form layer underneath. The formation of
112 a reddish violet colour indicates the presence of terpenes.

113 2.4 Pharmacognosy

114 **2.4.1 Determination of Moisture Content:** First, the crucible was washed, dried and weighed. Three
115 (3) g of the plant sample was weighed into the crucible. The crucible and its content were then placed in
116 the oven at a temperature of $105^{\circ}C$ for 4 hours. The sample was then dried to a constant weight. The loss
117 in weight is calculated.

118 **2.4.2 Determination of Total Ash:** Two (2g) of the ground plant sample was weighed into a platinum
119 crucible after which the crucible and its content were placed in a muffle furnace at a temperature of about
120 580° -600 C for about 6 hours. It was later weighed and the ash content calculated.
121

122 2.5 Antibacterial Test

123 **2.5.1 Preparation of concentrations of solvents soluble fractions of extracts:** Stock solutions of
124 10mg/ml concentrations of solvent soluble fractions of extracts of the plants were prepared by dissolving
125 5mg of each fraction extract in 0.5ml of dimethyl sulphoxide (DMSO). From the stock solution,
126 concentrations of 2mg/ml were made. Concentrations of ciprofloxacin standard antibiotic were similarly
127 prepared as those of the plant extracts. They were stored in sterilized vials and kept at the lower
128 compartment of a refrigerator until required for use [4].

129 **2.5.2 Preparation of Mueller-Hinton Agar (Medium):** An amount of 19.0g of Mueller-Hinton agar was
130 weighed in a 500ml media bottle. Five hundred (500) mls of distilled water was measured, added to it and
131 agitated for a while [14, 15]. The content was boiled on a hot plate fitted with magnetic stirrer until the

132 powder completely dissolved to ensure uniformity. The pH of the media was adjusted to 7.0±0.1. The
133 bottle was well corked and autoclaved at 121 °C for 15minutes at 1atm.

134 **2.5.3 Antibacterial sensitivity test:** The bacterium was cultured on nutrient agar and incubated at 37 °C
135 for 24hours. The bacterium was repeatedly cultured to obtain pure isolates. Morphological and
136 biochemical reactions were carried out to ascertain proper identification. They were inoculated into
137 nutrient agar slants and stored at 4 °C. The agar diffusion method was used [16]. Mueller-Hinton agar
138 plates were inoculated with standard test inocula by direct streaking, and plates were properly labeled. A
139 sterile cork borer (5mm in diameter) was used to make wells in the plates for the extracts. Then 0.1ml of
140 the fractions was dispensed in to each well. The plates were left to allow diffusion of extracts before being
141 placed in the incubator at 37 °C for 24hours [17]. The relative susceptibility of the organism to the extracts
142 was indicated by zones of inhibition produced after incubation.

Comment [C2]: Did you culture one of four microorganisms

143 **3. RESULTS AND DISCUSSION**

144 **3.1 Results**

145 The phytochemical screening of the ethanolic extracts of the stem bark of *E. africana* and *S. latifolus* were
146 presented in Table 1. This comparatively showed the phytochemicals present in each plant sample.
147 Tannin, alkaloids, anthraquinone, terpenes, sterols, phenols, resins and saponins were present in *E.*
148 *africana* while tannin, alkaloids, flavonoids, glycosides, anthraquinone, terpenes, phenols, resins and
149 saponins was found to be present in *S. latifolus*.

Comment [C3]: You did not give us the qualitative results of the zone of inhibition. From this I could deduced that you only carry out a sensitivity test of the anitbacteria

150 **Table 1: Phytochemical Screening of the ethanolic extract of the stem bark of *Entada***
151 ***africana* and *Sarcocephalus latifolus***

Phytochemicals	Stem bark	
	<i>Entada africana</i>	<i>Sarcocephalus latifolus</i>
Tannins	++	+
Alkaloids	++	++
Flavonoids	-	+
Glycosides	-	+
Anthraquinone	+	+
Terpenes	++	+
Sterols	+	-
Phenols	+	+
Resins	++	+
Saponins	+	++

152 ***key:** ++ = Highly present; + = Slightly present; - = Absent
153

154 Similarly, the result of the pharmacognosy performed on the stem bark of both plant sample were
155 presented in Table 2. The % moisture content in *E. africana* and *S. latifolus* is 9.47 and 10.72 respectively
156 while the % ash content is 5.67 and 6.21 respectively.

157 **Table 2: Pharmacognosy of the Stem Bark of *Entada africana* and *Sarcocephalus latifolus***

Parameter	<i>Entada africana</i>	<i>Sarcocephalus latifolus</i>
Moisture (%)	9.47	10.72
Ash (%)	5.67	6.21

158
159 Anti-bacterial activities of the stem bark of the plant samples investigated using four strains of bacteria:
160 *Staphlococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Bacillus subtilis* presented in Table 3
161 showed that the ethanolic extract of the stem bark of *Entada africana* exhibited anti-microial activity on

162 *Salmollena typhi* and *Bacillus subtilis* while the ethanolic extract of *Sarcocephalus latifolus* inhibited the
 163 growth of *Escherichia coli* and *Bacillus subtilis*.

164 **Table 3: Antibacteria activity of the ethanolic extract of the stem bark of *Entada africana* and**
 165 ***Sarcocephalus latifolus***

Strains of Bacteria	Ethanolic Extract of <i>Entada africana</i>	Ethanolic Extract of <i>Sarcocephalus latifolus</i>
<i>Staphylococcus aureus</i>	-	-
<i>Salmollena typhi</i>	+	-
<i>Escherichia coli</i>	-	+
<i>Bacillus subtilis</i>	+	+

*key: + = Activity; - = No Activity

3.2 Discussion

The result of the phytochemical analysis presented in Table 1 revealed that eight (8) out of ten (10) phytochemicals screened were present in the stem bark of *Entada africana*. They included tannins, alkaloids, terpenes and resins in high quantity. This was consistent to those reported by [4]. Sterols, anthraquinone, phenols, and saponins were also present but in low amount. However, the phytochemical constituent of the stem bark of *Sarcocephalus latifolus* (Table 1) include alkaloids and saponins in high amount, flavonoids, glycosides, anthraquinone, terpenes, phenols, resins and tannins in low amount.

The result of the quantitative pharmacognosy of *E. africana* and *S. latifolus* presented in table 2, revealed the % moisture content in *E. africana* to be 9.47 and *S. latifolus* as 10.72. The % Ash content of *E. africana* and *S. latifolus* are 5.67 and 6.21, respectively. This result suggests that there is high water content in the stem bark of *S. latifolus* to compare with that of *E. africana* which however implies that the plant sample (*S. latifolus*) may be prone to microbial attack and should be dried properly before storing for a long time. The high ash content in *S. latifolus* however indicates that there could be high mineral content than that of *E. africana*.

The analysis of the anti-bacteria activity of the stem bark of *Entada africana* and *Sarcocephalus latifolus* presented in Table 3, revealed that the ethanolic extract of *Entada africana* inhibited the growth of *Salmollena typhi* which was consistent as those reported by Mbatchou *et al.*, [4]. *Entada africana* also has anti-bacteria activity on *Bacillus subtilis*. This could be attributed to the presence of tannins which have shown potential antiviral [18], antibacterial [19] and antiparasitic effects (Kolodziej and Kiderlen, 2005). The ethanolic extract of *Sarcocephalus latifolus* inhibited the growth of *Escherichia coli* and *Bacillus subtilis* which was in conformity with what was demonstrated by [21] that hot aqueous and ethanolic extract of *S. latifolus* showed antibacterial property.

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 *Salmollena typhi* and *Bacillus subtilis* while *S. latifolus* inhibited the growth of *Escherichia coli* and *Bacillus subtilis*. This medicinal property of *E. africana* could serve as an antidote in the control of Typhoid fever, an ailment caused by *Salmonella typhi*.

Comment [C4]: At what concentration are you recommending for usage, and what is the most active concentrations from all the two concentration.

Comment [C5]: Please discuss the factor responsible for the zone of inhibition and give references to other authors who have reported such before.

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