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Full Length Research Paper

Assessment of antimicrobial activity of crude extracts of stem and root barks from *Adansonia digitata* (Bombacaceae) (African baobab)

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This study seeks to validate the antimicrobial activity of crude extracts of stem and root barks from *Adansonia digitata* (Bombacaceae), investigate the minimum inhibitory concentrations (MICs) of the extracts and bioactive constituents present in the barks. Crude ethanolic and aqueous extracts of stem and root barks were evaluated for antimicrobial activities using agar-well method. Bioactive constituents were investigated using standard procedures for phytochemical analysis. The MICs were investigated using broth micro dilution method. The results showed that the extracts had significant antibacterial activity. The MICs of the plant extracts ranged from 6 to 1.5 mg/ml. Tannins, phlobatannins, terpenoids, cardiac glycosides and saponins were found to be present in the stem bark. Terpenoids only were found in the root bark. It is concluded that stem and root barks of *A. digitata* contain bioactive constituents which are responsible for antimicrobial activity of the crude extracts.

Key words: Antimicrobial activity, bioactive constituents, *Adansonia digitata*, minimum inhibitory concentrations.

INTRODUCTION

A wide range of benefits to man, from trees and shrubs amongst others include medicine and foods (Mbuya et al., 1994; Davis et al., 1995; Edeoga et al., 2005; Kala, 2007; Sharief and Rao, 2007). A good number of trees and shrubs have been claimed by ethnoveterinary practitioners, ethnomedical practitioners and other local people to have medicinal benefits against infectious and/or non infectious animal and/or human diseases. Examples of animal diseases are newcastle disease, fowl cholera, fowl pox (Guèye, 1999), trypanosomiasis (Atawodi et al., 2002), coccidiosis and helminthosis (Saimo et al., 2003). Examples of human diseases reported to be managed using ethnomedicine include malaria, menstrual disorders, wound, asthma, kidney stones, eczema, diabetes and blood pressure (Ananil et al., 2000). Some of medicinal plants include *Acacia albida (Faidherbia albida), Acokanthera schimperi, Adansonia digitata* (Baobab)*, Afzelia quanzensis, Albizia gummifera, Albizia schimperiana subsp. schimperiana, Anacardium occidentale, Annona senegalensis (A. chrysophylla), Artocarpus heterophyllus (A. integrifolius), Balanites aegyptica, Bauhinia petersiana, Berchemia discolor, Bersama abyssinica, Terminalia brownie, Vangueria madascariensis (V. acutiloba), Warburgia ugandensis (W. salutaris), Ximenia americana, Zanthoxylum chalybeum* and *Ziziphus mucronata* (Mbuya et al., 1994). Phytochemical studies have shown that plants with antimicrobial activity contain bioactive constituents such as tannins, flavonoids, alkaloids and saponins (Dall'Agnol et al., 2003; Ogunleye and Ibitoye, 2003; Edeoga et al., 2005; Latha and Kannabiran, 2006; Awoyinka et al., 2007; Biradar et al.,

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Abbreviations: ARBE, Aqueous root bark extract; **ASBE,** aqueous stem bark extract; **ERBE,** ethanolic root bark extract; **ESBE,** ethanolic stem bark extract; **FVM,** Faculty of Veterinary Medicine; **MHA,** Mueller-Hinton agar; **MICs,** minimum inhibitory concentrations; **NB,** nutrient broth; **SDA,** Sabouraud dextrose agar; **SUA,** Sokoine university of agriculture.

2007).

A. digitata is among many plant species which have been reported to have ethnomedicinal uses and has been widely used in the traditional systems of medicine. According to the UN (2005) the fruit pulp of *A. digitata* is traditionally used for the treatment of fever, diarrhea, dysentery, haemoptysis and small pox in humans. Leaf infusions are used as treatment for diarrhea, fever, inflammation, kidney and bladder diseases, blood clearing and asthma in humans. The bark is used for treatment of fever, especially that caused by malaria. As far as ethnoveterinary medicine is concerned, reports indicate that bark of *A. digitata* is used for treatment of diarrhoea in poultry (Guèye, 1999) and fruits are used for treatment of Newcastle disease in poultry (Guèye, 1999; Wynn and Fougère, 2006).

Some work has been done to validate the efficacy of *A. digitata* and identify its bioactive constituents. For example experiments performed on rats showed that administration of fruit pulp extract at a dose between 400 and 800 mg/ml had a marked anti-inflammatory effect and reduce formalin-induced oedema in the animals. These effects are comparable with those produced by 15 mg/ml of phenylbutazone, a common anti-inflammatory drug used as an internal standard (UN, 2005). Equally, extracts from *A. digitata* fruit pulp have analgesic (pain killing) and antipyretic (temperature reducing) activities. This activity could be due to be presence of sterols, saponins and triterpenes in the fruit pulp (UN, 2005). Moreover, a study conducted on 160 children demonstrated that an aqueous solution of *A. digitata* fruit is almost as significant as the traditional "WHO solution" for dehydration of children affected with diarrhea. Tannins, mucilage, cellulose, citric acid and other typical constituents of the fruit pulp may be responsible for its effect against diarrhoea (UN, 2005). According to Ananil et al. (2000) methanolic extract of root bark has antiviral activity against herpes simplex virus, polio virus and sindbis virus while methanolic extract from leaves showed antiviral activity against herpes simplex virus and sindbis virus. Results of antibiotic studies show that methanolic extract of *A. digitata* root bark or leaves has antibacterial activity against *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis*, *Escherichia coli* and *Mycobacterium phlei* (Ananil et al., 2000). Methanolic extract from root of *A. digitata* has also been reported to have antitrypanosomal activity against *Trypanosoma congolense* and *T. brucei brucei* (Atawodi et al., 2003).

However, it appears that no study has been conducted to investigate bioactive constituents present in the stem and root barks of *A. digitata* and antimicrobial activity of crude aqueous and ethanolic extracts from stem and root barks of *A. digitata*. The present study investigated the bioactive constituents present in the extracts of stem and root barks from *A. digitata* and the antimicrobial activity of the extracts.

The main objective of this study was to validate the antimicrobial activity of crude extracts from stem and root barks of *A. digitata* against some selected species of bacteria and fungi of veterinary and/or public health importance. The specific objectives were to investigate the spectrum of activity of the crude extracts of stem and root barks of *A. digitata* against bacteria, to investigate the MICs of crude extracts of stem and root barks of *A. digitata*, to compare the antimicrobial activity of the crude extracts of stem and root barks of *A. digitata* to that of selected standard antibiotic or antifungal agent, and to investigate bioactive constituents in the stem and root barks of *A. digitata*.

MATERIALS AND METHODS

Study location

Field work (collection of stem and root barks, reduction of the barks to small chips and air-drying of the barks chips) was conducted at Mpwapwa, in the semi arid central zone of Tanzania. Laboratory work was carried out at the Faculty of Veterinary Medicine (Fvm), Sokoine University of Agriculture (SUA), Morogoro, Tanzania and the Institute of Traditional Medicine of Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania.

Collection of stem and root barks

Stem and root barks were collected from *A. digitata*, previously botanically identified by a botanist working at the National Livestock Research Institute, Mpwapwa, Tanzania, in collaboration with the authors of this paper. Stem barks were collected during the dry season (in September 2007) by debarking part of the stem using an axe. Root barks were collected towards the end of the rain season (in April 2008) using a hoe and an axe. The collected plant materials were cleaned, chopped into small chips using a bush knife then air-dried under shade for 20 days.

Preparation of crude stem and root barks powder

The dry chips of stem and root barks were powdered at the department of animal science and production, SUA, Tanzania using a Lab mill (Christy Hunt Engineering Ltd, England).

Preparation of the plant extracts

Ethanolic root bark extract (ERBE) and ethanolic stem bark extract (ESBE) were prepared by soaking 250 g of root bark powder and 200 g of stem bark powder in 80% ethanol, respectively, for 24 h. Thereafter each mixture was filtered and the filtrate was concentrated using a rotavapor (BÜCHI Labortechnik AG, Switzerland). Then the extracts were freeze dried using a freeze drier (Edwards High Vacuum International, England) leading to collection of 3 and 4 g of dry ERBE and ESBE, respectively. The extracts were kept in closed containers and stored in the refrigerator at 4ºC until required. The basis for the choice of ethanol as an extraction solvent was a pilot study which was carried out in advance and showed that 80% ethanol if mixed with powdered stem or root bark sample yield crude extracts with antimicrobial activity.

The aqueous stem bark extract (ASBE) or aqueous root bark extract (ARBE) was prepared by soaking 75 g of powdered stem or root bark sample in 525 ml of hot water which was initially at 100ºC

but was later left to cool to room temperature of 25 - 27ºC gradually. The basis for the choice of the above volume of the solvent and weight of the powdered plant sample was a pilot study conducted in advance to find out the volume of hot water and amount (in grams) of powdered stem or root bark sample that, when mixed, yield crude aqueous extracts with antimicrobial activity. Each extraction process was done in duplicate and the process continued for 42 h. After 42 h the mixtures were filtered using Whatman filter paper No 1 (Whatman International Ltd, England). The filtrates obtained were 200 ml of a clear orange stem bark extract and 160 ml of a clear light-orange root bark extract. Each filtrate was concentrated using a rotavapor (BÜCHI Labortechnik AG, Switzerland). Then the extracts were freeze dried using a freeze drier (Edwards High Vacuum International, England) leading to collection of 2 and 1.92 g of dry ASBE and ARBE, respectively. The extracts were kept in closed containers and stored in the refrigerator at 4ºC until required. Hot water was chosen as an extraction solvent basing on a pilot study conducted in advance and the fact that hot water is often used by traditional healers as a solvent in the traditional systems of medicine.

Preparation of stock extract solutions

Stock solutions of ASBE and ARBE (at a concentration of 480 mg/ml) were prepared by dissolving 1920 mg of each extract in a vial containing 4 ml of hot (60ºC) distilled water. Stock solutions of ESBE and ERBE were prepared by dissolving 1920 mg of each extract in a vial containing 4 ml of dimethyl sulfoxide (SIGMA-ALD RICH®, USA). The basis for the choice of the above dose (concentration) for this investigation was a pilot (*in vitro*) study which was carried out in advance on the test microorganisms.

Test microorganisms

Selected species of fungi and bacteria of veterinary and/or public health importance used in the antimicrobial assay were *Escherichia coli*, *Salmonella* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus anthracis, Candida albicans* and *Mucor* sp. These organisms were clinical isolates obtained from the Microbiology Laboratory, Department of Veterinary Microbiology and Parasitology, FVM, SUA, Tanzania.

Growth media

Mueller-Hinton Agar (MHA) (Merck, Postfach, Darmstadt FR, Germany), nutrient broth (NB) (HiMedia Laboratories Limited, Mumbai, India) and Sabouraud dextrose agar (SDA) (HiMedia Laboratories Pvt. Ltd, Mumbai, India) were used. The media were prepared according to the manufacturers' instructions.

Microbial sensitivity test against extracts of stem and root barks

The bacteria and fungi were grown in the MHA and SDA, respectively. The extracts were tested for activity against bacteria and fungi using agar-well diffusion method using procedures described by Biradar et al. (2007) except that the microorganisms were inoculated in their respective agar by streaking using a sterile wire loop; 45 µl of each extract was dispensed in each well (8 mm diameter). The plates inoculated with *Mucor* sp. were kept at room temperature (25-27ºC) for 5 days. Then the zone of inhibition around each well was measured using a transparent ruler and recorded. Each test was conducted in triplicate.

Determination of MICs of the crude plant extracts and a standard antibiotic, gentamicin (100 µg/ml)

Each stock extract solution was diluted ten times by adding 90 ml of NB in a vial containing 10 ml of the extract to make a 48 mg/ml solution. The MICs of the plant extracts and gentamicin against the microorganisms which were sensitive to the plant extracts were determined by using broth micro-dilutiom method. 50 µl of NB was put into each well (in rows A, B, C, D, E and F) in a plate using a micro pipette. In the first wells of rows A and B 50 µl of gentamicin was added. In each row a serial dilution was made from the first to the last (12th) well, the last 50 µl was discarded. Then 50 µl of a test organism was added in each well (only one type of organism was tested per plate). This served as a positive control. The concentration of gentamicin in the first wells of rows A and B was reduced from 100 to 25 µg/ml after the dilution by NB and the test organisms.

In the first wells of rows C and D 50 µl of a plant extract (ASBE, ARBE, ESBE or ERBE) was added. In each row a serial dilution was made from the first to the last (twelveth) well; the last 50 µl was discarded. Then 50 µl of test organism was added in each well. The concentration of each extract in the first wells of rows C and D was reduced from 48 to 12 mg/ml after the dilution by NB and the test organisms.

In the first wells of rows E and F 50 µl of test organism was added in each well (neither gentamicin nor crude plant extract was added). This served as a negative control.

Thereafter the plates were incubated at 37ºC for 24 h. 2 h before reading the results 25 µl of the indicator, 0.2% Iodonitrotetrazolium chloride (SIGMA-ALDRICH® Co, USA) was added in each well of row A, B, C, D, E and F. Then the plates were incubated for 2 h. The concentration of the last well without colour change (that is, the well next to a well with violet colouration) in a row was recorded as the minimum inhibitory concentration of a tested plant extract or the standard antibiotic, gentamicin (100 µg/ml), against the test microorganism.

Microbial sensitivity test against standard antibiotics

The bacteria which showed sensitivity to the plant extracts were tested for sensitivity against gentamicin (10 µg) and nitrofurantoin (300 µg) (Span Diagnostics Ltd, India) using disc diffusion method as described by Biradar et al. (2007). The diameter of each paper disc used was 7 mm.

Phytochemical screening

The presence of bioactive constituents particularly tannins, phlobatannins, saponin, flavonoids, steroids, terpenoids and cardiac glycosides in the stem and root barks of *A. digitata* was investigated by using standard procedures described by Edeoga et al. (2005).

Data analysis

Data (diameter of inhibition zones) were analyzed using STATVIEW (SAS Institute Inc. Copyright© 1992 - 1998, Version 5.0.1) statistical software and 0.05 level of significance.

RESULTS

Antimicrobial activity of the plant extracts

Both ASBE and ARBE showed significant antimicrobial activity against *B. anthracis*, *B. subtilis* and *S. aureus*.

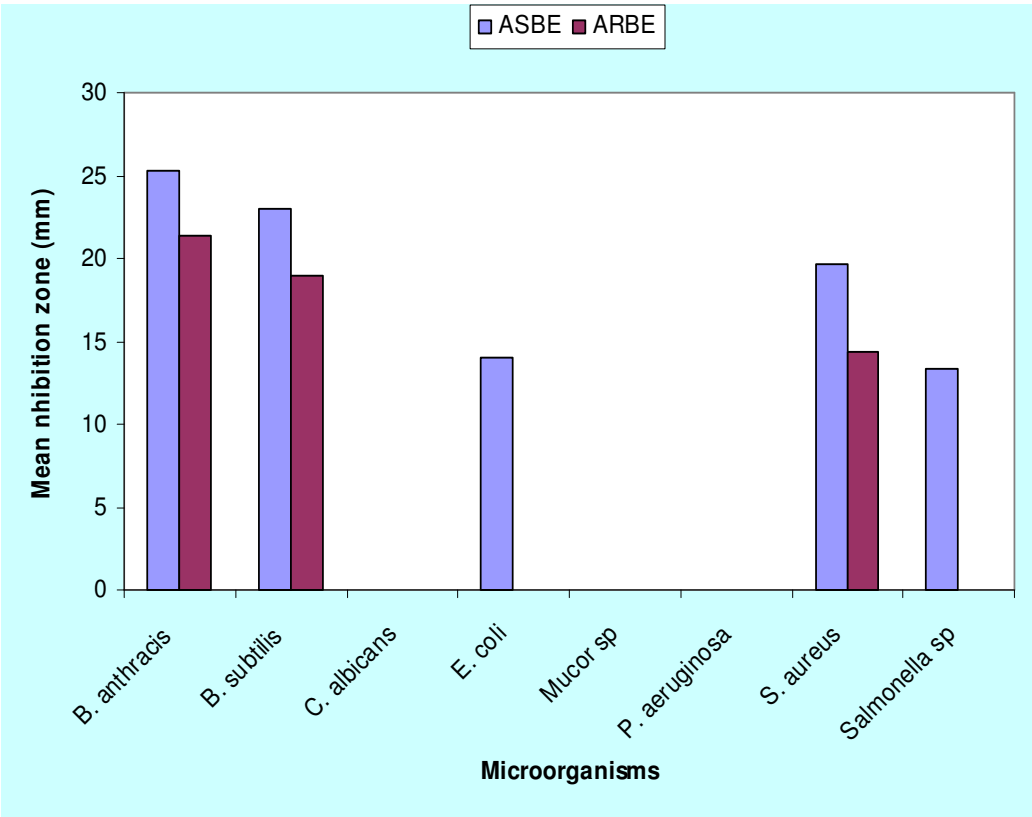

Figure 1. Mean diameter of inhibition zones after *in vitro* exposure of microorganisms to 45 µl of ASBE or ARBE for 24 h. Each inhibition zone includes the diameter of a well (8 mm) in which the extract was dispensed.

ASBE was also active against *E. coli* and *Salmonella* sp. which were resistant against ARBE. Both ERBE and ESBE showed activity against *B. subtilis*. ESBE showed activity against *E. coli* and *S. aureus* which were resistant to ERBE. The antimicrobial activity was indicated by a clear zone of inhibition around a well where the crude extracts were dispensed. Both aqueous and ethanolic stem and root bark extracts did not show any antimicrobial activity against *P. aeruginosa* and *C. albicans*.

The mean diameters of inhibition zones due to exposure of the microorganisms to the aqueous extracts and ethanolic extracts are indicated in Figures 1 and 2, respectively.

MICs of the crude plant extracts and the standard antibiotic, gentamicin (100 µg)

The MICs of the crude ASBE, ARBE, ESBE, ERBE and the standard antibiotic are indicated in Tables 1, 2, 3, 4 and 5, respectively.

Microbial sensitivity to standard antibiotics

Figure 3 shows mean diameter of inhibition zones due to exposure of microorganisms to gentamicin (10 µg) and nitrofurantoin (300 µg).

Phytochemical screening

Phytochemical analysis indicated the presence of tannins, phlobatannins, terpenoids, cardiac glycosides and saponins in the stem bark powdered sample or aqueous extract as well as the presence of terpenoids in the aqueous extract of root bark. Flavonoids and steroids were absent both on stem and root bark powdered samples or aqueous extracts. Tannins, phlobatannins, saponin and cardiac glycosides were also absent in the root bark powdered sample or aqueous extract.

DISCUSSION

In this study, results of phytochemical analysis indicate that of the screened bioactive constituents terpenoids only was found to be present in the root barks of *A. digitata* while terpenoids, tannins, phlobatannins, saponins and cardiac glycosides were found to be present in the stem barks.

The antimicrobial activity of crude stem bark extract

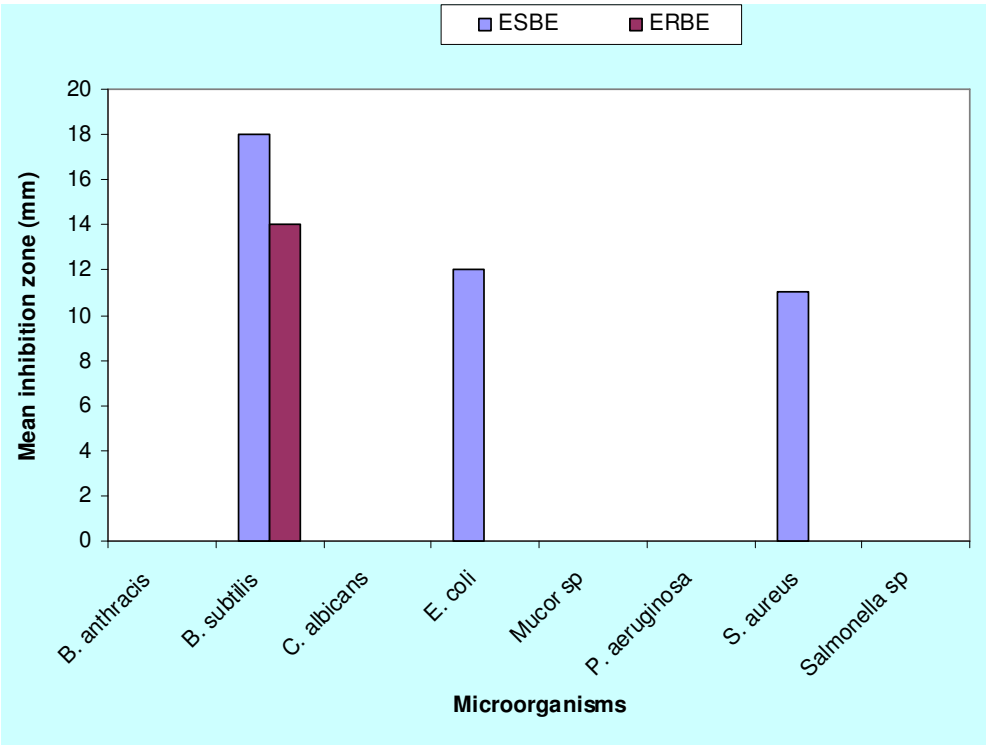

Figure 2. Mean diameter of inhibition zones after *in vitro* exposure of microorganisms to 45 µl of ESBE or ERBE for 24 h. Each inhibition zone includes the diameter of a well (8 mm) in which the extract was dispensed.

Table 1. MICs of the ASBE against the sensitive microorganisms.

Microorganism	Concentration of ASBE (mg/ml)							Inference
	12	6	3	1.5	0.75	0.375	0.1875	
B. anthracis	+	+	+	+	-	-	-	Sensitive at 1.5 mg/ml
B. subtilis	+	+	+	+	-	-	-	Sensitive at 1.5 mg/ml
E. coli	+	+	+	-	-	-	-	Sensitive at 3 mg/ml
S. aureus	+	+	+	-	-	-	-	Sensitive at 3 mg/ml
Salmonella sp.	+	+	+	-	-	-	-	Sensitive at 3 mg/ml

+ = Microbial growth inhibited; - = no inhibition of microbial growth.

Table 2. MICs of the ARBE against the sensitive microorganisms.

Microorganism	Concentration of ARBE (mg/ml)							Inference
	12	6	3	1.5	0.75	0.375	0.1875	
B. anthracis	+	+	+	-	-	-	-	Sensitive at 3 mg/ml
B. subtilis	+	+	+	-	-	-	-	Sensitive at 3 mg/ml
S. aureus	+	+	+	-	-	-	-	Sensitive at 3 mg/ml

+ = Microbial growth inhibited; - = no inhibition of microbial growth.

against bacteria could be attributed to the presence of tannins, phlobatannins, terpenoids and saponins in the stem bark. The attributing factor for antibacterial activity of crude root bark extract could be the presence of terpenoids in the root bark. These bioactive constituents are associated with the antibacterial activity of the crude ex-

Table 3. MICs of the ESBE against the sensitive microorganisms.

Microorganism	Concentration of ESBE (mg/ml)							Inference
	12	6	3	1.5	0.75	0.375	0.1875	
B. subtilis	+	+	+	+	-	-	-	Sensitive at 1.5 mg/ml
E. coli	+	+	+	-	-	-	-	Sensitive at 3 mg/ml
S. aureus	+	+	-	-	-	-	-	Sensitive at 6 mg/ml

+ = Microbial growth inhibited; - = no inhibition of microbial growth.

Table 4. MIC of the ERBE against the sensitive microorganism.

Microorganism	Concentration of ERBE (mg/ml)							Inference
	12	6	3	1.5	0.75	0.375	0.1875	
B. subtilis	+	+	-	-	-	-	-	Sensitive at 6 mg/ml

+ = Microbial growth inhibited; - = no inhibition of microbial growth.

Table 5. MICs of gentamicin against the sensitive microorganisms.

Microorganism	Concentration of gentamicin (µg/ml)							Inference
	25	12.5	6.25	3.125	1.56	0.78	0.39	
B. anthracis	+	+	+	+	-	-	-	Sensitive at 3.125 µg/ml
B. subtilis	+	+	+	+	-	-	-	Sensitive at 3.125 µg/ml
E. coli	+	+	-	-	-	-	-	Sensitive at 12.5 µg/ml
Salmonella sp.	+	+	+	+	-	-	-	Sensitive at 3.125 µg/ml
S. aureus	+	+	+	+	+	+	-	Sensitive at 0.78 µg/ml

+ = Microbial growth inhibited; - = no inhibition of microbial growth.

tracts based on the fact that certain types of tannins, terpenoids, saponins and phlobatannins have been previously reported to be responsible for antimicrobial activities of crude plant extracts (Ogundare et al., 2006). Furthermore, studies have confirmed that certain types of purified tannins, saponins and terpenoids have antimicrobial activity: Ikigai et al. (1993) reported that tannins, particularly epicatechin or epigallocatechin gallate, are effective against both *S. aureus* and *E. coli*. Other findings (Ho et al., 2001) indicate that epicatechin-($4^{\beta\rightarrow}8$)-epicatechin-($4^{\beta\rightarrow}8$, $2^{\beta\rightarrow}0^{\rightarrow}7$)-catechin extracted from *Vaccinium vitis-idaea* L has strong antimicrobial activity against *Porphyromonas gingivalis* and *Prevotella intermedia*. According to Lim et al. (2006) hydrolysable tannin extracted from barks of *Rhizophora apiculata* has significant antibacterial and anti-yeast activities.

The antimicrobial activity of saponin, crude saponin extracted from *Sorghum bicolor* L has been reported to be effective *in vitro* against *S. aureus* (Soetan et al., 2006). Khanna and Kannabiran (2008) reported saponin fractions of leaves of *Gymnema sylvestre* and *Eclipta prostrata* to have antimicrobial activity against *P. aeruginosa*, *E. coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Proteus mirabilis* (gram-negative bacteria), *S. aureus* (gram-positive bacteria), *Aspergillus fumigatus*, *A. niger* and *A. flavus* (fungi).

As far as terpenoids are concerned, Villarreal et al. (1994) reported antimicrobial activity of a terpenoids (taraxasterol) extracted from *Asteraceae* species against *S. aureus*. Other reports (Roberto et al., 2004) indicate that a terpenoid, 2-epi-jatrogrossidione (a rhamnofolane diterpine), extracted from roots of *Jatropha gaumeri*, has significant activity against *B. subtilis* ATCC-6633.

With regard to the spectrum of antibacterial activity, results show that ASBE and ESBE have a broad-spectrum of activity. This could be due to the presence of tannins and saponins which have been reported to have broad spectrum antibacterial activity, Ikigai et al. (1993) and Khanna and Kannabiran (2008), respectively. On the other hand ARBE and ERBE showed a narrow spectrum of activity, the antimicrobial activity being restricted against gram-positive bacteria only. The narrow spectrum of activity of root bark extracts could be attributed to the presence of terpenoids in the root barks of *A. digitata*, terpenoids have been reported to be effective against gram-positive bacteria (Villarreal et al., 1994; Roberto et al., 2004).

As far as the degree of susceptibility is concerned results (diameter of inhibition zone) clearly indicate that gram-positive bacteria are more susceptible to aqueous

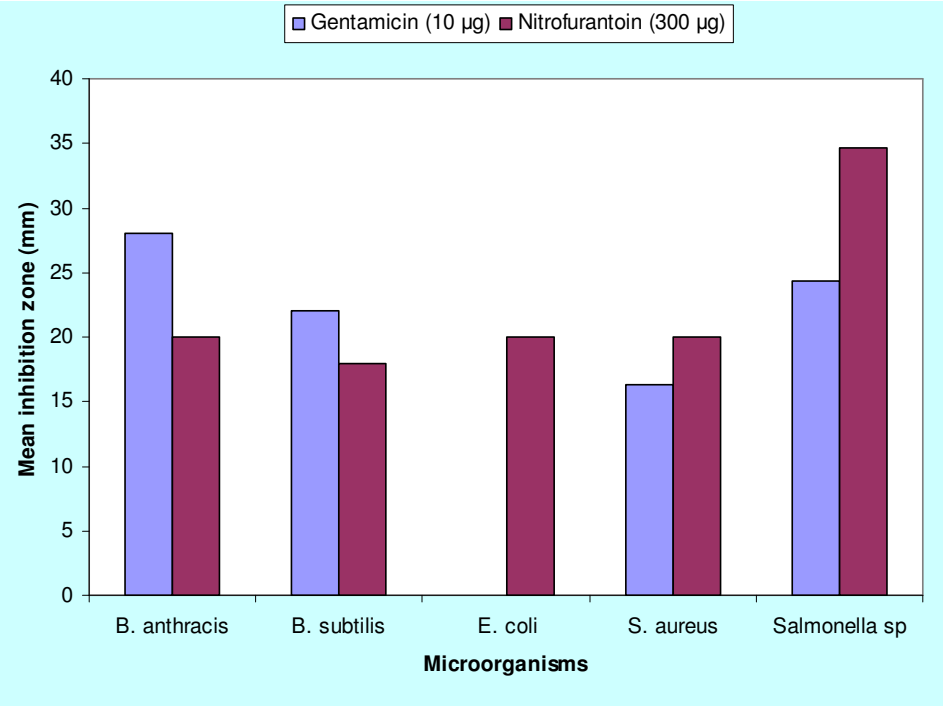

Figure 3. Mean inhibition zone following exposure of microorganisms to gentamicin (10 µg) and nitrofurantoin (300 µg). Each inhibition zone includes 7 mm diameter of the disc.

stem or root bark extract as compared to gram-negative bacteria and fungi. Among the sensitive test microorganisms a wide inhibition zone was recorded against *B. anthracis* implying that ASBE and ARBE are more effective against *B. anthracis* as compared to other sensitive tested microorganisms. A narrow inhibition zone was recorded against *Salmonella* sp implying that the activity of ASBE and ARBE against *Salmonella* sp. is low as compared to other sensitive tested microorganisms.

Among the tested gram-positive bacteria only *B. subtilis* was susceptible to ERBE. These findings suggest that the susceptibility of *B. subtilis* to terpenoids could be higher as compared to that of other gram-positive bacteria such as *S. aureus*. Regarding the antimicrobial activity of ESBE, a wide inhibition zone was recorded against *B. subtilis*.

Although results of phytochemical analysis indicated that the stem barks of *A. digitata* contain tannins and saponins which, according to Lim et al. (2006) and Khanna and Kannabiran (2008) have antifungal/antiyeast activity; in this study both ASBE and ESBE did not show atifungal/antiyeast activity. The explanation for this disagreement is that probably the types tannins and saponins present in the stem barks of *A. digitata* do not have antifungal activity.

When compared to the activity of a standard antibiotic, gentamicin (10 µg), against the tested bacteria an interesting finding is the activity of crude ASBE and ESBE against *E. coli*, a bacterial isolate which is resistant against the standard broad-spectrum antibiotic, gentamicin (10 µg).

Conclusion

Based on the findings of this study it is concluded that stem and root barks of *A. digitata* contain bioactive constituents which are responsible for antimicrobial activity of the crude aqueous and ethanolic extracts from stem or root barks of *A. digitata*. This explains the scientific basis for the use crude stem and/or root bark extracts in the traditional system of medicine for treatment of diseases such as white bacillary diarrhea (Pullorum disease) in poultry (Guèye, 1999) and symptoms of diseases such as fever especially that caused by malaria in humans (UN, 2005) and diarrhoea in poultry due to Newcastle disease (Wynn and Fougère, 2006).

However, toxicological analysis of the crude extracts is recommended in order to assess their safety in the body of a patient when administered. Further studies are also recommended in order to isolate and identify each bioactive constituent present in the stem and/or root barks of *A. digitata*.

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