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Studies on the biocidal and cell membrane disruption potentials of stem bark extracts of *Afzelia africana* (Smith)

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ABSTRACT

We had recently reported antibacterial activity in the crude extract of the stem bark of *Afzelia africana* (Akinpelu et al., 2008). In this study, we assessed the biocidal and cell membrane disruption potentials of fractions obtained from the crude extract of the plant. The aqueous (AQ) and butanol (BL) fractions exhibited appreciable antibacterial activities against the test bacteria. The minimum inhibitory concentrations of the AQ and BL fractions ranged between 0.313 and 2.5 mg/ml, while their minimum bactericidal concentrations varied between 0.625 and 5.0 mg/ml. Also, the AQ fraction killed about 95.8% of *E. coli* cells within 105 min at a concentration of 5 mg/ml, while about 99.1% of *Bacillus pumilus* cells were killed by this fraction at the same concentration and exposure time. A similar trend was observed for the BL fraction. At a concentration of 5 mg/ml, the butanol fraction leaked 9.8 µg/ml of proteins from *E. coli* cells within 3 h, while the aqueous fraction leaked 6.5 µg/ml of proteins from the same organisms at the same concentration and exposure time. We propose that the stem bark of *Afzelia africana* is a potential source of bioactive compounds of importance to the pharmaceutical industry.

Key terms: *Afzelia africana*, antibacteria, phytochemistry, killing rate, protein leakage.

INTRODUCTION

The prevalence of multiple antibiotics resistance developed by microorganisms against the available synthetic antibiotics has increased astronomically in the last decade. This has prompted tremendous effort to explore for more potent antimicrobial agents, especially of natural origin to combat this development. Literature reports and ethnobotanical records suggest that plants have tremendous potentials in the pharmaceutical industry as important sources of new compounds for antimicrobial drugs syntheses.

Afzelia africana belongs to the family Caesalpiniaceae. The English name of the plant is mahogany. The tree is widely distributed in Africa and Asia (Keay, 1989), where it is used as food and for plank wood, as well as widely used as folklore remedies among many tribes in Africa. Previous studies have reported the plant to exhibit anti-inflammatory and analgesic bioactivities (Akah *et al*., 2007). Atawodi (2005) reported trypanocidal activities of the leaves and stem bark extract of the plant against *Trypanosoma brucei*. Powdered root of the plant mixed with millet beer has been used as treatment for hernia among some tribes in Cote d'Ivoire (Dalziel, 1937).

In this paper, we report the biocidal regimes of the fractions obtained from the crude extract of the stem bark of *A. africana,* as well as their potential for cell membrane disruption in our attempt to explore for new bioactive molecules of antimicrobial importance.

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METHODS

Plant materials

Fresh stem bark of *A. africana* used in this work was collected in Abeokuta, Nigeria in the month of April 2008. The stem bark was air-dried to constant weight, powdered and stored in an air-tight container for further use.

Preparation of extract

Exactly 750 g of the powdered bark of *A. africana* was extracted in cold using methanol and sterile distilled water in a 3: 2 ratio for 4 days. The mixture was then filtered and the filtrate was first concentrated *in vacuo* using rotary evaporator to remove the organic solvent. The remaining aqueous residue was lyophilized to obtain the crude extract. The extract was brown in colour and the yield collected was 105 g.

Preparation of microorganisms for the experiment

The following test bacteria obtained from the culture collection of the Applied and Environmental Microbiology Research Group (AEMREG), Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa were used for this study: *Escherichia coli* (ATCC 8739), *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 19582), *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 10702), *B. pumilus* (ATCC 14884), *Pseudomonas aeruginosa* (ATCC 7700), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC 10031), *K..pneumoniae* (ATCC 4352), *Proteus vulgaris* (ATCC 6830), *P. vulgaris* (CSIR 0030), *Serratia marcescens* (ATCC 9986), *Acinetobacter calcaoceuticus* (UP), *Acinetobacter calcoaceticus anitratus* (CSIR), *K. pneumoniae* (LIO), *Bacillus subtilis* (LIO) *Shigella dysenteriae* (LIO), *Staphylococcus epidermidis* (LIO), *Pseudomonas aeruginosa* (LIO), *P. vulgaris* (LIO), *Enterococcus faecalis* (LIO), *Staphylococcus aureus* (LIO) *Micrococcus kristinae* (LIO) and *Kocuria rhizophila* (LIO). The bacterial isolates were first sub-cultured in nutrient broth (Oxoid Ltd.) and incubated at 37 ^{o}C for 18-h before use.

Fractionation of the crude extract of A. africana with organic solvents of different polarities

The crude extract was successfully partitioned using organic solvents in order of their polarity, that is, n-hexane, chloroform, diethyl ether and butanol. Exactly 20 g of the crude extract was resolved in 100 ml of sterile distilled water in a 500 ml separatory funnel. This was allowed to dissolve properly and about 100 ml of n-hexane was added to the mixture for the extraction. The funnel was shaken vigorously and allowed to stand for 15 min to allow for the separation of the organic and aqueous layers. The n-hexane layer was thereafter separated from the aqueous layer and collected in a clean flask. This process was repeated thrice until the aqueous layer became colourless. The n-hexane fractions collected were bulked and concentrated to dryness *in vacuo* using a rotary evaporator and kept in the freezer for further use. The remaining aqueous layer was re-concentrated to eliminate the residual n-hexane and then lyophilized. The same procedure was performed sequentially with the chloroform, diethyl ether and butanol solvents.

Antibacterial Sensitivity Tests

The antibacterial activities of the fractions from crude extract of *A. africana* stem bark were determined in accordance with the agar-well diffusion method described by Russell and Furr (1977); and Irobi *et al.* (1994). The bacterial isolates were first grown in nutrient broth for 18-h and later standardized to OD_{600nm}0.1 before use. One hundred microlitre of the standardized bacterial suspension was evenly spread on Mueller-Hinton agar using a glass spreader. Wells were then bored into the agar media using a sterile 6 mm cork borer and were filled with the solution of the fractions, taking care not to allow spillage of the

solution to the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 h to allow proper diffusion of the extract into the media. The plates were thereafter incubated at 37 ºC for 24 h, after which they were observed for zones of inhibition. The effects of the fractions on the test bacterial isolates were compared with those of tetracycline and ampicillin standard antibiotics at a concentration of 1 mg/ml and 10 µg/ml respectively.

Determination of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the active fractions

The MICs of the active fractions were determined using the method of Akinpelu and Kolawole (2004). Two-fold dilutions of each active fraction was prepared and 2 ml aliquots of different concentrations of the solution were added to 18 ml of pre-sterilized molten nutrient agar at 40 ºC to give final concentrations regimes of 0.040 and 5.0 mg/ml. The medium was then poured into sterile Petri dishes and allowed to set. The surfaces of the media were allowed to dry under a laminar flow before streaking with 18 h old bacterial cultures. The plates were later incubated at 37 ºC for up to 72 h, after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration of the fraction that will prevent the growth of the susceptible test bacteria. The MBCs of the fractions were determined as described by Olorundare *et al*. (1992), with few modifications. Samples were taken from plates with no visible growth in the MIC assay and subcultured onto freshly prepared nutrient agar plates and later incubated at 37 ºC for 48 h. The MBC was taken as the lowest concentration of the extract that did not allow bacterial growth on the surface of the agar plates.

Determination of the rate of killing and protein leakage

Assay for the rate and extent of killing of bacterial isolates by the active fractions was determined in accordance with the method of Odenholt *et al*. (2001). Representatives of each Gram positive and Gram negative bacterial isolates were used viz., *Bacillus pumilus* (Gram positive) and *Escherichia coli* (Gram negative). The turbidity of the 18 h old test organism was first standardized to 10^8 cfu/ml. A 0.5 ml volume of known cell density from each organism suspension was added to 4.5 ml of different concentrations of the solution of the fractions. These were held at room temperature and the killing rate was determined over a period of 2 h. Exactly 0.5 ml volume of each suspension was withdrawn at time intervals and transferred to 4.5 ml of nutrient broth recovery medium containing 3% "Tween 80" to neutralize the effects of the antimicrobial compound carry-overs from the test organisms. The suspension was then serially diluted and plated out for viable counts. The plates were later incubated at 37 ºC for 48 h. The control plates contained the bacterial cells without the fractions. The emergent bacterial colonies were counted and compared with the counts of the culture control. Protein leakage was determined in accordance with the method in our previous description (Aiyegoro et al., 2008) by treatment of various concentrations of the extracts (relative to MIC) with bacterial cells washed three times in physiological saline by centrifugation at 10,000 rpm for 10 min followed by re-suspension in physiological saline. At intervals, each suspension was centrifuged at 7000 rpm and the supernatant obtained was assayed for protein using the Bradford method (1976). The concentration of protein was estimated from the established standard curve obtained using bovine serum albumin (BSA). *B. pumilus* (Gram positive) and *E. coli* (Gram negative) were also used for this assay.

RESULTS

Five fractions were obtained from the crude extract of the test stem bark of *A. africana* used in this study, and these includes aqueous (AQ), butanol (BL), chloroform (CL), diethyl ether (DE) and hexane (HX)

fractions. These five partially purified fractions exhibited antibacterial activities against most of the tested bacterial isolates comprised of both Gram-positive and Gram-negative organisms. Two of the five fractions, that is, aqueous (AQ) and butanolic (BL) fractions, exhibited appreciable antibacterial activities at a screening concentration of 10 mg/ml. The zones of inhibition exhibited by the two fractions (AQ and BL) ranged between 15 mm and 28 mm, while those exhibited by the crude extract (25 mg/ml) ranged between 13 mm and 22 mm (Table 1). The chloroform (CL), diethyl ether (DE) and n-hexane (HX) exhibited limited activities when compared with the aqueous and butanolic fractions. The antibacterial activities of the AQ and BL fractions compared favourably with those of the two standard antibiotics - ampicillin and tetracycline used in this study and appeared to be broad spectrum in action. The minimum inhibitory concentrations (MICs) of AQ and BL along with that of ampicilin were determined. The MICs of the two fractions ranged between 0.313 mg/ml and 2.50 mg/ml (Table 2). The minimum bactericidal concentrations (MBC) of the AQ fraction ranged between 0.625 mg/ml and 5.0 mg/ml while that of BL fraction ranged between 0.625 mg/ml and 2.5 mg/ml (Table 3). From all indications, the BL fraction showed better antibacterial activities than AQ fraction, thus suggesting that butanol could be a preferred solvent for the extraction of antibacterial compounds from the stem back of *A. africana*.

TABLE 1

Sensitivity patterns of zones of inhibition exhibited by *A. africana* stem bark fractions and standard antibiotics against the test bacterial isolates

Test bacteria	Zones of inhibition (mm**)						
	AQ	BL	CL	DE	HX	TET	AMP
Escherichia coli ATCC 8739	15.00	21.00	0.00	15.00	21.00	17.00	18.00
E. coli ATCC 25922	16.00	18.00	0.00	15.00	21.00	28.00	25.00
Staphylococcus aureus ATCC 6538	0.00	0.00	0.00	0.00	0.00	0.00	24.00
Enterococcus faecalis ATCC 29212	0.00	0.00	0.00	0.00	0.00	19.00	17.00
Bacillus cereus ATCC 10702	0.00	0.00	0.00	0.00	0.00	24.00	24.00
Bacillus pumilus ATCC 14884	15.00	18.00	0.00	11.00	15.00	27.00	23.00
Klebsiella pneumoniae ATCC 10031	15.00	18.00	0.00	0.00	26.00	23.00	14.00
Proteus vulgaris ATCC 6830	20.00	18.00	0.00	0.00	0.00	17.00	18.00
Serratia marcescens ATCC 9986	15.00	18.00	0.00	0.00	0.00	13.00	17.00
Acinetobacter calcaoceticus anitratus CSIR	16.00	20.00	11.00	15.00	16.00	17.00	13.00
K. pneumoniae (LIO)	15.00	18.00	0.00	0.00	0.00	15.00	13.00
Bacillus subtilis (LIO)	0.00	0.00	0.00	0.00	0.00	22.00	27.00
Shigella flexineri (LIO)	0.00	15.00	0.00	0.00	0.00	0.00	0.00
Staph. epidermidis (LIO)	0.00	0.00	0.00	0.00	0.00	10.00	17.00
Proteus vulgaris (LIO)	16.00	18.00	0.00	0.00	0.00	22.00	19.00
Enterococcus faecalis (LIO)	15.00	18.00	0.00	0.00	0.00	22.00	21.00
Staphylococcus aureus (LIO)	18.00	22.00	16.00	15.00	18.00	0.00	22.00
Staph. aureus OKOH2B (LIO)	16.00	20.00	0.00	0.00	20.00	0.00	13.00
Staph. aureus OKOH 3 (LIO)	20.00	25.00	0.00	15.00	26.00	0.00	17.00
Micrococcus kristinae (LIO)	18.00	22.00	0.00	0.00	24.00	18.00	18.00
Kocuria rhizophila (LIO)	20.00	26.00	0.00	0.00	28.00	17.00	13.00

AQ- aqueous fraction (10 mg/ml); BL- butanol fraction (10 mg/ml); CL- chloroform fraction (10 mg/ml); DE- diethyl ether fraction (10 mg/ml); HX- n-hexane fraction (10 mg/ml); TET- tetracycline (1 mg/ml); AMP- ampicilin (10 µg/ml); (mm**) - mean of three readings; LIO- locally isolated organism.

TABLE 2

MIC of aqueous and butanol fractions of *Afzelia africana* stem bark and ampicilin

Bacteria	Aqueous fraction (mg/ml)	Butanol fraction (mg/ml)	Ampicilin (μg/ml)
Escherichia coli ATCC 8739	2.5	2.5	2.00
E. coli ATCC 25922	0.313	0.313	4.00
Bacillus pumilus ATCC 14884	2.5	1.25	0.03
Klebsiella pneumoniae ATCC 10031	1.25	0.625	1.60
Proteus vulgaris ATCC 6830	2.5	1.25	4.00
Serratia marcescens ATCC 9986	2.5	1.25	3.20
Acinetobacter calcaoceticus anitratus CSIR	0.313	0.313	5.12
K. pneumoniae (LIO)	2.5	1.25	5.12
Shigella flexineri (LIO)	ND	1.25	1.00
Proteus vulgaris (LIO)	2.5	1.25	4.00
Enterococcus faecalis (LIO)	1.25	0.625	2.00
Staphylococcus aureus (LIO)	1.25	1.25	0.06
Staph. aureus OKOH2B (LIO)	2.5	1.25	0.13
Staph. aureus OKOH3 (LIO)	2.5	1.25	1.00
Micrococcus kristinae (LIO)	2.5	1.25	0.13
Kocuria rhizophila (LIO)	2.5	1.25	0.25

ND- not done; LIO- locally isolated organisms.

TABLE 3

The MBC values of aqueous and butanol fractions of *Afzelia africana* stem bark

Microorganism	Aqueous fraction (mg/ml)	Butanol fraction (mg/ml)
Escherichia coli ATCC 8739	5.0	5.0
E. coli ATCC 25922	0.625	0.625
Bacillus pumilus ATCC 14884	5.0	2.5
Klebsiella pneumoniae ATCC 10031	2.5	1.25
Proteus vulgaris ATCC 6830	5.0	2.5
Serratia marcescens ATCC 9986	5.0	2.5
Acinetobacter calcaoceticus anitratus CSIR	0.625	0.625
K. pneumoniae (LIO)	5.0	2.5
Shigella flexineri (LIO)	ND	2.5
P. vulgaris (LIO)	5.0	2.5
Enterococcus faecalis (LIO)	2.5	1.25
Staphylococcus aureus (LIO)	2.5	2.5
Staph. aureus OKOH2B (LIO)	5.0	2.5
Staph. aureus OKOH3 (LIO)	5.0	2.5
Micrococcus kristinae (LIO)	5.0	2.5
Kocuria rhizophila (LIO)	5.0	2.5

ND- not done; LIO- locally isolated organisms.

The bactericidal efficacy of AQ and BL fractions were investigated against two of the tested bacterial isolates by determining the killing rate of the representative organisms and the protein leakage from their cells. Representative organisms included *E. coli* represents (Gram-negative) and *B. pumilus* (Gram-positive). Different concentrations of the aqueous (AQ) and butanolic (BL) fractions obtained from the crude extract of the plant exhibited a significant bactericidal activity against *E. coli* and *B. pumilus*. The AQ fraction was highly bactericidal against *E. coli* and *B. pumilus* over a period of time intervals ranging between 15-120 min at different concentrations (Figures 1a, b). At a concentration of 5 mg/ml, the aqueous fraction (AQ) reduced *E. coli* density by $6.3\log_{10}$ from initial population of $6.8\log_{10}$ within 60 min of interaction, thus amounting to about 58.3% of cells killed.

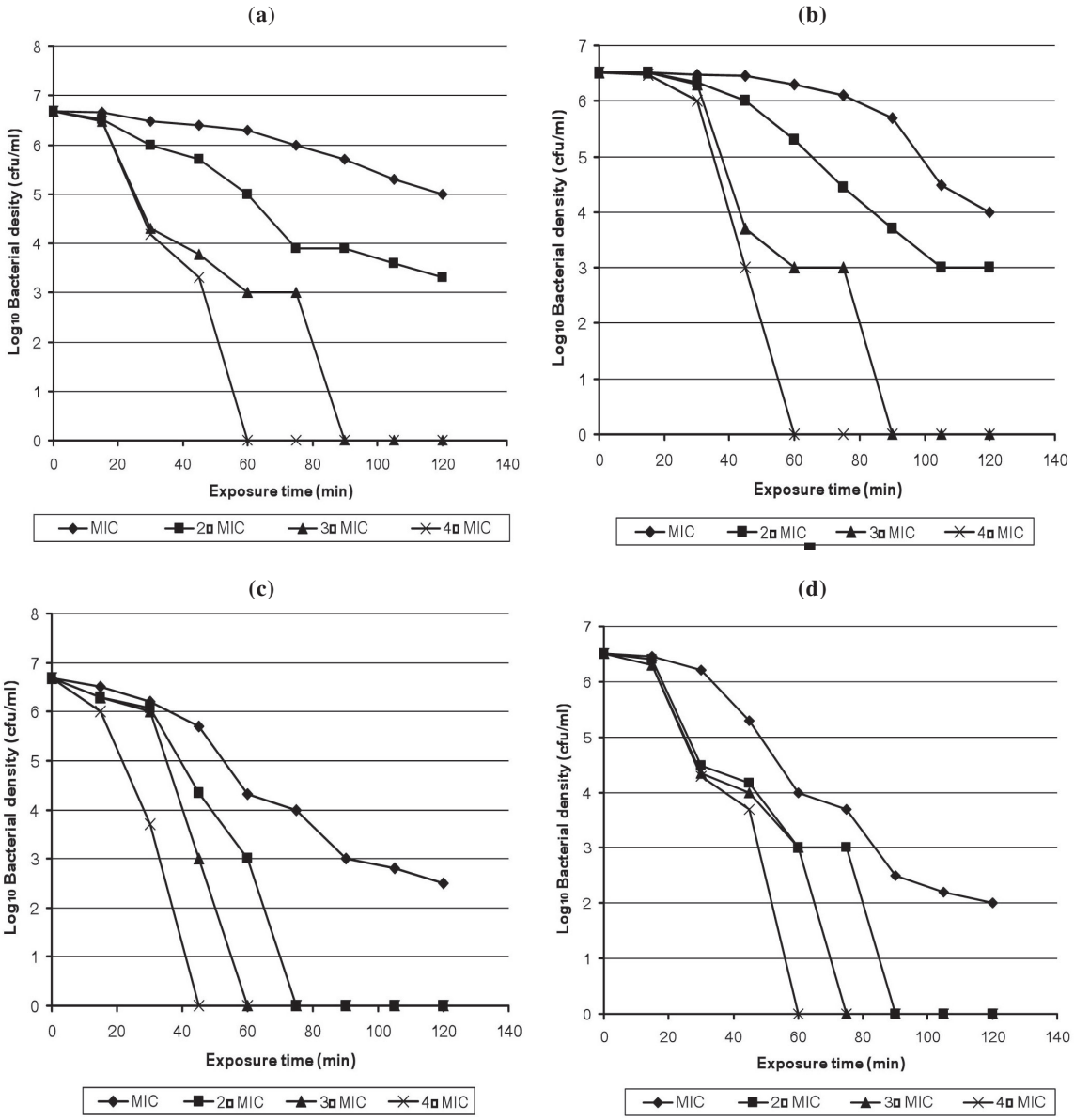

Figure 1: Profile of rate of kill of *Escherichia coli* (**a**) and *Bacillus pumilus* (**b**) by aqueous fraction of *Afzelia africana* stem bark as well as by the butanol fraction (**d** and **c** respectively).

When the time interval was increased to 105 min at the same concentration, the percentage population of the cells killed increased to 95.8%, that is, a reduction of 5.3 $\log_{10}$ in cell population (Figure 1a). On exposure of *B. pumilus* cells to the AQ fraction at a concentration of 2.5 mg/ml for a period of 60 min the percentage of the cells killed was 37.5%, that is, a reduction of 6.3$\log_{10}$ in cells population from an initial cell population of 6.51$\log_{10}$. When the time interval was increased to 105 min at the same concentration, about 99.1% of the cells was killed (Figure 1b). At a concentration of 5.0 mg/ml of the AQ fraction, the percentage of the population of *E. coli* cells killed within 60 min was 97.9% and up to 99.9% at 105 min. A similar trend was observed when *B. pumilus* cells were subjected to the same concentration exposure (Figures 1a, b).

The butanol (BL) fraction exhibited higher bactericidal activity compared to the AQ fraction based on the rate of kill assay. When *E. coli* cells were treated with the BL fraction at a concentration of 5 mg/ml for a time interval of 60 min, there was a reduction of 4.32$\log_{10}$ in the cell population, indicating about 99.6% cell death. When the contact time was increased to 105 min at the same concentration, the whole cell population was killed (Figure 1c). On the other hand, about 99.7% of *B. pumilus* cells were killed at a concentration of 5 mg/ml within 60 min of exposure to the extract. The organism was completely wiped out when exposure time was increased to 105 min (Figure 1d). From the observation, the kill rate exhibited by the fractions against the tested organisms appeared to be both concentration and time dependent.

E. coli and *B. pumilus* were used to test for the ability of the two fractions AQ and BL to leak protein from these cells. The BL fraction at a concentration of 2.5 mg/ml (1 × MIC) caused more protein leakage in *B. pumilus* (Figure 2d) than *E. coli* (Figure 2b) over a 2 h exposure period. For example, the amount of protein leaked out of *B. pumilus* and *E. coli* in 2 h were 8.0 and 2.8 μg/ml respectively. Over the same period, the amount of protein leaked when the two organisms were exposed to 2.5 mg/ml of AQ fraction were 2.5 μg/ml for *E. coli* (Figure 2a) and 3.5 μg/ml for *B. pumilus* (Figure 2c). A similar trend was observed when the concentrations of the two fractions were varied. Overall, the BL fraction showed the highest leakage of total protein from the test bacteria than the AQ fraction, and this was corroborated by the higher proportion of cell death observed with the BL fraction.

Discussion

Our investigations revealed that *E. coli*, *Staph. aureus*, *Shigella sp.*, *K. pneumoniae* and Serratia *marcescens,* all of which are known pathogens of man, were susceptible to the extracts of the stem bark of *A, africana*. *Staphylococcus aureus* is known to cause septicemea and boils (Colque-Navarro *et al.*, 2000; Weber, 2005), while *E. coli* plays an important role in dysentery (Kaper*,* 2005). The susceptibilities of these pathogens corroborate the use of the plant in folklore remedies for the treatment of microbial infections. All the Gram-negative bacterial isolates used in this study were susceptible to the plant extract. Gram-negative bacteria are known to be inherently more resistant to the antimicrobial compounds than Gram-positive bacteria. The resistant capability of Gram-negative bacteria to antimicrobial compounds is associated with their cell wall, which is made up of three structures - cytoplasmic membrane that contains lipids and protein; a thin peptidoglycan layer and an outer membrane composed of lipopolysaccharide (Plesiat and Nikaido, 1992; Nakaido, 1998). Thus, the stem bark of *A. africana* appers to be a potential source of antibacterial compounds that could be relevant in the treatment of infections caused by Gram-negative bacteria. Furthermore, a reduction in microbial population to 99% of the initial population of the organisms within the shortest period of time, as observed in the rate of kill test, is the generally accepted definition of bactericidal activity in antibiotics (Pankey and Sabath, 2004).

Thus, the bactericidal potentials of fractions obtained from *A. africana* observed in this study showed a significant therapeutic potential of this plant and hence support its usefulness in folklore remedies.

From our observations, leakage of proteins from the cells of the test organisms by the BL and AQ fractions is an indication that their mode of action is probably related to the disruption of cell membrane and eventually death of the organism (Sanati *et al*., 1997). From our findings there appears to be a relationship between the amount of total protein released and the proportion of test organisms killed (Figures 1a-d).

In our previous study (Akinpelu et al., 2008), we reported the presence of a number of phytochemical compounds in the crude extract of the test plant, and these include tannins, flavonoids, steroids,

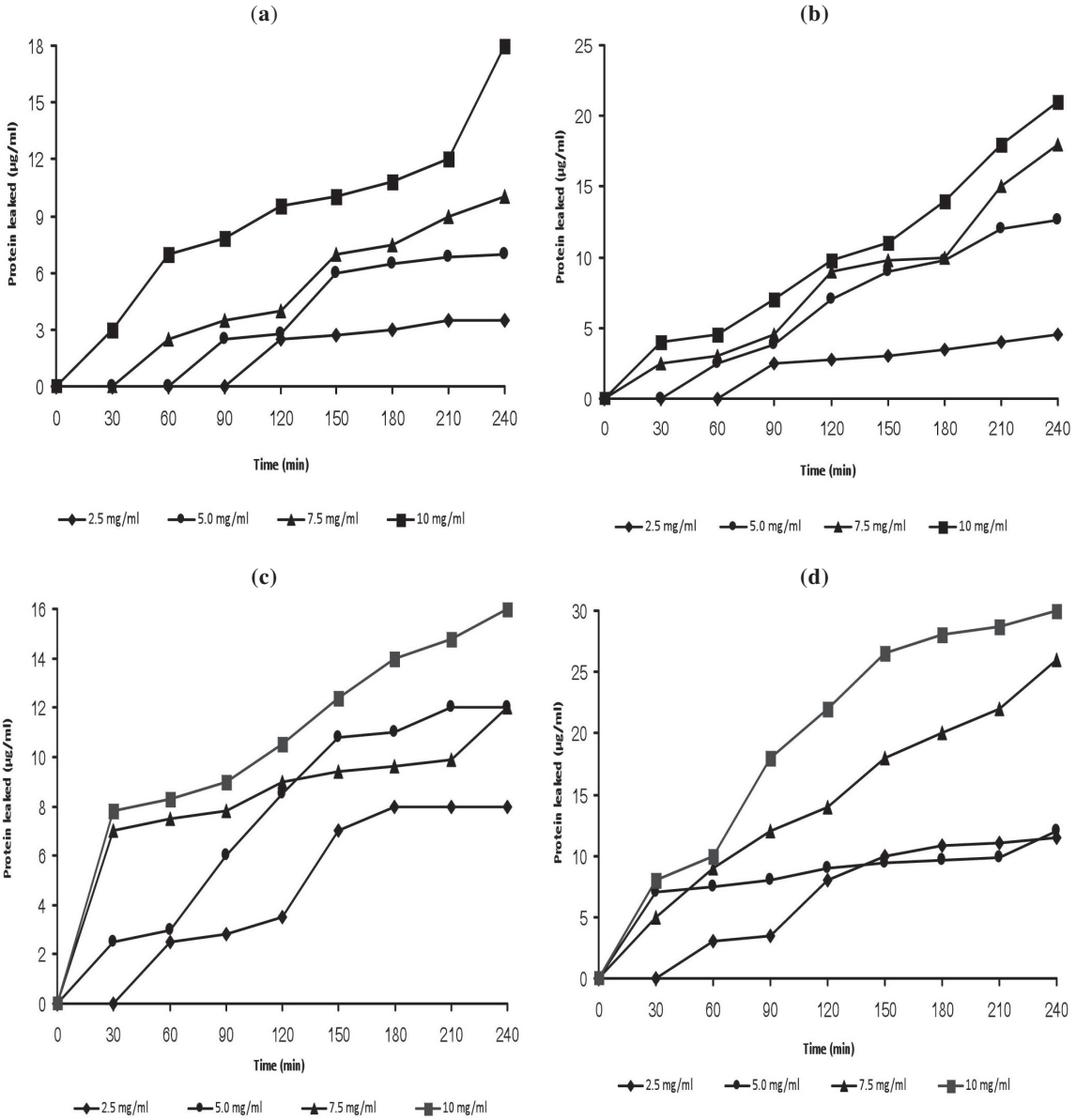

Figure 2: Effects of aqueous ((a) and (c)) and butanol ((b) and (d)) fractins of Afzellia africana stem bark on protein leakage from *Escherichia coli* ((a) and (b)) and *Bacillus pumilus* ((c) and (d)) cells.

alkaloids and saponins. These phytochemical compounds are known to be biologically active and thus aid the antimicrobial activities of *A. africana*. Phytochemicals exert antimicrobial activity through different mechanisms; tannins for example, act by iron deprivation, hydrogen bounding or specific interactions with vital proteins such as enzymes (Scalbert, 1991) in microbial cells. Herbs that have tannins as their component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003), thus exhibiting antimicrobial activity. The presence of tannins in *A. africana* supports the traditional medicinal use of this plant in the treatment of different ailments. Motal *et al.* (1985) revealed the importance of tannins for the treatment of inflamed or ulcerated tissues. Li *et al.* (2003) reviewed the biological activities of tannins and observed that tannins (whether total or pure compound) have remarkable activity in cancer prevention and anticancer. This implies that *A. africana* can serve as a source of drug for the treatment and prevention of cancer. In addition to its antimicrobial, anticancer activities, tannins have roles such as stable and potent antioxidants (Trease and Evans, 1983).

Alkaloid is another phytochemical compound observed in the stem bark extract of *A. africana*. Alkaloids have been associated with medicinal uses for centuries and other possible roles have not been examined. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines (Nobori *et al*., 1994). In addition, alkaloids possess anti-inflammatory, anti-asthmatic, and anti- anaphylactic properties with consequences of altered immunological status *in vivo* (Ganguly and Sainis, 2001; Gopalakrishnan *et al.,* 1979; Staerk *et al.,* 2002). Furthermore, alkaloid, which is one of the largest groups of phytochemicals in plants, has amazing effect on humans and this has led to the development of powerful pain killer medications (Raffauf, 1996). These observations on alkaloids corroborate the use of *A. africana* in folklore remedies.

Flavonoids, which are also one of the constituents of *A. africana* stem bark extract, exhibit antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties (Hodek *et al*., 2002). The ability of flavonoids to scavenge hydroxyl radicals, superoxide anion radicals and lipid peroxyradicals highlights some of their health-promoting functions in organisms, which are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA (Ferguson, 2001) Flavonoids in the human diet may reduce the risk of various cancers, as well as preventing menopausal symptoms (Hodek *et al*., 2002). All these facts support the usefulness of *A. africana* in folklore remedies and is one of the reasons this plant is widely used for the treatment of many diseases among many tribes in Africa. *A. africana* exhibited antimicrobial properties and flavonoids act in this way. In addition to the antimicrobial activities exhibited by flavonoids, they also exhibit antitrypanosomal and antileishmanial activities (Tasdemir *et al*., 2006). Epidermiological studies suggest that the consumption of flavonoids is effective in lowering the risk of coronary heart diseases (Rice-Evans and Miller, 1996). All these facts suggest that A. *africana* can as well be used to treat coronary heart disease. Furthermore, several flavonoids exhibit antiviral activities (Xu *et al*., 2000). Lastly, saponins, which are responsible for numerous pharmacological properties (Estrada *et al.,* 2000), also tested positive in *A. africana* stem bark extract. Saponins are considered key ingredients in traditional Chinese medicine and are responsible for most of the observed biological effects in medicinal plants (Liu and Henkel, 2002). Saponins are known to produce an inhibitory effect on inflammation (Just *et al*.,1998). These observations cited on phytochemical compounds support our findings on the usefulness of *A. africana* in traditional medicament. Therapeutic effects of some medicinal plants commonly used in folklore remedies can therefore be

attributed to the antioxidant properties of their phytoconstituent

CONCLUSION

The aqueous and butanol extracts of the stem bark of *Afzelia africana* showed significant antibacterial properties. The butanol fraction was more active than the aqueous fraction, thus suggesting that butanol could be a preferred solvent for the extraction of antibacterial compounds from the plant. As well, activities of the two extracts suggest cell membrane disruption as a probable mechanism of biocidal activity of the extract. It is necessary to isolate and characterize the active principle(s) of the plant, including its/their *in vivo* potencies, and these are subjects of intensive ongoing investigation in our group.

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