

Antibacterial Studies of the Stem Bark of Detarium Microcarpum Guill. & Perr. (Fabaceae)

Abubakar S^{*1}, Ibrahim H¹, Adeshina G. O², Olayinka B. O²

¹Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria

²Department of Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria

Corresponding Author: Abubakar S, Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria. **Tel:** 08065619861, 08073873876, **E-mail:** sanimazab@gmail.com

Citation: Abubakar S et al. (2017), Antibacterial Studies of the Stem Bark of Detarium Microcarpum Guill. & Perr. (Fabaceae). Int J Nano Med & Eng. 2:4, 32-40. DOI: [10.25141/2474-8811-2017-4.0032](https://doi.org/10.25141/2474-8811-2017-4.0032)

Copyright: ©2017 Abubakar S et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Received: March 7, 2017; **Accepted:** March 29, 2017; **Published:** May 02, 2017

Abstract:

Bacteria isolates comprising Staphylococcus aureus and Escherichia coli from urine samples of patients from Nasara clinic of the Ahmadu Bello Teaching hospital (ABUTH), Zaria were identified to genera level using the conventional biochemical tests and were further characterized to species level using the appropriate microscopic procedures and confirmatory tests. The isolates were then tested for their antibiotic sensitivity against eight different standard antibiotics and against three samples from the stem bark of Detarium microcarpum. The zones of inhibition, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and the Rate of Kill of the three samples were measured. The isolates were generally resistant to amoxicillin and augmentin but susceptible to nitrofurantoin, gentamicin and ofloxacin. Most of the E. coli isolates were resistant to Tetracycline as compared to the samples of S. aureus isolates. The zones of Inhibition showed by the plant samples ranged from 6 mm to 45 mm, the MIC ranged from 0.625 mg/ml to 50 mg/ml and MBC ranged from 1.25 mg/ml to 50 mg/ml. The antimicrobial activity of the extract and fractions of the stem bark of the plant was compared with that of a standard antibiotic (ciprofloxacin). The traditional medicinal use of the plant stem bark was justified by the result of this study

Keywords : Antimicrobial, Detarium Microcarpum, Minimum-Inhibitory-Concentrations, Minimum-Bactericidal-Concentrations, Rate-of-Kill, Susceptibility

1.0 INTRODUCTION

1.1 Detarium. microcarpum Guill & perr.

Detarium. microcarpum belongs to the family Fabaceae. It is commonly called Tallow tree in English and Taura in Hausa. It is a savannah tree, growing up to 9.114 m, with reddish-brown scaly bark; leaves glaucous beneath; flowers cream in dense inflorescences; fruits are edible, branchlets deciduous; it is a plant of the drier savannahs. Leaflets rounded and usually emarginated at apex, oblong-elliptic, 6-12, alternate: calyx in bud densely pubescent outside; inflorescences congested; leaflets thickly coriaceous, usually about 7-11cm broad, but sometimes smaller, with numerous translucent gland-dots; fruits sub orbicular and flattened, about 4cm in diameter and 2.5cm thick, not very fleshy; often forming small abortive or galled fruits (Hutchinson et. al, 1966). With the increasing antimicrobial resistance in many

bacterial populations and consequently rendering most commonly prescribed, conventional orthodox antibiotics ineffective, plants still remains the source of potent antimicrobial/anti-infective agents. In general, natural drug substances offer some vital and appreciable roles in modern system of medicine thereby adequately justifying their legitimate presence in the prevailing store of therapeutic substances (Kar, 2007). This is because of the earth's rich biodiversity. This abundance and diversity of African flora, especially in the tropical rain forests, has prompted enormous efforts to investigate these plant resources for lead compounds in the development of new pharmaceutical agents and other compounds with economic potentials (Nkunya, 1994). This has also made the source of phytomedicines cheap and readily available especially to rural dwellers. This helps the researchers to ultimately come up with ways in which such plants could be used in treating diseases and infections or develop drugs for the

orthodox medical practice (Evans, 2008). The bark leaves and roots of *Detarium microcarpum* are widely used throughout its distribution area as diuretics and astrigents. They are prepared as infusions or a decoction to treat rheumatism, venereal diseases, urogenital infections, haemorrhoids, carries, biliousness, stomach-ache, intestinal worms and diarrhea including dysentery (Kouyaté, 2006). The Fresh bark or leaves of *Detarium microcarpum* have been applied to wounds, in Burkina Faso to prevent and cure cutaneous, subcutaneous parasitic infections. In Mali the bark is also used to treat measles, and itch, while a decoction of the leaves or roots is taken against, cramps and difficult delivery. The powdered seeds are applied to skin infections and inflammations, whereas the fruit is eaten to cure meningitis and malaria. In Senegal a mixture of the leaves of *Detarium microcarpum*, *Sclerocarya birrea* and *Acacia macrostachya* Rchb. ex DC. Pounded in milk is considered very efficient for snakebites. In veterinary medicine the leaves and roots are used to treat diarrhoea in cattle in southern Mali, and in Benin to treat constipation. The plant as a whole is used in the treatment of arthritis, rheumatism, etc.; genital stimulants/depressants; leprosy; liver, etc. (Kouyaté, 2006). The antimicrobial activity of this plant was therefore investigated to validate the claims that *Detarium microcarpum* is used for these reported medicinal uses

2.0 MATERIALS AND METHODS

2.1 Sample Collection

Forty (40) mid-stream urine samples (about 10mls each) from 20 different HIV positive patients and 20 different HIV negative patients who had not taken any antibiotic or HAART at least two weeks before sampling period were collected from Nasara clinic, ABUTH, Zaria. The samples were collected in 25ml sterile bottles. These were taken immediately to the laboratory and cultured on bacteriological media

2.2 Isolation and Identification of Bacteria from Urine Samples

Sterile bijoux bottles containing 20 ml of sterile nutrient broth were prepared prior to samples collection and 5 ml of each urine sample was inoculated into each bottle containing. These were kept in an incubator at 37 °C for 18 hours. Two sets of Clean and sterile Petri dishes with one set containing 20 ml of MacConkey Agar –Oxoid LTD and the other set, 20 ml each of Manitol Salt Agar (MSA) – Oxoid LTD were prepared. Each sample earlier grown in Nutrient Broth was cultured in the plates using a sterile inoculating loop by the streaking method and labeled appropriately. These were kept in an incubator at 37 °C for 48 hours. The viable colonies from the selective media were then sub cultured into prepared 5ml Nutrient agar slants. Each colony was sub cultured triplicates and labeled appropriately. These were stored in a refrigerator for further tests such as Gram staining and biochemical tests.

2.3 Collection and Preparation of Plant Material

The plant material was collected from the wild in Giwa local Government Area located in Kaduna State, Nigeria. The collection was done on December 16th, 2008. About 1 Kg of the stem cuttings was collected. Another fresh twig comprising all the essential parts used in the identification of plants was collected and taken to the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria for authentication. A voucher number,

900676 was assigned to the plant sample on 17th December, 2008 by the taxonomists in charge. Preliminary processing of the plant material was done. This includes elimination of undesirable materials and contaminants, washing to remove soil, sorting and cutting. The bark was removed from the stem cuttings dried in an open air under shade. This was then powdered using mortar and pestle (Brdi et. al., 2008).

2.3.1 Extraction of Plant Samples

The powdered crude drug (1000 g) was first of defatted using 5 litre of petroleum ether (60-80 °C). The marc obtained from this was subjected to extraction by cold percolation using 1 litre of 70 % methanol. The filtrate obtained was divided into two portions. Tannins were precipitated from one portion using 1 % solution of gelatin containing 10% NaCl. This was filtered to separate the precipitated tannins and a filtrate is obtained. The tannins were recovered from the residue by adding another 1 ltr of 99 % MeOH to the precipitate obtained and filtered. This dissolves the tannins leaving a residue of gelatin and NaCl. The filtrate contains the tannins while the gelatin and the NaCl remain in the precipitates as the residue (or the marc). The filtrate was concentrated over water bath at a controlled temperature. The resulting extract constitutes the tannin fraction (TF). The filtrate obtained after precipitating the tannins was also concentrated in an evaporating dish over a water bath. This constitutes the Residual Fraction (RF).

2.4 Phytochemical Screening of the plant Samples

Standard screening tests of the crude extract Crude Extract (MCE), Tannin Fraction (TF) and Residual fraction (RF) were carried out for the presence of various phytochemical constituents of the stem bark.

2.5 Antibiotic Susceptibility Testing (AST)

2.5.1 Disc Agar Diffusion (DAD) Technique:

The susceptibility of the test organisms, *Escherichia coli* and *Staphylococcus aureus* to a panel of eight antibiotics in a multidisc (Abtek® Biologicals LTD) was determined by the modified Kirby-Bauer's method. The test organisms were standardized by dilution of their overnight NB culture with sterile normal saline; *E. coli* (1 in 5,000) and *S. aureus* (1 in 1,000). The multidisc contained the following antibiotics: Amoxycillin (25 µg), Cotrimoxazol (25 µg), Nitrofurantoin (300 µg), Gentamicin (10 µg), Nalidixic Acid (30 µg), Ofloxacin (30 µg), Augmentin (30 µg), Tetracycline (30 µg). Twenty-one sterile plates were prepared and 20ml of sterile Mueller Hinton agar was aseptically dispensed into each plate and allowed to cool and solidify (Prescott, 2002). The surface of each agar was inoculated with 5 ml of each sample of standardized bacterial culture by flooding the surface of the solidified agar using a micro pipette and then the excess discarded. The setups were allowed to stay on the plate for 10 minutes at room temperature with the top (lid) in place.

The multidiscs were then placed on the inoculated Mueller-Hinton agar plates using a pair of sterile forceps. The plates were allowed to stay for about 30 mins then incubated for 18 hours at 35 °C. The diameters of the zones of growth inhibition were measured to the nearest mm for each of the antibiotics. The tests were carried out in duplicates hence an average of the diameters of each pair was calculated and recorded (Prescott, 2002). The results obtained

were also subjected to statistical analysis to compare the activities of the antibiotics against the test organisms.

2.6 Determination of Antibacterial Activity of Plant Samples

Here, the three samples (MCE, TF and RF) from the plant and a standard drug (ciprofloxacin) were used. The surface of each sterile Mueller-Hinton agar plate was inoculated with 5ml of an appropriate standardized bacterial culture by flooding the surface of the solidified agar. Each plate was labeled appropriately and allowed to stay for 10 minutes at room temperature with the top (lid) in place. Four holes per plate were punched-out in the agar using a borer. The borer was flamed before and after use on each plate. A concentration of 100 mg/ml each for the three plant samples and 50 µg/ml for the standard drug (ciprofloxacin) were aseptically put into the holes using sterile syringes. A control (without antibiotics) was also set up alongside the experiment. The entire set up was allowed to stay for about 30 minutes to allow for the diffusion of the extract/fractions; then incubated for 18 hours at 35 °C. After incubation, diameters of the zones of growth inhibition were measured for each of the plant samples tested (the extract and two fractions) to the nearest mm. Each concentration was set up in triplicates and the average of each triplicate diameter was calculated and recorded (Prescott, 2002). The results obtained were also subjected to statistical analysis to compare the activities of the plant samples against the test organisms.

2.7 Determination of Minimum Inhibitory Concentration (MIC)

Mueller Hinton Agar (500 ml double strength) was prepared according to the manufacturer's instructions. This was dispensed in 10ml each into 50 clean sample bottles and sterilized by autoclaving at 121 °C for 15 mins.

A total of 10 different concentrations of each plant sample were prepared. Firstly, stock concentration of 100 mg/ml. About 10ml of this stock concentration dissolved in 10 ml of the prepared Mueller Hinton agar (MHA) gives a concentration of 50 mg/ml. This represents the first concentration and labeled as concentration –A. The remaining nine concentrations were then prepared from the stock concentration by serial dilution. Thus from Concentrations A-J, we have: (50, 40, 30, 20, 10, 5, 2.5, 1.25, 0.625, and 0.3125) mg/ml respectively

Exactly 10 ml of prepared molten sterile Mueller Hinton agar was mixed with 10 ml of each concentration (A-J) and aseptically poured into a sterile plate labeled with the corresponding concentration and allowed to set. This procedure was done in quadruplicates for each concentration. The plates were allowed to solidify at room temperature with the lid of each plate in place. An adequate quantity of paper discs punched-out from Whatman No. 1 filter paper were placed in a clean Petri dish and kept under UV light for 2-5 hours to be sterilized. A pair of these discs was gently and aseptically placed on the agar plates where the organisms will be inoculated. An overnight broth culture of the test organisms was standardized and 10 µl of these were dispensed onto the paper discs in the agar plates and allowed to stay on the plate for 5 to 10 minutes at room temperature with the top (lid) in place. These

were then incubated for 16 to 18 hours at 35 °C. This procedure was carried out for the three different extracts (Prescott, 2002).

After incubation, the series of plates were observed for microbial growth. The lowest concentration of the test agent that did not show any visible growth was considered the minimum inhibitory concentration (MIC) of the microbial agent. A positive control was also set up to ensure viability of test organisms and sterility of the media used (Prescott et. al., 2005).

2.8 Determination of Minimum Bactericidal Concentration (MBC)

The paper discs that did not show any growth on or around them at the end of the MIC test procedure were carefully and aseptically transferred into bottles containing 5 ml Nutrient Broth (NB) with 3 % tween-80 using sterile forceps. Each bottle containing a pair of the paper discs was labeled appropriately with test organisms and the concentration of test antimicrobial agent. The bottles were then incubated at 37 °C for 72 hours. The lowest concentration of the test agent that did not show any visible growth in the recovery broth medium was considered the minimum bactericidal concentration (MBC). A negative control was also set up to ensure viability of test organisms and sterility of the media used (Prescott et. al., 2005).

2.9 Determination of the Rate of Kill

The organism with the highest MBC was used to determine the rate of kill; therefore Isolate number 5 (E. coli) was used to determine the rate of kill as this showed the highest value of MBC. This also implies that it has the highest resistance as compared to the other isolates. About 450 ml of Mueller Hinton agar with 3% tween-80 was prepared according to the manufacturers' instructions and dispensed in 10 mls into clean macatney bottles and sterilized in an autoclave at 121 °C for 15 mins. The bacterial isolate was sub-cultured in 5ml nutrient broth and incubated for 18hrs. This was standardized based on 1 in 5,000 dilutions using sterile normal saline to obtain approximately 105 cfu/ml. A fixed concentration of 100 mg/ml of the plant extract (i.e. 5 x MIC for crude extract and 2 x MIC for the tannin and Residual Fractions) was prepared. About 1ml of the standardized culture was added to the prepared concentration of the extract. 1ml sample was removed from the reaction mixture using a micropipette at 0,10, 30, 60, 120, 180 and 240 minutes, diluted serially in 9ml sterile distilled water containing 30% tween-80 and was mixed with 10 ml of the prepared sterile double strength MHA. This was poured aseptically in clean sterile plates, allowed to set and incubated at 37°C for 24 hrs. The control comprised Mueller Hinton Agar without plant samples but inoculated with the test inoculums (Ehinmidu, 2003).

3.0 RESULTS

3.1 Yields of Plant Extract and Fractions From the 1000g of the powdered plant sample, the extract and various fractions had the following yields: the petroleum ether fraction (8.6%), Methanolic Crude Extract (41.2%), tannin fraction (10.4%), and the residual fraction (6.5%). The residue and other substances left after the extraction and fractionation processes makes up the remaining 33.3 %. this is also shown in table 1 below

Plant Samples	Yield of Concentrated Sample (g)	Percentage Yield (%)
Pet ether Fraction (PEF)	86	8.6
Methanolic Crude Extract (MCE)	412	41.2
Tannin Fraction (TF)	104	10.4
Residual Fraction (RF)	65	6.5
TOTAL	667	66.7

Table 1. Yields of the Extract and Fractions

3.2 Phytochemical Screening of the plant Samples

The result of the screening tests revealed the presence of carbohydrates, cardiac glycosides, saponins, flavonoids, tannins and alkaloids

CONSTITUENTS	OBSERVATION	Inferences		
		A	B	C
Carbohydrates	A ring of purple colour	+	+	+
	Brick red precipitate of cuprous oxide	+	+	+
Anthraquinones derivatives	No change	-	-	-
Cardiac glycosides	A reddish brown colour gradually turns blue	+	+	-
	A deep red colour, which fades to brownish yellow	+	+	-
Saponins	A honey comb froth persists for about 10- 15 minutes	+	+	+
	presence of haemolysis	+	+	+
	pink colour indicates the presence of triterpenoids	+	+	+
Flavonoids	A pink or red colour	+	+	+
	A yellow solution which becomes colourless in HCl	+	+	+
Tannins	blue coloured precipitate	+	+	+
Alkaloids	Turbidity or precipitation	+	+	-

Table 2. Phytochemical Constituents of the Powdered Stem Bark of *D. microcarpum*

KEY: A = Methanolic Crude Plant Extract (MCE)
 B =Residual Fraction (RF)
 C =Tannins fraction (TF)
 + = Present
 - = Absent

3.3 Antibiotic Susceptibility Testing (AST) using Disc Agar Diffusion (DAD) Technique:

In these studies, virtually all cultured bacterial cells grew indicating a high frequency of occurrence and viability of the cells particularly in vivo as they are clinical isolates. When grown against antibiotic discs, they were generally resistant to amoxicillin and augmentin but showed a high level of susceptibility to nitrofurantoin, gentamicin and ofloxacin. Out of the 7 samples of the E. coli isolates, most of them were resistant to Tetracycline as compared to the 14 samples of S. aureus isolates which had most of them susceptible to it. See tables 2 and 3

S/№ of Test Organisms	Diameter of Zones of Growth Inhibition for each Antibiotic Disc (mm)							
	AMX (25µg)	COT (25µg)	NIT (300µg)	GEN (10µg)	NAL (30µg)	OFL (30µg)	AUG (30µg)	TET (30µg)
1	NZ	NZ	20.0	18	19	18	NZ	NZ
2	NZ	16	11	15	19	NZ	NZ	NZ
3	NZ	NZ	18	NZ	NZ	25	NZ	NZ
4	NZ	NZ	23	NZ	NZ	NZ	NZ	NZ
5	NZ	NZ	25	19	NZ	11	NZ	NZ
6	NZ	NZ	NZ	14	NZ	14	NZ	NZ
7	NZ	NZ	NZ	20	NZ	NZ	NZ	NZ

Table 3. Antibiotic Susceptibility Testing (AST) Using Disc Agar Diffusion (DAD) Technique on E. coli isolates

S/№ of Test Organisms	Diameter of Zones of Growth Inhibition for each Antibiotic Disc (mm)							
	AMX (25µg)	COT (25µg)	NIT (300µg)	GEN (10µg)	NAL (30µg)	OFL (30µg)	AUG (30µg)	TET (30µg)
1	NZ	22	NZ	NZ	NZ	28	NZ	16
2	NZ	6	NZ	17	NZ	24	NZ	7
3	NZ	NZ	28	NZ	17	28	NZ	8
4	NZ	NZ	NZ	NZ	NZ	20	NZ	10
5	NZ	NZ	8	12	NZ	NZ	NZ	NZ
6	NZ	NZ	27	NZ	NZ	30	NZ	10
7	NZ	NZ	NZ	NZ	NZ	NZ	NZ	7
8	NZ	24	26	18	24	32	NZ	12
9	NZ	NZ	15	19	NZ	29	NZ	12
10	NZ	NZ	7	10	NZ	28	NZ	NZ
11	NZ	NZ	25	NZ	NZ	21	NZ	NZ
12	NZ	NZ	15	NZ	NZ	NZ	NZ	NZ
13	NZ	NZ	15	NZ	NZ	NZ	NZ	NZ
14	NZ	NZ	NZ	12	NZ	22	NZ	14

Table 4. Antibiotic Susceptibility Testing (AST) Using Disc Agar Diffusion (DAD) Technique on S. aureus isolates

KEY:

- AMX = Amoxicillin
- COT = Cotrimoxazole
- NIT = Nitrofurantoin
- GEN = Gentamicin
- NAL = Nalidixic acid
- OFL = Ofloxacin
- TET = Tetracycline

NZ = No Zone (Complete Drug resistance)

The zones of growth inhibition had a highest value of 45 mm in one of the *S. aureus* isolates and a lowest value of 6 mm from one of the *E. coli* isolates.

3.4 Antibacterial Activity Using Three Plant Samples and a Standard Drug

In the determination of antibacterial activity using three plant samples and a standard drug on *E. coli* isolates, there were no growth inhibitions for isolates 1 and 3 in both the crude extract and the tannin fraction. However, others had inhibitions ranging from 6-11.5mm. The residual fraction had growth inhibitory effect on all test organisms (23-35mm). There was no growth inhibition at all in the control.

S/№ Organisms	of Zones of Growth Inhibition (mm)			
	A 100mg/ml	B 100mg/ml	C 50µg/ml	Control
1	-	-	23	-
2	17	11.5	31	-
3	-	-	24	-
4	17	17	25	-
5	07	06	35	-
6	16	7.5	32	-
7	19	6	23	-

Table 5. Antibacterial Activity Using Plant Samples and a Standard Drug on *E.coli*

KEY:

- A = Crude Extract
- B = Tannin Fraction
- C = Ciprofloxacin
- = No Zone of Growth inhibition

In the case of *S. aureus* isolates, the 12th isolate showed complete drug resistance against all samples. However there were growth inhibitions in all other isolates ranging from 12-45 mm. Again, there was no growth inhibition at all in the control.

S/№ of Organisms	Zones of Growth Inhibition for Each Antibiotic (mm)			
	A 100 mg/ml	B 100 mg/ml	C 50 µg/ml	Control
1	18.5	25.5	26	-
2	16.5	12	35	-
3	22	26	35	-
4	19	12.5	36	-
5	17	13	24.5	-
6	18	12	15.5	-
7	17	15.5	25	-
8	16	12	32	-
9	19.5	14	35	-
10	14	13	37.5	-
11	20	18	45	-
12	-	-	-	-
13	17.5	12	25	-
14	19	18	38.5	-

Table 6. Antibacterial Activity Using Plant Samples and a Standard Drug on Staph aureus

KEY: A = Crude Extract, B = Tannin Fraction, C = Ciprofloxacin,
 - = No Zone of Growth Inhibition

3.5 MIC and MBC

The MIC and MBC showed activities against some of the bacterial isolates however isolate 5 (an E.coli), had MICs of 5 mg/ml, 50 mg/m and 50 mg/ml for the crude, tannin and Residual Fractions respectively and MBCs of 30 mg/ml, 50 mg/ml and 50 mg/ml for the crude extract, tannin and Residual Fractions respectively. The E. coli 5 showed the highest MBCs of 30 mg/ml, 50 mg/ml and 50 mg/ml for the crude extract, tannin and Residual Fractions respectively and was therefore used for the determination of the rate of kill (Ehinmidu, 2003).

1	5.0	-	50	10.0	-	-
2	2.5	50	30	5.0	-	-
3	5.0	-	50	10.0	-	50
4	2.5	40	10	2.5	-	-
5	5.0	50	50	30.0	50	50
6	10.0	50	30	20.0	50	-
7	10.0	10	10	10.0	-	-
8	2.5	30	10	10.0	50	50
9	2.5	10	30	10.0	50	-
10	5.0	10	30	10.0	-	-
11	10.0	40	10	10.0	-	-
12	2.5	20	10	5.0	-	-
13	5.0	10	20	20.0	-	-
14	5.0	20	10	10.0	50	-
15	5.0	10	20	5.0	50	-
16	10.0	30	20	10.0	-	-
17	0.625	30	20	1.25	50	50
18	0.625	20	10	1.25	-	-
19	1.25	20	10	10.0	-	-
20	0.625	10	10	10.0	-	-

Table 7. MIC and MBC results for Crude, Residual and Tannin Samples

KEY:

MCE = Methanolic Crude Extract

TF = Tannin Fraction

RF = Residual Fraction

- = No Minimum concentration (Growth occurred even at highest concentration of 50mg/ml)

3.6 The Rate of Kill

The rate of kill gave a graph pattern when the log of viable counts was plotted against the time intervals. There was a general gradual onset of kill for the three fractions. The rate of kill became rapid at about 10 minutes for the crude extract, and at about 120 minutes for both the TF and RF. However there was a little rise in the population in the tannin fraction before the crash while that of the MeOH extract had a slightly irregular pattern. The drop in population of the bacterial cells continued until there were no viable counts at later times of the experiment. The shapes of the death curve produced are quite similar to the normal death curve pattern produced by most anti-bacterial drugs (Ehinmidu, 2003).

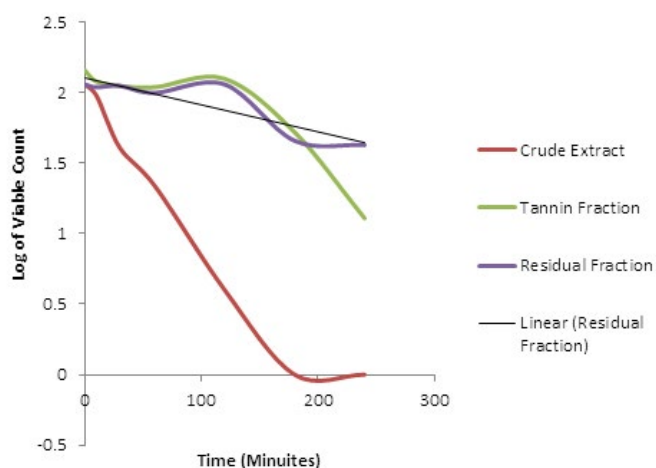


Fig 2. Death/Survival rate of E.coli on exposure to Crude Extract, Tannin Fraction and Residual Fraction of the Stem Bark of D. microcarpum

Discussion

In these studies, the yield of 412 g of the crude extract shows that the solvent used is quite suitable for the extraction of the extract. The value of the fractions being less than this justifies the fact that they are fractions of the powdered sample see (table 1).

The outcome of the phytochemical screening tests is an indication of the possible active therapeutic agents in the plant D. microcarpum eg Tannin-containing drugs has been used traditionally as styptics and internally for the protection of inflamed surfaces of the mouth and throat and as antidiarrheal and as antidotes in poisoning by heavy metals, alkaloids and glycosides. Recent studies have concentrated on the antitumor activity of tannins. Anti-HIV activity has also been demonstrated (Evans, 2008). Others like saponins and flavonoid not only justifies the medicinal uses of the plant but it also confirms the findings of other work done on this plant (Ebi et al, 2011) and also Carbohydrates had been reported (Abdalbasit et al, 2009)

In the susceptibility tests, the total absence of zones of growth inhibition with Amoxycillin and Augmentin is an indication that these strains of organisms have possibly developed total resistance to these two antibiotics. The E. coli isolates were also all resistant to Tetracycline . thus, this may not be a suitable antibiotic in the treatment of diseases associated with E. coli. The isolates showed a high level of susceptibility to nitrofurantoin, gentamicin and ofloxacin. Out of the 7 samples of the E. coli isolates, most of them were resistant to Tetracycline as compared to the 14 samples of S. aureus isolates which had most of them susceptible to it. Tetracycline should therefore be better used against S. aureus than the E. coli. A multiple comparisons of the activity of the antibiotics, where only two antibiotics are compared at a time, showed a significant difference. ANOVA test performed at 0.05 shows that there is generally a significant difference in their activity.

In the antibacterial activity of the plant extracts and the standard drugs, the statistical analysis showed that there was a significant difference between the standard drug and the samples (MCE, RF and TF) used. However the difference between any of the two samples was not significant. Also, for the well diffusion technique using only the plant extracts, only the multiple comparisons between the crude plant extract and the methanolic extract showed a significant difference. The difference between the other two fractions (Tannins and methanol) was not significant.

The MIC and MBC showing activities against most of the bacterial isolates supports the claims of the medicinal uses of the plant. The crude extract has an MBC of 1.25mg/ml for isolates 17 and 18, 2.5mg/ml for isolate 4 and 5mg/ml for isolates 2, 12 and 15. The plant has shown anti-bacterial (bactericidal) activity. Thus supporting and establishing that the plant, D. microcarpum has antimicrobial activity (Kouyaté, 2006).

In the rate of kill, The rapidity and intensity of this kill is a proof of the bactericidal action of this plant. The little rise in the population in the tannin fraction before the crash suggests a possible synergistic action with some other compounds for complete action.

Conclusion

In conclusion, the isolated organisms in this study and their sensitivity profile have great implication on the health status of individuals with infection related to the organisms. Some of the isolates showed resistance to one or two of the antibiotics tested. This is indeed worrisome.

References

- 1.Abdalbasit, A. M. and Mohamed E. S. (2009). *Detarium microcarpum* Guill and Perr. fruit proximate chemical analysis and sensory characteristics of concentrated juice and jam. *African Journal of Biotechnology* Vol. 8 (17), pp. 4217-4221.
- 2.Birdi, T. J., Brijesh S. and Daswani P. G. (2008). *Approaches Towards the Preclinical Testing and Standardization of Medicinal Plants*. Foundation for Medical research, india.

3. Canell, Richard J.P. (1998). Natural Products Isolation Glaxo Wellcome Research and Development, Stevenage, Herts, UK. Humana Press Totowa, New Jersey pp 349-352.
4. Ebi. G. C. and Afieroho O. E.. (2011). [Phytochemical and antimicrobial studies on Detarium microcarpum Guill and Sperr \(Caesalpinioceae\) seeds coat](#) African Journal of Biotechnology Vol. 10(3), pp. 457-462,
5. Ehinmidu, J. O. (2003). [In-vitro antifungal activity of single and combined doses of some antifungal agents against clinical Candida albicans isolates in Zaria, Nigeria.](#) Nigerian Journal of Experimental and Applied Biology. 5(2) 127-131.
6. Evans, W. C. (2008). [Trease and Evans pharmacognosy. 15th Edition. Rajkamal Electric press, Delhi, India pp. 516 - 536.](#)
7. Hutchinson, j. and Daziel, J. N. (1966) Flora of Tropical West Africa. First Edition, Vol.1, part 1 Crown Agents for Overseas Government And Administrations, millbank, London. pp. 457
8. Kar, A. (2007). Pharmacognosy and Pharmacobiotechnology (Revised-Expanded Second Edition): New Age International Publishers Limited. pp ix & 1c
9. Kouyaté, A.M. and van Damme, P. (2006). Detarium microcarpum Guill. & Perr. In: Schmelzer, G.H. & Gurib-Fakim, A. (Editors). Prota 11(1): Medicinal plants/Plantes médicinales 1. [CD-Rom]. PROTA, Wageningen, Netherlands. Retrieved April 06, 2011 from: <http://www.prota.org>
10. Nkunya, M.H.H. (1994). Unusual Metabolites From Tanzanian Annonaceous Plants: the genus Uvaria. Department of Chemistry, University of Dar es Salaam, P.O.Box 35061 Dar es Salaam, Tanzania. Pp. 268-270
11. Prescott, H (2002). [Laboratory Exercises in Microbiology. Fifth edition. The McGraw-hill Companies, pp 154](#)
12. Prescott, L. M., John, P. H. and Donald. A. K. (2005). General Microbeic Fifth edition. McGraw-Hill Newton. Pp 5 & 282.