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Evaluation of Antimicrobial Activities of *Albizia zygia* DC Leaf Extracts against Some Clinically Important Pathogens

Oluyemi Odeyemi[1], Anthonia Olufunke Oluduro[*1], Oluwole Moses David[2]
1. Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria
2. Department of Microbiology, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria
* E-mail of the corresponding author: *aoluduro2003@yahoo.co.uk.*

Abstract
In vitro antimicrobial effects of aqueous, ethanolic and methanolic extracts *of Albizia zygia* dc leaf against some clinically important bacterial and fungal pathogens were reported. Following extraction of air dried *A. zygia* dc leaf by different solvents (water, ethanol and methanol), the filtrates were concentrated *in vacuo* using rotary evaporator. The antibacterial and antifungal activities were assayed by agar diffusion method on Muller-Hinton Agar (Himedia Laboratories Pvt. Ltd, Vadhani) and Potato dextrose agar (Oxoid, Ltd, Bashingstoke, Hampshire, England) plates, respectively. Standard methods were used to determine the time-kill assay of methanolic extract, the amount of protein and potassium ion leaked in the test bacteria. All the extracts (aqueous, ethanolic and methanolic) did not possess any antifungal property. The aqueous and ethanolic extracts were not active against the test bacteria. Methanolic extract showed significant antibacterial effect on greater percentage of the test bacteria with diameter of zones of inhibition ranging from 3.0 to 21.12 mm at 30 mg/ml and 5.2 to 25.4 mm at 50 mg/ml of the extract. The minimum inhibitory concentration (MIC) of the methanolic extract ranged between 3.75 and 15.3 mg/ml. The methanolic extract of *A. zygia* leaf showed a significant bactericidal and bacteriostatic activity against *Bacillus subtilis* and *Klebsiella pneumoniae* over the time range (15-120 min) at different MIC concentrations. The time-kill assay of methanolic extract of *A. zygia* against *K. pneumoniae* was dose dependent. The amount of protein leaked was higher in *B. subtilis* than *K. pneumoniae* at 30 µg/ml (P = 0.05). There was no significant difference in the level of K^+ leaked at 15 µg/ml (1 X MIC) and 30 µg/ml (2 X MIC) of the extract.
The methanolic leaf extract of *A. zygia* showed a considerable inhibitory effect on greater percentage of the test bacterial pathogens, but did not possess antifungal property. The antibacterial potential could be harnessed in the folklore management of infections caused by the susceptible test bacteria.

1.0 Introduction

Bacteria develop resistance to antibiotics in a variety of ways, including methods that may decrease the intracellular concentrations of the antibiotic, deactivate the antibiotic, change the binding sites for the antibiotic, and develop adaptations that bypass the need for the binding site targeted by the antibiotic (Kaye *et al*., 2000). The increasing rate of resistance to antibiotics with its high cost has foster the search for new antimicrobials.

Nature has always provided therapeutic means of treating microbial diseases. Nature has endowed several plants with metabolites which are of medicinal importance (Cowan, 1999). Medicinal plants are widely accepted and relatively cheap when compared to orthodox medicines. Apart from their accessibility, medicinal plants have been reported to be the bedrock of modern medicine. However, the shortcomings of traditional medicine include lack of scientific proof of their efficacies, lack of hygienic preparation (in most cases) and lack precise dosage. In Africa, like other developing nations, people rated the advantages of these pharmaceutics far above their shortcomings.

Albizia zygia is a deciduous tree 9-30 m tall with a spreading crown and a graceful architectural form (Petzke, 1997). The bark is grey and smooth. Its leaves pinnate, pinnae in 2-3 pairs and broadening towards the apex, obliquely rhombic or obviate with the distal pair largest, apex obtuse, 29-72 by 16-43 mm (Chudnoff, 1984; Petzke, 1997). *Albizia zygia* is a cheap substitute to some of the known synthetic drugs (Schultes, 1978; Chudnoff, 1984; Ndjakou *et al*., 2007). It is useful in the treatment of allergy-caused respiratory disorders (Ndjakou *et al.,* 2007). The *in vitro* anti -*Neisseria gonorrhoeae* activity of *A. zygia* has been reported by Mesfin *et al.* (2012). Different parts of *Albizia zygia* (bark, fruit, flowers and leaves) have been reported to be used as medicinal remedies (Ndjakou *et al*., 2007). The study evaluates the antimicrobial potential of *A. zygia* and its effects on the bacterial cell components

2.0 Materials and Methods

2.1 Source of Microorganisms

Microorganisms used in this study were collected from the stock culture unit of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. The isolates includes Gram positive bacteria: *Bacillus cereus* OAU 84*, Bacillus subtilis* OAU 81*, Clostridium sporogenes* NCTC 532*, Enterococcus faecalis* NCTC 775*, Micrococcus luteus* NCIB 196 and *Staphylococcus aureus* NCIB 8588, while the Gram negative bacteria

include: *Acinetobacter baumanni* OAU 234, *Salmonella* Typhi OAU 152, *Escherichia coli* OAU 382, *Klebsiella pneumoniae* OAU 49, *Pseudomonas aeruginosa* OAU 27, *Pseudomonas fluorescens* OAU 47 and *Shigella dysenteriae* OAU 221. The fungal isolates comprise *Aspergillus fumigatus* OAU 002, *Candida albicans* OAU 026, *Microsporum canis* OAU 071, *Mucor indicus* OAU 93 and *Rhizopus oryzae* OAU 72. Bacterial isolates were standardized to match (0.5 McFarland Standard) at optical activity of 625 nm.

2.2 Source and preparation of plant materials

Fresh mature leaf of *A. zygia* tree located at ENPOST Farms, Ilesa, Osun State, Nigeria, was used for the study. The leaf was harvested and identified at the herbarium unit of Botany Department, Obafemi Awolowo University, Ile-Ife, Nigeria, and voucher specimens were deposited. The *A. zygia* leaf was air-dried for 3 weeks at room temperature and ground to fine powder. A 200 g of ground leaf sample was soaked separately in 500 ml of each of the solvents (methanol, ethanol and water) for 48 h for aqueous extraction and 96 h for both ethanol and methanol extractions. The sample was filtered using Whatman Number 1 filter paper. Each of the filtrates was concentrated to dryness *in vacuo* using a rotary evaporator (Heldoph, Germany). The percentage yields of extracts were calculated and stored in the refrigerator at 4^{o}C for further use. The leaf extracts were re-dissolved in 4% DMSO.

2.3 Determination of antibacterial and antifungal activity of the crude leaf extracts of *A. zygia*

The antibacterial and antifungal activities of *A. zygia* leaf extracts (Aqueous, ethanolic and methanolic) were determined using agar-well diffusion method described by Irobi *et al.* (1994). For the determination of antibacterial activity, 0.1ml of standardized bacterial inoculum suspension (10^6 CFU/ml) was seeded on Mueller-Hinton agar (Himedia Laboratories Pvt.Ltd, Vadhani) plates. Wells were bored with sterile cork borer (6mm) on the plates and filled with 0.1 ml of 30 and 50 mg/ml of the plant extracts, 0.1 ml of streptomycin was used as positive control and 0.1 ml of the extraction solvents as negative control. The plates were incubated at 37^{o}C for 24 h. The antifungal activity was determined by flooding potato dextrose agar plates with 0.1ml spore suspension (adjusted to 0.1 absorbance at 590 nm wavelength) of a 5-day old fungal culture using the spread plate technique. The wells bored on the PDA plates were separately filled with 0.1ml of plant extract (25 mg/ml, 50 mg/ml), 0.1 ml solution of griseofulvin (2 mg/ml) and 0.1 ml of extraction solvent (control). The plates were allowed to stand on bench for 1 h and incubated at 28^{o}C for 3-5 days. Diameter of zones of growth inhibition were measured to the nearest millimeter with a ruler. This experiment was done in duplicate.

2.4 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the plant extract

The MIC of the leaf extract of *A. zygia* was determined using the method of Akinpelu and Kolawole (2004). Different concentrations of the plant extract solution were obtained by double-fold dilutions. The plant extract solution (2 ml) was added to 18 ml of pre-sterilized molten nutrient agar (Oxoid, Ltd, Bashingstoke, Hampshire, England) at 40^{o}C. The medium was poured into Petri dish and allowed to set. A 18-h old broth culture of test organism (10^6 CFU/ml) was streaked on the agar plate and incubated at 37^{o}C for 48 h. The MIC was taken as the lowest concentration that prevents visible bacterial growth.

The MBC of the plant extract was determined as described by Olorundare *et al.* (1992). A loopful of sample from the plate that did not show visible growth in the MIC assay above was sub-cultured on freshly prepared nutrient (Oxoid, Ltd, Bashingstoke, Hampshire, England) agar plates and incubated at 37^{o}C for 24 h. The MBC was taken as the lowest concentration of the plant extract solution that did not show any visible bacterial growth on the agar plates.

The MIC index (MICI) of extract was calculated as the ratio of MBC and MIC and interpreted as follow: MICI $\leq$ 2.0 was considered bactericidal, >2 but <16- bacteriostatic, and $\geq$16.0- ineffective (Shanmughapriya *et al*., 2008).

2.5 Time-kill assay of *A. zygia* leaf extract

The death rate of bacterial isolates by methanolic leaf extract of *A. zygia* was determined in accordance with the method of Odenholt *et al*. (2001). *Bacillus subtilis* and *K. pneumoniae* representing both Gram positive and Gram-negative bacteria, respectively were selected for the time- kill assay of the extracts. The death rate was determined over a period of 4 h at room temperature. One milliliter of standardized culture (10^6 CFU/ml) of *B. subtilis* and *K. pneumoniae* was separately added to 9 ml of different MIC concentrations of methanolic extract (15 µg/ml (1×MIC), 30 µg/ml (2×MIC), 60 µg/ml (4×MIC) and left for one hour at room temperature. The inoculum suspension (0.5 ml) was withdrawn at time intervals and transferred to 4.5 ml of nutrient broth recovery medium containing 3% "Tween 80". To determine the number of viable bacteria left after treatment with the extract, 1 ml of the suspension was serially diluted and plated on nutrient agar plates and incubated at 37^{o}C for 24 h. Colonies were counted and expressed as colony forming unit per ml (Cfu/ml). Test bacterial suspension in 5% methanol was used as control.

2.6 Determination of Protein and Potassium Ions Leakage from Bacterial Cells

The amount of protein leaked from *B. subtilis and K. pneumoniae* by different MICs of methanolic extract was determined by the method of Bradford (1976). The bacterial cells were washed thrice in physiological saline by

centrifuging at 4000 rpm for 10 min, re-suspended in sterile physiological saline and standardized. The suspended cell (1 ml) was added to 9 ml of the plant extract solution to make the desired concentrations. At intervals of 0 min, 30 min, 60 min, 120 min, 180 min and 240 min, the mixture was centrifuged at 4000 rpm and the supernatant was collected. About 0.1 ml of each supernatant was added to 5 ml Bradford reagent to determine the amount of protein leaked (Bradford, 1976). The supernatant obtained after centrifugation of the reaction mixture was also analyzed for potassium ion (K+) content using a spectrophotometer (flame photometer). The procedure was repeated using different MICs of the methanolic extract (1 × MIC, 2 × MIC and 4 × MIC). Test bacterial suspension alone without the plant extract and plant extract solution prepared in 5% methanol were used as controls.

2.8 Determination of the phytochemicals in *A. zygia* leaf

The phytochemical groups present in *A. zygia leaf* such as cardiac glycosides, flavonoids, tannins and triterpenes were evaluated by the method of Sofowora (1993). Saponins and steroids were determined according to Hammer *et al*. (1999).

3.0 Results

The methanolic extract of *A. zygia* had the highest percent yield (25%), followed by ethanolic extract (10%) and aqueous extract (6%) (Table 1). All the extracts (aqueous, ethanolic and methanolic) did not possess any antifungal property The aqueous and ethanolic extracts were not active against the test bacteria. The diameter of the zones of inhibition of bacterial isolates by the methanolic leaf extract of *A. zygia* is shown in Table 2. Methanolic extract showed significant antibacterial effect on greater percentage of the test bacteria with diameter of zones of inhibition ranging from 3.0 to 21.12 mm at 30 mg/ml and 5.2 to 25.4 mm at 50 mg/ml of the extract.

Table 1. Percentage weight yield of *Albizia zygia* leaf extracts

Albizia zygia Extract	**Dry weight (g)**	**Extracted weight (g)**	**Weight loss (g)**	**Percentage yield (%)**
Methanolic	200	50	150	25
Ethanolic	200	20	180	10
Aqueous	200	12	188	6

The zones of growth inhibition increased in diameter with increased concentration of extract. The largest diameter of zone of inhibition was against *B. subtilis* (25 mm) while the least was against *S. dysenteriae* (3 mm). Meanwhile the methanolic extract did not show any zone of growth inhibition against *Staphylococcus aureus*, *Enterococcus faecalis*, *and Pseudomonas aeruginosa* at 30 mg/ml. The antibacterial activity of the leaf extract compared positively with the reference antibiotic (streptomycin) (Table 2).

Table 2. Inhibition zone diameter (mm) of test bacterial isolates to the different extracts of *A. zygia* (mg/ml)

Microorganisms	**Methanol**		**Ethanol**		**Aqueous**		**Streptomycin (10µg/ml)**	**DMSO (4%)**
	30	**50**	**30**	**50**	**30**	**50**		
B. cereus OAU 84,	11±2.1	14±2.8	0	0	0	0	24±4.8	0
B. subtilis OAU 81	21±1.8	25±3.8	0	0	3±2.1	0	28±2.9	0
S. aureus NCIB 8588	0	15±3.8	0	0	0	0	15±1.4	0
C. sporogenes NCTC 532	16±2.7	18±3.1	0	0	0	0	10±3.2	0
M. luteus NCIB 196	0	0	0	0	0	0	12±2.8	0
E. faecalis NCTC 775	0	0	0	3	0	0	0	0
P. aeruginosa OAU 27	0	0	0	0	0	0	22±7.1	0
P. fluorescens OAU 47	13±3.1	16±3.6	0	0	0	0	28±3.6	0
S. Typhi OAU 152	11±4.1	11±4.1	0	0	0	0	13±3.8	0
K. pneumoniae OAU 49	15±2.7	19±3.9	0	0	0	0	28±4.1	0
E. coli OAU 382	12±4.1	12±3,7	0	0	0	0	21±3.6	0
S. dysenteriae OAU 221	3±1.0	5±3.6	0	3±	0	0	15±4.4	0
A. baumanni OAU 234	8±2.1	9±2.7	0	0	0	0	19±5.4	0

Table 3 shows the MIC and MBC of the methanolic extract of *A. zygia* against the test bacteria. The MIC of the methanolic extract ranged between 7.5 and 30.0 mg/ml while the MBC was between 15.0 and >120.0 mg/ml. The MIC index (MBC/MIC) ranged between 2.0 and 8.0 (Table 2). The MIC of the methanolic extract against *B. subtilis* and *B. cereus* were 15.3 and 15.04 mg/ml, respectively. *Clostridium sporogenes* had MIC of 7.5mg/ml while *Kl. pneumoniae* had 3.75 mg/ml. The minimum bactericidal concentration (MBC) ranged between 7.5 mg/ml and 30 mg/ml. The MBC against *B. subtilis* and *B. cereus* was 30 mg/ml, while the MBCs of *Clostridium sporogenes* and *Klebsiella pneumoniae* were 15 mg/ml and 7.5 mg/ml, respectively (Table 3).

Table 3. The Minimum Inhibitory Concentration (MIC) and Minimum bactericidal concentration (MBC) of the methanolic extract of *A. zygia* against test bacteria

Bacteria	MIC (µg/ml)	MBC (µg/ml)	MBC/MICMIC index	Interpretation
B. cereus OAU 84,	15.30	30.00	2.00	Bactericidal
B. subtilis OAU 81	15.00	30.00	2.00	Bactericidal
S. aureus NCIB 8588	30.00	120.00	4.00	Bacteriostatic
C. sporogenes NCTC 532	3.75	7.50	2.00	Bactericidal
M. luteus NCIB 196	30.00	120.00	4.00	Bacteriostatic
E. faecalis NCTC 775	30.00	120.00	4.00	Bacteriostatic
P. aeruginosa OAU 27	30.00	120.00	4.00	Bacteriostatic
P. fluorescens OAU 47	15.00	60.00	4.00	Bacteriostatic
S. Typhi OAU 152	15.00	60.00	4.00	Bacteriostatic
K. pneumoniae OAU 49	7.50	15.00	2.00	Bactericidal
E. coli OAU 382	15.00	120.00	8.00	Bacteriostatic
S. dysenteriae OAU 221	15.00	60.00	4.00	Bacteriostatic
A. baumanni OAU 234	15.00	30.00	2.00	Bactericidal

Table 4 shows the death rate of *B. cereus* and *K. pneumoniae* by methanolic extract of *A. zygia*. The methanolic crude extract of *A. zygia* leaf showed a significant bacteriocidal and bacteriostatic activity against *B. cereus* and *K. pneumoniae* over the time range (15-120 min) at different MIC concentrations.

The kill- rate by methanolic extract of *A. zygia* on *B. subtilis* was dose dependent. At 60 µg/ml (4 x MIC), the rate of killing of the bacterium was 45.3% at the first 30 min of contact. This rate continued with time. At 90 min of exposure of the bacterium to the extract, the death rate was 100%. Meanwhile, at 120 min of exposure, the population of the bacterium was reduced by 73 % and 68.82 % at MICs- 30 µg/ml and 15 µg/ml, respectively (Table 4).

Table 4. Total viable count of *Bacillus subtilis and Klebsiella pneumoniae* at different minimum inhibitory concentrations of methanolic extract of *A. zygia*

	Death rate (%)					
Exposure time (minutes)	4 X MIC (60 µg/ml)		2 X MIC (30 µg/ml)		1 X MIC (15 µg /ml)	
	B. subtilis	*K. pneumoniae*	*B. subtilis*	*K. pneumoniae*	*B. subtilis*	*K. pneumoniae*
0	0	0	0	0	0	0
15	45.30	79.33	33.86	75.71	30.00	28.0
30	48.82	85.33	70.0	75.86	33.53	32.22
45	81.76	86.33	70.71	79.29	36.47	33.33
60	94.71	90.0	70.71	80.0	50.58	35.56
75	96.47	91.0	70.71	83.57	57.64	41.11
90	100	98.0	71.43	84.29	67.06	46.67
105	100	98.0	73.0	85.71	67.06	47.78
120	100	98.0	73.0	85.71	68.82	47.78

The time-kill assay of methanolic extracts of *A. zygia* against *K. pneumoniae* showed that the bacterial response to the extract was dose dependent. The highest rate of kill was observed at 60 µg/ml (4 X MIC) as there was total reduction in cells population starting from 90 min of exposure (Table 4). The activity of the MIC-15 µg/ml of the extract was the least. After the first 30 min of exposure of *K. pneumoniae*, the rate of killing was 32.22, 77.86 and 85.33 % 15 µg/ml, 30 µg/ml and 60 µg/ml, respectively (Table 4).

The effect of the methanolic leaf extract of *A. zygia* on protein leakage in *B. subtilis* and *K. pneumoniae* is shown in table 5. The protein leaked was higher in *B. subtilis* than *K. pneumoniae* at 60 µg /ml and 30 µg/ml when exposed for 2 h. The difference in the protein leakage from the two selected bacteria was not statistically significant using unpaired t test ($F = 2.203$, $P = 0.0565$).

Table 5. Protein leakage from *B. cereus* and *K. pneumoniae* by methanolic extract of *A. zygia*

Time (Min)	Organisms	Minimum Inhibitory Concentrations (µg/ml)		
		30 µg/ml	15 µg/ml	7.5 mg/ml
0	*B. subtilis*	0	0	0
	K. pneumoniae	0	0	0
30	*B. subtilis*	545.53±46.94	384.66±24.93	198.07±15.45
	K. pneumoniae	282.25±52.38	292.91±36.93	209.94±19.46
60	*B. subtilis*	517.14±23.12	486.57±46.93	291.33±23.57
	K. pneumoniae	361.31±82.56	240.32±34.33	222.27±46.52
120	*B. subtilis*	587.97±41.94	540.74±45.91	360.44±47.93
	K. pneumoniae	370.74±56.83	340.57±60.00	358.38±37.22
180	*B. subtilis*	593.78±65.24	529.92±40.09	354.93±43.92
	K. pneumoniae	423.22±51.99	401.66±39.42	381.87±65.31
240	*B. subtilis*	671.26±42.75	641.70±92.73	370.92±40.05
	K. pneumoniae	457.18±56.94	432.43±38.92	329.69±85.57

The amount of potassium ion leaked out of the cytoplasm of the bacteria was concentration dependent but not time dependent (Figures 1 and 2). From the *B. subtilis,* the amount of K^+ leaked were 35.74% at 15 µg/ml (1 X MIC), 46.93% at 30 µg/ml (2 X MIC) and 77.80% at 60 µg/ml (4XMIC) at the 1 h of exposure of the bacterium to the extracts (figure 1).

Exposure of *K. pneumoniae* to *A. zygia* methanolic extract at 2 h led to potassium leakage of 63% at 15 µg/ml (1 X MIC), 60% at 30 µg/ml (2 X MIC) and 83% at 60 µg/ml (4 X MIC) (Figure 2). There was no significant difference between K^+ leaked at 15 µg/ml (1 X MIC) and 30 µg/ml (2 X MIC) from both *B. subtilis* and *K. pneumoniae*.

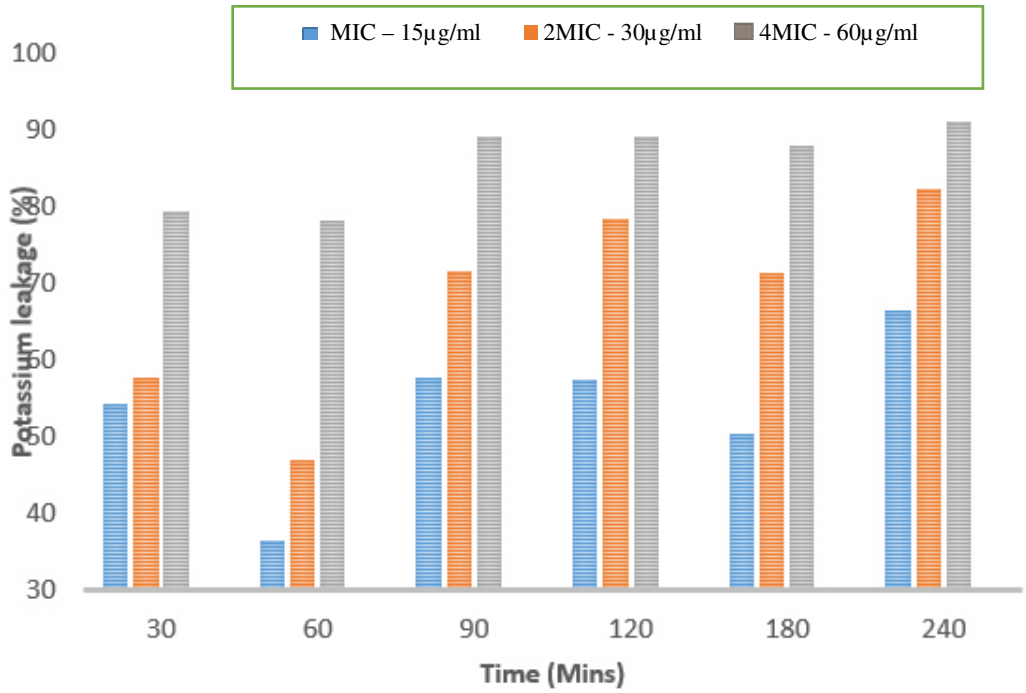

Figure 1: The percentage of potassium leakage from the *B. subtilis* after exposure to different concentrations of *A. zygia*

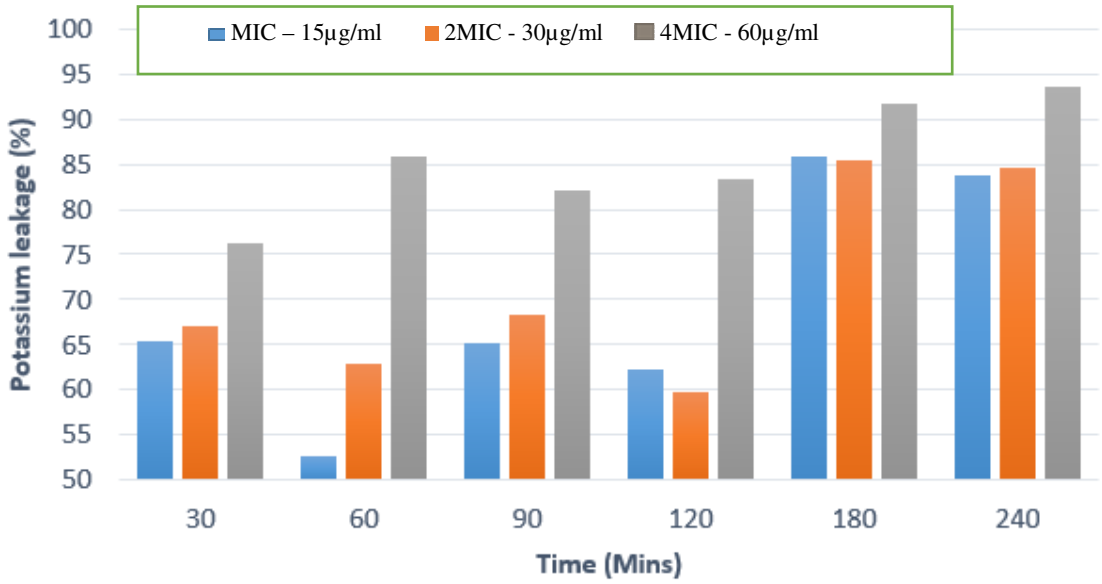

Figure 2: The percentage of potassium leakage from the *K. pneumoniae* after exposure to different concentrations of *A. zygia*

One way analysis of variance showed no significant statistical difference (P=0.629) between potassium ion (K^{+}) leaked at 15 µg/ml (1 X MIC) and 30 µg/ml (2 X MIC) from both *B. subtilis* and *K. pneumoniae*.

Phytochemical analysis of the leaf showed the presence of alkaloid, flavonoid, tannin, saponin anthraquinone glycosides, while anthocyanosides and cyanogenic glycosides were absent (Table 6).

Table 6. Phytochemical groups in *Albizia zygia* leaf

Chemical constituent	Leaf
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Anthraquinone	+
Cyanogenic glycosides	–
Anthocyanosides	–
Cardiac glycoside	+

4.0 Discussion

Methanolic leaf extract of *A. zygia* shows a considerable antibacterial activity as it produced a wide range of diameter of zone of inhibition against most of the test bacteria at 30 and 50 mg/ml. This indicates that the methanolic leaf extract of *A. zygia* possesses some inhibitory properties. The non-antibacterial effect of both the aqueous and ethanolic leaf extract of *A. zygia* may probably due to insolubility of the bioactive components in water and ethanol. Similarly, the non -antifungal activity of all the extracts may be due to impermeability of complex fungal wall.

Albizia zygia has traditionally been used in the treatment of many types of ailments like pain, inflammation and microbial infections (Ndjakou *et al.*, 2007; Achinto and Munirrudin, 2009).

The methanolic extract of *A. zygia* was effective against both Gram-positive and Gram negative test bacteria thereby conferring on it a broad spectrum antibiotic activity. The inhibitory effect of the extract on Gram-negative and Gram-positive test bacteria varies. This may likely be because the antimicrobial may not be targeting the cell wall that makes the major difference between the two groups of the organisms. Its site of action may be either inhibition of DNA synthesis through topoisomerase or attack on other sites (Guittat *et al.*, 2003). The methanolic extract shows a considerable inhibitory effect on *Salmonella* Typhi. This collaborates the work of Ndjakou *et al.* (2007) which reported the inhibitory effect of *A. zygia* on *Salmonella* sp in mice. The MIC (15 mg/ml) of the *A. zygia* against *B. subtilis* was similar to the MIC- 15 mg/ml reported by Achinto and Munirrudin (2009). The antibacterial activity of the leaf extract compared positively with the reference antibiotic (streptomycin) particularly against *B. subtilis* and *Clostridium sporogenes.* The MIC index shows that the extract has both bactericidal and bacteriostatic effects on the susceptible test bacteria. Achinto and Munirrudin (2009)

reported a MIC values of *A. zygia* extract (15 mg/ml) on *Bacillus* sp., 50 mg/ml on *Corynebacterium diphtheriae* and > 400 mg/ml on *Proteus mirabilis* and *Vibrio cholera.* Meanwhile, the MIC recorded against the test bacteria in this study ranged from 7.5 to 30.0 mg/ml. A low MIC indicates a better antibacterial agent.
The death rate of the susceptible test bacteria was high. This is a clear evidence that the extract has different phytochemicals that could be responsible for this action. Certain phytochemicals were present in the leaf extract. Phytochemicals have been found to be effective against bacteria *in vitro* and *in vivo.* They are the sole factor that responsible for the bacteriostatic and bacteriocidal effects against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls, lyses of the cell wall and cell membranes of the bacteria (Omulokoli *et al*., 1997).

Medicinal plants have been reported to have several mechanisms of actions and the leakage in the protein from the test bacteria in the study indicates destabilization of cytoplasmic and plasma membranes as indicated by Puupponen-Pimia *et al.* (2004). Handa *et al.* (2008) also reported that some phytochemicals can disrupt the plasma membrane by localized hyper-acidification and disruption of membrane transport and/or electron transport. They could also cause structural and functional damages to plasma membrane (Tsuchiya and Iinuma, 2000).

The leakage of the electrolyte and protein from the cytoplasmic of the two bacteria is dose dependent. The higher the concentration, the higher the amount of potassium ion and protein leaked out. The leakage of electrolyte and protein from the cells is clear evidence of leakages of ions, adenosine triphosphate (ATP), nucleic acids and amino acids from the cell (Tassou *et al.*, 2000).

Conclusion

The methanolic leaf extract of *A. zygia* shows a considerable inhibitory effect on some of the test bacterial pathogens, but did not possessed any antifungal property. Antimicrobial potential of methanolic leaf extract of *A. zygia* could be harnessed and complemented with synthetic drug therapy against infections caused by the susceptible test bacteria. The mechanism(s) of action and the active compound is still open to investigation.

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