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ANTIBACTERIAL PROPERTIES OF AQUEOUS STEM BARK EXTRACT OF *Ekebergia senegalensis* A. Juss. (Meliaceae)

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ABSTRACT

The stem bark of *Ekebergia senegalensis* which has some traditional medicinal applications was investigated for its antibacterial properties. The in-vitro antibacterial activity of the aqueous stem bark extract of *Ekebergia senegalensis* was conducted. Disc diffusion method was used to assess the effect of the extract on *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, *Corynebacterium glutanicum*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli* at concentrations of 1000, 800, 600, 400 and 200 mg/ml, respectively. Varying degrees of antibacterial activities were observed against *Staphylococcus aureus, Salmonella typhi*, *Bacillus subtilis*, *Corynebacterium glutanicum*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* while *Enterobacter aerogenes*, *Streptococcus pyogenes*, and *Escherichia coli* were resistant to the extract at all the concentrations used. The highest antibacterial activity was observed against *Corynebacterium glutanicum* and *Proteus mirabilis* with a diameter zone of inhibition of 15 mm while the lowest activities were observed in *Salmonella typhi* and *Pseudomonas pyogenes* with a diameter zone of inhibition of 8 mm. The minimum inhibitory concentration showed that very low concentrations of $0.05x10^3$mg/ml (*Bacillus subtilis*, *Corynebacterium glutanicum*, and *Proteus mirabilis*) and $0.1x10^3$mg/ml (*Salmonella typhi* and *Pseudomonas aeruginosa*) of the extract can inhibit growth. The minimum bactericidal concentrations of $0.1x10^3$mg/ml and $0.15x10^3$mg/ml for the aqueous stem bark extract showed that the plant's stem bark is bactericidal to *Bacillus subtilis*, *Corynebacterium glutanicum*, *Proteus mirabilis*, *Salmonella typhi* and *Pseudomonas aeruginosa*. Conclusively, the aqueous stem bark extract of *Ekebergia senegalensis* exhibited broad spectrum antibacterial activity, especially at high doses.

Keywords: antibacterial, aqueous stem bark, *Ekebergia senegalensis*

INTRODUCTION

The extensive use of plants as the primary source of health remedies due to their pharmacological properties is quite common. There has been increasing interest in the use of medicinal herbs for meeting the goals of primary health care delivery worldwide (Elujoba *et al.*, 2005). Scientific scrutiny of these herbs' therapeutic potential and safety has also increased, thereby providing physicians with data to help patients make wise decisions about their use (O' Hara *et al.*, 1998). Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune suppression, and allergic reactions. This

situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance, there is a need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants. This prompted the present study which investigated the antibacterial properties of the aqueous stem bark extract of *Ekebergia senegalensis*.

MATERIALS AND METHODS

Plant Material and Extraction

The stem bark of mature *Ekebergia senegalensis* were collected from Hong local government area of Adamawa state and were identified and authenticated by a plant taxonomist of the Department of Biology, Faculty of Science, University of Maiduguri. The barks of the plant were thoroughly washed and dried in laboratory for three weeks. The dried barks were ground to a fine powder using pestle and mortar and then stored in a dry cylinder. Two hundred (200 g) grams of the powdered stem bark was mixed with one liter of distilled water in 5-liter beaker and stirred at 65^{o}C for 30 minutes. It was allowed to cool and shaken vigorously before filtration using Whatman filter paper. The filtrate was concentrated in a rotary evaporator and stored at 4^{o}C, the yield was 12.2 g that is 6.1% (W/W).

Antimicrobial Activity

Preparation of Inoculant:

Clinical and pure isolates of *Streptococcus pyogenes*, *Staphylococcus aureus*, *Bacillus subtilis*, *Corynebacterium glutamicum*, *Klebsiella planticola*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella typhi*, and *Escherichia coli* were obtained from the Department of Veterinary Medicine Laboratory, University of Maiduguri, Borno, Nigeria. All the cultures were tested for purity. Each inoculum was prepared by inoculating the stock culture that have been suspended in a saline solution (0.9%) into a freshly prepared nutrient agar at 37^{0}C for 18-24 hours and maintained in a sterile condition.

Preparation of Discs:

Five concentrations of the plant extracts were prepared: (1000, 800, 600, 400 and 200 mg/ml). Disc of Whatmann filter paper with a diameter of 6 mm, were impregnated with the different concentrations of the extract. Commercially available antibiotic diffusion disc was used as standards for comparism.

Disc Diffusion Assay

The Disc Whatmann filter paper impregnated with a known volume and appropriate concentration of the extracts were placed on a plate of sensitivity testing agar, uniformly inoculated with the test organism using tetracycline as the standard antibiotic is then incubated at 37^{0}C for 18-24 hours.

The antimicrobial substance diffuses from the Disc of Whatmann filter paper into the medium and the growth of the test organism was inhibited at a distance from the disc, which is related to the sensitivity of the organism. Organisms sensitive to the antimicrobial substance are inhibited at a distance from the disc while the resistant organism have smaller zones of inhibition or grow up to the edge of the disc.

Determination of Minimum Inhibitory Cncentration (MIC).

The MIC was determined by the micro-dilution method as recommended by (Ellof, 1998) and (NCCLS, 2001). For each bacterium, five test tubes were used each containing the culture medium, inoculums and the extract. The concentrations of the extracts employed were 200, 150, 100, 50 and 25 mg/ml, respectively.

The test tubes were covered and incubated overnight at 37^{0}C for 24 hours. Inhibition of bacterial growth were judged by the turbidity of the medium. The MIC was defined as the lowest concentration at which no visible growth was observed.

Determination of Minimal Bactericidal Concentration (MBC).

MBC was determined at the concentration defined as the lowest extract

concentration at which 99.9% of the bacterial population were killed after 18-24 hours incubation at 37^0C. MBC was carried out by sub culturing the growth in the test tubes into another plate and incubated at 37^0C for 18-24 hours.

Data Analysis

The data collected were measured from the zone of inhibition. The inhibition zone diameters were evaluated in millimeters using a meter rule. The concentrations of the extract inducing inhibition were considered as antimicrobial as compared to that obtained from tetracycline which was the standard antibiotic.

RESULTS AND DISCUSSION

Antimicrobial Analysis

In the present study, the aqueous stem bark extract of *E. senegaliensis* possesses an in vitro antibacterial activity against gram-positive (*Bacillus subtilis*, *Corynebacterium glutanicum*, and *Staphylococcus aureus*) and gram-negative bacteria (*Salmonella typhi*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*) (Table 1). This finding is in contrast to the observations of Doss *et al.,* 2011, in which no inhibition zone was observed for the aqueous leave extract of Mimosa pudica and Panicum maximum against similar test organisms (*Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Klebsiella pneumoniae*). However, no antibacterial activity was observed in the present study against (*Streptococcus pyogenes*, *Enterobacter aerogenes* and *Escherichia coli)* (Table 1). The absence of antibacterial activity indicates that there was no zone of inhibition against these test organisms (*Streptococcus pyogenes*, *Enterobacter aerogenes*, and *Escherichia coli*). This implies that the dose level of the extract could be so low to be effective against the test microbes (Taylor *et al.* 2001, Adegboye *et al.* 2008).

Table 1: Diameter zone of inhibition (mm) of the test organisms at different concentrations of the aqueous stem bark extract of *E. senegaliensis.*

	Aqueous stem bark extract (mg/ml)					
Test Organisms	Standard drug	1000	800	600	400	200
Streptococcus pyogenes	15	-	-	-	-	-
Staphylococcus aureus	20	13	10	9	-	-
Bacillus subtilis	40	14.5	13	12	11	11
Corynebacterium glutamicum	45	15	13	12	12	11
Klebsiella planticola	32	12	14	13	10	-
Enterobacter aerogenes	18	-	-	-	-	-
Pseudomonas aeruginosa	18	12	11	9	9	8
Proteus mirabilis	42	15	13	12	11	10
Salmonella typhi	45	13	11	9	9	8
Escherichia coli	16	-	-	-	-	-

Table 2: Minimum Inhibitory Concentration (MIC) of the aqueous stem bark extract of *E. senegaliensis.*

	Aqueous Stem Bark Extract (mg/ml)				
Test Organisms	200	150	100	50	25
Bacillus subtilis	-	-	-	+	+
Corynebacterium glutamicum	-	-	-	+	+
Pseudomonas aeruginosa	-	-	+	+	+
Proteus mirabilis	-	-	-	+	+
Salmonella typhi	-	-	+	+	+

= Susceptibility, + = Absense of susceptibility

Table 3: Minimum Bactericidal Concentration (MBC) of the aqueous stem bark extract of *E. senegaliensis.*

	Aqueous stem bark extract (mg/ml)				
Test Organisms	200	150	100	50	25
Bacillus subtilis	-	-	+	+	+
Corynebacterium glutamicum	-	-	+	+	+
Pseudomonas aeruginosa	-	+	+	+	+
Proteus mirabilis	-	-	+	+	+
Salmonella typhi	-	+	+	+	+

- = Susceptibility, + = Absense of susceptibility.

The minimum inhibitory concentration (MIC) showed that very low concentrations ($0.05x10^3$mg/ml and $0.1x10^3$mg/ml) of the aqueous stem bark extract of *E. senegaliensis* can inhibit the growth of *Bacillus subtilis*, *Corynebacterium glutanicum*, *Proteus mirabilis*, *Salmonella typhi* and *Pseudomonas aeruginosa* (Table 2). The minimum bactericidal concentrations (MBC) of $0.1x10^3$mg/ml and $0.15x10^3$mg/ml for the aqueous stem bark of *E. senegaliensis* can kill *Bacillus subtilis*, *Corynebacterium glutanicum*, *Proteus mirabilis*, *Salmonella typhi* and *Pseudomonas aeruginosa* (Table 3).

CONCLUSION AND RECOMMENDATIONS

Demonstration of antibacterial activity of aqueous stem bark extract of *E. senegaliensis* against the test organisms (*Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, *Corynebacterium glutanicum*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli*) is an indication that there's the possibility of sourcing alternative antibiotic substances in this plant for the development of newer antibacterial agents. The aqueous stem bark extract of *E. senegaliensis* has a broader spectrum of activity, being active against both Gram-positive and Gram-negative organisms.

However, attempts should be made to conduct an invivo findings, as the diameter zone of inhibition is not only affected by sensitivity of the microorganisms alone but concentration of the extract in the disc used and its rate of diffusion in the media as well. This study also revealed that the aqueous stem bark extract of *E. senegalensis* may be use to manage some infectious diseases which calls for further experiments on isolation and characterization of the active component as well as determining the toxicity of the component. This will therefore provide means of standardizing its use by traditional users especially with respect to dose rate and route of administration.

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