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Antibacterial Activity of the Stem bark Extracts of *Detarium senegalense* JF Gmelin

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ABSTRACT

Background: The global trend in the upsurge of antibiotic resistance has necessitated an urgent need to develop new antimicrobial agents. Plants have long been successfully used in traditional medical practice in the treatment of infectious diseases. The use of *Detarium senegalense***,** a widely distributed tropical plant in the folkloric treatment of infectious diseases in Nigeria has not been fully investigated**. Objective:** This study was therefore designed to investigate the effect of various organic solvent fractions of the aqueous extract of the stem bark of *D. senegalense* on both gram-positive and gram-negative bacteria. **Results:** The result showed that the aqueous extract was active against only *Staphylococcus aureus* and *Pseudomonas aeruginosa* at the concentration range of 200 – 1000 mg/ml while the organic solvent fractions were active against all the bacteria tested. The methanolic fraction exhibited relatively strong inhibitory activity against all the organisms tested, with the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 50 mg/ml and 100 mg/ml respectively against all the organisms tested, except *Streptococcus pyogenes* that had higher MIC of 100 mg/ml and MBC of 200 mg/ml. This result compared closely with the standard antibiotic (tetracycline 250 mg/ml) used in this work**. Conclusion:** In conclusion, the broad spectrum inhibitory activity of the various extracts of stem bark of *D. senegalense* against both gram-negative and gram-positive bacteria recorded in this study strongly support the traditional use of the plant in the treatment of diseases.

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INTRODUCTION

Pathogenic bacteria have always been considered a major cause of morbidity in animals and humans (Djeussi *et al*., 2013). Antibiotic resistance and the fast developing multi-drugs resistance micro-organism like *Salmonella* species have become a source of global concern in the field of medicine (Prasannabalaji *et al*., 2012). The global trend in the upsurge of antibiotic resistance is largely attributed to the indiscriminate use of antibiotics for the treatment of human and animal diseases, which in turn is causing a significant failure (Hancock, 2005), giving rise to an urgent need to develop new innovative antimicrobial agents for treating infectious diseases.

The World Health Organisation reported that 75 – 95% of the people living in developing countries chiefly rely on traditional medicine involving the use of plant extracts or their active constituents (WHO, 2011).

Plants have long been investigated as potential sources of new antimicrobial agents. Random screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotic research. The potential of higher plants as sources for new drugs are still largely unexplored. Among the estimated 250,000 – 500,000 plant species only a small percentage has been scientifically investigated (Mahesh and Satish, 2008).

Detarium senegalense (J. F Gmelin) is one of the plants used in north eastern part of Nigeria for the treatment of infectious diseases and other ailments. *Detarium senegalense* belongs to the family *Leguminoseae* and subfamily *Ceasalpinocea*. It is a native of Africa and mostly found in Senegal, Gambia, Sudan, Democratic Republic of Congo and Nigeria along water course (Wang *et al*., 1996). The plant serves as fruit and ornamental shade tree within its area of distribution (Akah *et al*., 2010). *Detarium*

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senegalense is reported to have considerable use in food and pharmaceutical industries (Wang *et al*., 1996). A decoction of the bark is claimed to be effective in cases of anaemia, pneumonia, diarrhea, stomach ache, digestive disorder and in expelling the placenta after childbirth (Burkill, 1995; Sowemimo *et al*., 2011). Scientific information on the antimicrobial potential of this plant is still inadequate. Therefore this study was designed to investigate the effect of various organic solvent fractions of the aqueous extract of the stem bark of *D. senegalense* on both gram-positive and gram-negative bacteria.

MATERIALS AND METHODS

Collection of Plant Material and Extraction:

Fresh leaves and stem bark of *D. senegalense* were collected in April, 2009 from Biu Local Government Area of Borno State, Nigeria. The plant was identified and authenticated at the Department of Biological Science, University of Maiduguri, Nigeria, where a voucher specimen (BCH 178A) was deposited. The stem was washed with tap water and dried under shade. The dried stem was pulverized to a fine powder and stored in a glass container at 4°C. One hundred grams of the powder sample was exhaustively extracted with distilled water using soxhlet extractor. The extract was evaporated to dryness in hot air oven at 40 - 50°C. The dried crude aqueous extract obtained was suspended in cool distilled water and then filtered using Whatman No.1 filter paper. The filtrate fractionated successively with chloroform, ethyl acetate, n-butanol and methanol. The fractionation with the organic solvents which are of different polarity was done until the organic solvents layer were visibly clear to obtain chloroform, ethyl acetate, n-butanol and methanol soluble fractions and residual aqueous fraction in sequence as described by Cho *et al*. (2003); Motohashi *et al*. (2004). The organic solvents were evaporated to dryness using rotary evaporator, and stored at 4°C until required.

Microbial cultures:

Pure cultures of gram-positive; *Staphylococcus aureus* (*Staph. aureus*), *Streptococcus pyogenes* (*Strep. pyogenes*) and gram-negative; *Salmonella typhi* (*S. typhi*), *Klebsiella pneumoniae* (K. *pneumoniae*), *Escherechia coli* (*E. coli*), and *Pseudomonas aeruginosa* (*Ps. aeruginosa*) bacteria were obtained from the Department of Veterinary Medicine Research Laboratory, University of Maiduguri, Nigeria. The isolates were propagated on nutrient agar (Oxoid Ltd, England) plates at 37°C overnight and then stock-cultured on nutrient agar slants. The stock cultures were incubation at 37°C overnight and then stored at 4°C for further studies.

Extracts Concentration:

Stock solutions of the different organic solvents soluble fractions were prepared by dissolving 200, 400, 600, 800 and 1000 mg of the extract in 1 ml of distilled water. The following concentrations of each fraction were prepared, 200, 400, 600, 800 and 1000 mg per ml. Standard antibacterial agent (tetracycline, Cipla Ltd, Mumbai, India) at a concentration of 250 mg/ml was also used on all bacteria organisms and their zones of inhibition were compared with those of the extract.

Antibacterial Sensitivity Testing:

The disc diffusion method as described by the National Committee of Clinical Laboratory Standards (1993) was adopted to determine the antibacterial activity of the various organic solvent fractions of *D. senegalense* stem bark. Discs containing different concentrations of dissolved extracts was prepared with sterilized filter paper (Whatman No. 1; 6 mm in diameter), soaked in different beakers containing different concentrations (200, 400, 600, 800 and 1000 mg/ml) of the extracts. The discs were dried at 50°C.

Bacterial samples were re-cultured from the stock-cultures and grown overnight at 37°C. After incubation, each isolate was suspended in sterile normal saline and subsequently diluted using sterile normal saline to give an inoculum size of about 10^6 CFU/ml. The inocula were spread on the surface of dried nutrient agar plates with sterile swab sticks which have been dipped in the diluted suspensions of the test organisms. The seeded plates were incubated at 37°C for 30 minutes before the impregnated discs (2 discs for each concentration) were applied aseptically on the surface of the plates. The treated plates were then incubated at 37°C for 48 hr. The same procedure was carried out using tetracycline (10 mg/ml) as the positive control. Plates without the antibiotic or extract discs were set up as the negative control experiment. The diameters of the zones of inhibition were measured to the nearest mm using a rule. The zone of inhibition above 6 mm in diameter for each isolate was used as a measure of susceptibility to the extracts and this was compared to the standard antibiotic.

Minimum Inhibitory Concentration:

The minimum inhibitory concentration (MIC) of the aqueous extract of *D. senegalense* (JF Gmelin) stem bark was determined using the method of Greenwood (1989). Six sterilized test tubes were arranged in 6 rows in

a test tube rack, each row for one of the 6 micro-organisms used for the test. Half a milliliter of sterilized nutrient broth was pipetted into the first tubes of the 6 rows to obtain a concentration of 100 mg/ml, thereafter there was a serial dilution of the extract in each row to obtain concentrations of 50, 25, 12.5, 6.25, 3.13 and 1.57 mg/ml, respectively. The test organism (0.5 ml) were pipetted into each of the test tubes and incubated at 37°C for 24 hr. The MIC was recorded as the least concentration of the extract that completely inhibited the growth of the test organisms. The contents of the tubes were further sub-cultured for 24 hr. to determine bactericidal or bacteriostatic activity. Bactericidal effect was demonstrated when no growth occurred on the sub-cultured medium after MIC determination.

Antibacterial effect of the organic solvent fractions of Detarium senegalense on bacterial activity:

The bacterial cultures used were similar to those used in the estimation of the effect of aqueous extract of *D. senegalense* on bacterial activity, and was obtained from the Department of Microbiology, University of Maiduguri, Nigeria. The disc-diffusion method of National Committee of Clinical Laboratory Standards (1993) was also used, however, organic solvents of different polarities including ethyl acetate, n-butanol, chloroform, methanol and water residue were used for determination of antibacterial activity.

The minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) were according to the method of Greenwood (1989).

RESULTS AND DISCUSSION

In vitro antibacterial effect of the aqueous stem bark extracts of Detarium senegalense:

The *in vitro* antibacterial effect of the aqueous extract is shown in Table 1. The extract at 1000, 800 and 600 mg/ml only, produced a zone of inhibition of 9, 8 and 7 mm respectively on *Staph. aureus.* At concentrations of 1000, 800, 600, 400 and 200 mg/ml, the extract produced zones of inhibition of 13, 11, 10, 8 and 7 mm respectively on *Pseudo. aeruginosa*. The inhibitory activities of the extract on these microorganisms are in dose dependent manner. All other organisms tested were resistant to the extract at all tested concentrations. The standard agent, tetracycline, at 250 mg/ml produced zones of inhibition of 30, 20, 20, 25, 30 and 25 mm on *Staph. aureus, Strep. pyogenes, S. typhi*,

E. coli, *Ps. aeruginosa* and *K. pneumoniae* respectively. The MIC and MBC of the extract were found to be 100 and 200 mg/ml respectively for *Ps. aeruginosa* (Table 2). This result indicates that the crude extract is only active against *Ps. aeruginosa* and *K. Pneumoniae* which may explain the traditional use of decoction of *D. senegalense* stem bark in the treatment of wound infection and pneumonia. The crude extract was reported to contain carbohydrate, saponins, glycosides, tannin, flavonoid, alkaloids and steroids (Sanni, 2013). The antibacterial effect could be due to the presence of flavonoids and tannins in the extract that were reported (Okwu, and Uchegbu, 2012) to possess appreciable antimicrobial activities. However the presence of carbohydrate could facilitate the growth of bacteria and therefore may antagonise the antibacterial activity of the active principles. This may account for the decreased antibacterial activity of the aqueous crude extract.

In vitro antibacterial effect of D. senegalense stem bark organic solvent fractions:

The *in vitro* antibacterial effects of *D. senegalense* stem bark organic solvent fractions are presented in Table 3, while the MIC and MBC of the fractions are shown in Tables 4, 5 and 6. The ethyl acetate fraction at the concentrations used in this study inhibited the growth of *E. coli*, *Ps. aeruginosa*, and *K. pneumoniae*. *Strep. pyogenes* and *S. typhi* were only resistant to the extract fraction of 200 mg/ml. *Staph. aureus* was resistant to all the concentrations of the ethyl acetate fraction used in the study. The MIC and MBC of the ethyl acetate fraction for both *E. coli* and *Ps. aeruginosa* were found to be 100 mg/ml while that of *K. pneumoniae* were 25 mg/ml (Table 6).

The n-butanol fraction at 1000, 800 and 600 mg/ml concentration inhibited the growth of all the tested organisms while at 400 mg/ml concentration; it inhibited the growth of *Staph. aureus* and *K. pneumoniae* only. At 200 mg/ml concentration, all the organisms were resistant to the action of this fraction (Table 3).

The chloromform fraction at the concentration of 1000 to 600 mg/ml inhibited all the tested organisms(*Staph. aureus, S. typhi, E. coli, Ps. aeruginosa, K. pneumoniae* and *Strep. pyogenes*). However, at 400 mg/ml concentration only *E. coli* and *Pseudo. aeruginosa* were not inhibited. The fraction at the concentration of 200 mg/ml did not inhibit the growth *S. typhi, E. coli, Ps. aeruginosa* and *Strep. pyogenes* (Table 3). The MIC of the chloroform fraction for *K. pneumoniae* and *Staph. aureus* was 50 mg/ml and the MBC of the fraction for both organisms was 100 mg/ml (Table 5)

The methanolic fraction at the concentrations tested inhibited the growth of *Sta. aureus, S. typhi, E. coli, Ps. aeruginosa, K. pneuomoniae* and *Strep. pyogenes* (Table 3). The MIC of the methanolic fraction for *E. coli, S. typhi* and *Ps. aeruginosa* was 50 mg/ml and the MBC of the fraction for these organisms was 100 mg/ml. The MIC of the methanolic fraction for *K. pneumoniae* and *Staph. aureus* was 25 mg/ml and the MBC

of the fraction for these organisms was 50 mg/ml. The MIC of the methanolic fraction for *Strep. pyogenes* was 100 mg/ml and the MBC of the fraction for *Strep. pyogenes* was 200 mg/ml (Table 6).

The water residue fraction at 1000 mg/ml inhibited the growth all the tested bacteria except *Strep. Pyogenes*. At 800 mg/ml concentration only *E. coli*, *Ps. aeruginosa* and *K. pneumoniae* were inhibited. At 600 mg/ml concentration, it inhibited the growth of *Strep. pyogenes*, *Ps. Aeruginosa* and *S. typhi*. The fraction at 200 mg/ml concentration inhibited the growth of all organisms tested except *Staph. aureus* (Table 3). These inhibitions were however less than that produced by the control drug (tetracycline).

The methanol soluble fraction of the aqueous extract of *D. senegalense* in this study showed broad spectrum of activity against all the gram-positive and gram-negative bacteria used. This is an indication that the methanol extract fraction has better antibacterial properties as compared to the activity of the crude aqueous extract that only inhibited *Ps. aeruginosa* at the various concentrations used in this study.

The result of this study also shows that the organic solvent fractions of *D. senegalense* have better efficacy than the crude aqueous extract and the water residue fraction when the microbial inhibitions are compared. This is in agreement with the findings of McLaughlin, (1991); Geidam *et al*. (2007), who reported that fractionation ensures better biological activity.

The activities of the aqueous extract of *D. senegalense* and the ethyl acetate and methanol fractions against *Ps. aeruginosa* are very interesting since it is well known that most antibacterial agents are not active against this organism (Franklin, 1993). The effectiveness of the methanol extract fraction on *Staph. aureus* is surprising, since multiple antibiotic resistance strains of *Staph. aureus* exist in clinical setting worldwide as well as in Nigeria (Olukemi *et al*., 1997). The activities of ethyl acetate and methanol fractions against *E. coli* are also interesting since *E. coli* strains have developed resistance to antimicrobials commonly used in animal production (Yang *et al*., 2004) and even to frontline antimicrobials such as the fluoroquinolones (Angulo *et al*., 2000; Livermore *et al*., 2002).

The antibacterial properties of the organic solvent fractions could be due to the presence of flavonoids and tannins in the extracts; these secondary metabolites are known to possess appreciable antimicrobial activities (Narayana *et al*., 2001; Okwu and Uchegbu, 2012; Dwivedi *et al*., 2011). In an earlier study, flavonoids, tannins, glycosides among others are known to be present in the aqueous and organic solvent fractions of *D. senegalense* (Sanni, 2013). The present study appears to confirm earlier report by Burkill (1995) that *D. senegalense* stem bark has antibacterial activities. This may support the folkloric use of the plant in the treatment of diarrhoea, wounds and dysentery. The organisms tested in this study have been implicated in diarrhoea and/or dysentery (*Salmonella* serotypes, *E. coli* and *Klebsiella* species) and wound infections (*Staphylococcus*, *Streptococcus* and *Pseudomonas*).

In conclusion, this study has shown that *D. senegalense* crude extract and its fractions possess antibacterial activities; the methanol soluble fraction of *D. senegalense* aqueous stem bark extract possesses broader spectrum antibacterial properties. This fraction showed a good activity against the organisms at relatively lower concentrations and therefore could possibly be used against bacterial infections. However, further studies need to be carried out.

Table 1: Antibacterial activity of *D. senegalense* aqueous stem bark extract

Concentrations (mg/ml) Zone of Inhibition (mm)						
	Staph. aureus	*Strep. pyogenes*	*Salm.* Typhi	*Esch. coli*	*Pseudo. Aeruginosa*	*Kleb. Pneumonia*
Extract (1000)	9	R	R	R	13	R
Extract (800)	8	R	R	R	11	R
Extract (600)	7	R	R	R	10	R
Extract (400)	R	R	R	R	8	R
Extract (200)	R	R	R	R	7	R
Tetracycline (250)	30	20	20	25	30	25

R = Indicates the resistance to the test organism

Table 2: Minimum inhibitory and bactericidal concentration of *D. senegalense* stem bark aqueous extract on *Pseudomonas aeruginosa*.

Concentrations of extract (mg/ml)	Minimum Inhibitory Concentration (MIC) *Pseudomonas aeruginosa*	Minimum Bactericidal Concentration (MBC) *Pseudomonas aeruginosa*
200	−	−
100	−	−
50	+	+
25	+	+
12.5	+	+

Key: + = Indicates bacterial growth; − = Indicates no bacterial growth

Table 3: Antibacterial efficacy of *D. senegalense* stem bark organic solvent fractions

Fractions	Concentration (mg/ml)	Zones of inhibition (mm)					
		Sta. aureus	*Str. pyogenes*	*S.* Typhi	*E. coli*	*Ps. aeruginosa*	*K. pneumoniae*
Ethyl acetate	1000	R	12	12	13	18	20
	800	R	10	10	15	15	16
	600	R	8	8	9	12	15
	400	R	7	7	8	8	14
	200	R	R	R	7	7	12
N-butanol	1000 800 600 400 200	10 9 8 7 R	8 7 6 R R	8 6 6 R R	10 7 6 R R	8 7 6 R R	12 11 10 8 R
Chloroform	1000 800 600 400 200	15 13 11 10 9	10 9 8 6 R	14 12 10 8 R	10 8 6 R R	10 8 6 R R	14 13 12 10 9
Methanol	1000 800 600 400 200	15 13 12 11 10	10 9 8 7 7	14 13 12 10 8	14 13 11 9 8	18 16 14 12 10	15 14 12 11 10
Water residue	1000 800 600 400 200	11 8 9 9 7	R R R R R	7 R R R R	12 8 R 6 R	10 7 R 6 R	10 6 9 R R
Tetracycline	250	17	21	20	18	14	22

R – resistance by the organism

Sta. aureus = Staphylococcus aureus; Str. pyogenes = Streptococcus pyogenes; S. Typhi *=Salmonella* Typhi; *E. coli = Escherichia coli*; *Ps. aeruginosa = Pseudomonas aeruginosa*; *K.Pneumoniae = Klebsiella pneumoniae*

Table 4: Minimum inhibitory and bactericidal concentration of *D. senegalense* stem bark ethyl acetate fraction

Concentrations of extract (mg/ml)	Minimum Inhibitory Concentration (MIC)				Minimum Bactericidal Concentration (MBC)		
	E. coli	*Ps. aeruginosa*	*K. pneumoniae*		*E. coli*	*Ps. aeruginosa*	*K. pneumoniae*
200	–	–	–		–	–	–
100	–	–	–		–	–	–
50	+	+	–		+	+	–
25	+	+	–		+	+	–
12.5	+	+	+		+	+	+

Key: + = Indicates bacterial growth; – = Indicates no bacterial growth

Sta. aureus = Staphylococcus aureus; Str. pyogenes = Streptococcus pyogenes; S. Typhi *=Salmonella* Typhi; *E. coli = Escherichia coli*; *Ps. aeruginosa = Pseudomonas aeruginosa*; *K.Pneumoniae = Klebsiella pneumoniae*

Table 5: Minimum inhibitory and bactericidal concentration of chloroform fraction of *D. senegalense* stem bark

Extract Concentrations (mg/ml)	Minimum Inhibitory Concentration (MIC)		Minimum Bactericidal Concentration (MBC)	
	K. pneumoniae	*Sta. aureus*	*K. pneumoniae*	*Sta. aureus*
200	–	–	–	–
100	–	–	–	–
50	–	–	+	+
25	+	+	+	+
12.5	+	+	+	+

Key: + = Indicates bacterial growth; – = Indicates no bacterial growth

Sta. aureus = Staphylococcus aureus; K.Pneumoniae = Klebsiella pneumoniae

Table 6: Minimum inhibitory and bactericidal concentration of methanolic fraction of *D. senegalense* stem bark

Organism	Concentrations of Extract (mg/ml)									
	Minimum Inhibitory Concentration (MIC)					Minimum Bactericidal Concentration (MBC)				
	200	100	50	25	12.5	200	100	50	25	12.5
E. coli	−	−	−	+	+	−	−	+	+	+
S. Typhi	−	−	−	+	+	−	−	+	+	+
K. pneum	−	−	−	−	+	−	−	−	+	+
P. aerug	−	−	−	+	+	−	−	+	+	+
Staph.aureus	−	−	−	−	+	−	−	−	+	+
Strep.pyogenes	−	−	+	+	+	−	+	+	+	+

Key: + = Indicates bacterial growth; − = Indicates no bacterial growth
Staph. aureus = Staphylococcus aureus; Strep. pyogenes = Streptococcus pyogenes; S. Typhi *=Salmonella* Typhi; *E. coli = Escherichia coli*; *P. aerug = Pseudomonas aeruginosa*; *K.Pneum = Klebsiella pneumoniae*

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