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Full Length Article

Membrane-Active Antibacterial Compounds in Methanolic Extracts of *Jatropha curcas* and their Mode of Action against *Staphylococcus aureus* S1434 and *Escherichia coli* E216

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Abstract

This research presents the antibacterial potential and mode of action of related active compounds of kernel meal, leaves, stem bark, root bark and root wood extracts of *Jatropha curcas* Linn. plant on *Staphylococcus aureus* S1434 and *Escherichia coli* E216. At double MIC (minimum inhibitory concentration) value, cell viability of *S. aureus* S1434 was inhibited by all extracts, but only kernel meal and root wood extracts inhibited *E. coli* E216. At half MIC, the μ_{24} (decrease in cell viability after 24h) for *S. aureus* S1434 was 69 and 66%, while that of *E. coli* E216 were 44 and 42% in the presence of kernel meal and leaves extract, respectively. However at double MIC, less than 5% of viable cells of *S. aureus* S1434 were detected in the leaves and root bark extracts after 5h. Conversely, less than 5% of the viable cells of *E. coli* E216 were detected in the presence of kernel meal and root wood extract after 7.5h. Loss of 260_{nm} absorbing compounds and proteins from bacterial cells was directly proportional to the time of exposure of cells to the extracts. All extracts caused bacterial cells to lose their ability to tolerate salt (NaCl) at double MIC value. The loss of 260_{nm} absorbing compounds, proteins and the loss of tolerance to NaCl suggest that leaves, root bark and kernel meal damaged the bacterial cell membrane. The analysis of bioactive compounds by GC-MS confirmed the presence of acetic acid, hexadecanoic acid, citric acid, 9-octadecenoic acid as the major membrane-active antibacterial compounds.

Keywords: Antibacterial mechanism; Minimum inhibitory concentration; Membrane damage; XTT assay

Introduction

Jatropha curcas Linn. (*J. curcas*) belongs to the family of *Euphorbiaceae* and it is a drought resistant shrub which is widely grown in Central and South America, South-east Asia, India and Africa. It has gained importance in Malaysia, as a source of seed oil for biofuel production. In many African and Asian countries *J. curcas* plant has been initially considered a traditional herb to cure various ailments ranging from simple fevers to infectious diseases including sexually transmitted diseases (Pandey *et al.*, 2012).

The leaves have been used against cough and as an antiseptic after birth (Debnath and Bisen, 2008). The strong antimicrobial activities of the branches render it suitable as a chewing stick in Nigeria (Kayode and Omotoyinbo, 2009). The kernel and the oil are used as purgative and to treat syphilis (Thomas *et al.*, 2008). The ethnomedical practice in West African showed the application of leaves in different forms to cure various ailments like fever, mouth infections, jaundice, guinea worm sores and joint rheumatism (Thomas *et al.*, 2008; Aiyelaagbe *et al.*, 2011). The roots of *J. curcas* have been used after decoction as a mouthwash for bleeding gums, toothache, eczema, ringworm, and scabies, and to cure dysentery (Carels, 2009; Aiyelaagbe *et al.*, 2011).

Recently, Viswanathan *et al.* (2012) reported the stigmasterol, β-amyrin, friedelin and R (+) 4-hydroxy-2-pyrrolidinone as antimicrobial compounds present in the methanolic extract obtained from the leaf of *Jatropha tanjorensis*. Similarly, another study counducted by Oskoueian *et al.* (2011) reported the phenolics, flavonoids together with saponins and phorbol esters as antibacterial compounds detected in the methanolic extract of *Jatropha curcas* kernel. These information and evidences support the fact that this plant possesses antibacterial activity.

Despite of the reports indicating the antibacterial potential of *Jatropha curcas* plant, information on the mechanism of antibacterial action of extract and related

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bioactive compounds are still lacking. Hence, this study was conducted to determine how methanolic extracts of different parts of *J. curcas* Linn. plant affect pathogenic bacterial species like *Staphylococcus aureus* S1434 and *Escherichia coli* E216 and what are the active antibacterial compounds present in the extracts.

Materials and Methods

Collection of Plant Materials

Ripe *Jatropha curcas* Linn. seeds were obtained from the Malaysian Agricultural Research and Development Institute (MARDI), whereas the whole plant was freshly collected from Universiti Putra Malaysia farm (GPS location of 3°0'26.91"N latitude and 101°42'13.24"E longitude). A voucher specimen (SK1764/2010) was deposited in the Phytomedicinal Herbarium, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

Preparation of Extracts

The leaves, stem bark, root bark, root wood and kernel seeds were separated manually. The materials were cleaned with sterile distilled water, air dried and finely ground using a grinder mill. Ground kernels were defatted in a Soxhlet apparatus using petroleum ether (boiling point of 40-60°C) for 16 h at 40°C (AOAC, 1990). The kernel meal residue was dried in the oven at 50°C to remove the solvent. Thirty two grams of each sample were placed in 800 mL of methanol and refluxed at 50°C for 60 min (Chen *et al.*, 2007). The extracts were filtered through Whatman filter paper No. 1 and were then evaporated to dryness using a rotary evaporator (Buchi) at 40°C. The residues obtained were dissolved in 1% dimethyl sulfoxide (DMSO). Different concentrations (half, one and double MIC) of methanolic extracts of various plant parts used were derived from minimum inhibition concentration (MIC) values previously determined by broth microdilution (Namuli *et al.*, 2011). The MIC values for the leaves, stem bark, root bark, root wood and kernel against *S. aureus* were 3.13, 0.39, 0.78, 1.37 and 1.56 mg/mL, respectively. The MIC values for the leaves, stem bark, root bark, root wood and kernel against *E. coli* E216 were 0.78, 3.13, 1.95, 14.06 and 12.50 mg/mL, respectively.

Bacterial Species

The clinical isolates *S. aureus* S1434 and *E. coli* E216 were obtained from the Institute for Medical Research, Malaysia.

Preparation of Inocula

A 24 h bacterial culture was centrifuged at 12,000×g for 10min at 4°C and the pellet harvested was washed twice using phosphate buffer solution (PBS) at pH 7.0. After washing, the pellet was dissolved in PBS and the optical density (OD) of the suspension was adjusted to 0.8 at 600 nm using a spectrophotometer (Ultrospec® 2100 pro).

Cell Viability

Cell viability was assessed using calorimetric method based on 2,3-bis[2-methyloxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT) reduction assay (Moriarty *et al.*, 2005). The experiment was performed in sterile McCartney bottles containing 2 mL inoculum and extract (at half, one and double MIC values) and were filled with broth to a final volume of 20mL. Bacterial growth without plant extract and blank without inoculum were used as controls. The bottles were incubated in a water bath shaker at 37°C and 120 rpm. One millilitre of each sample was removed at 0, 2.5, 5, 7.5 and 24 h and processed for the XTT reduction assay.

A solution of 0.5 mg/mL XTT was prepared using PBS. The solution was filter sterilized using 0.2 µm filter and stored at -70°C. Prior to each assay, an aliquot of XTT was thawed and menadione (10 mM prepared in acetone) was added to a final concentration of 50 µM. Each 200 µL sample in a vial was centrifuged at 8000x*g* for 5 min and the supernatant was removed. Two hundred micro-litre of XTT was added to the pellets. The samples were vortexed and poured into a 96 microtitre plate, which was then incubated in the dark for 2h at 37°C to allow XTT reaction with the cells. The OD resulting from the change in XTT colour (yellow to orange) was measured at 492_{nm} using a microtiter plate reader (Mindray). The final OD was obtained by subtracting the OD values of the blank. The experiment was carried out in triplicates and the decrease in cell viability after 24 h was calculated as follows:

$$\mu_{24} = OD_{(positive\ control)} - OD_{(treatment)}/OD_{(positive\ control)}$$

Salt Tolerance

Preliminary tests were carried out to determine the salt tolerance of *S. aureus* S1434 and *E. coli* E216 (Sampathkumar *et al.*, 2003) because different bacterial species tolerate different salt concentrations, some are halo-tolerant while others are not. The concentrations of NaCl at 75 and 20 g L^{-1} were then selected for *S. aureus* S1434 and *E. coli* E216, respectively. The bacterial cells were treated with methanolic extracts and were then tested for their ability to tolerate salt. Suspensions of bacteria were treated with different plant extracts in broth at half, one and double MIC values for 1 h (Carson *et al.*, 2002). The cells from the broth were collected by centrifugation (12,000×*g*) and washed once with PBS. The cells were re-suspended in 1.0 mL of PBS and 200 µL of this suspension was added to (i) 2.0 mL of PBS and (ii) 2.0mL of PBS containing NaCl at the concentrations of 75 or 20 g L^{-1}. After mixing, the OD_{680} was measured using a spectrophotometer (Ultrospec® 2100 pro). The experiment was carried out in triplicates. The tolerance to NaCl was indicated by the reduction in OD_{680}, which was calculated by subtracting the mean value of three

measurements in PBS from the mean value of three measurements in PBS containing 75 or 20 g L^{-1} of NaCl for the respective test organisms. In each case, the change in OD obtained was expressed as a percentage of the mean value obtained with PBS alone (Sampathkumar *et al.*, 2003).

Cellular Leakage

Leakage of cytoplasmic contents was determined by using Ultrospec® 2100 pro spectrophotometer (Carson *et al.*, 2002; Sampathkumar *et al.*, 2003). The OD of the cell filtrates after exposure of the bacterial cells to the crude extracts was measured at 260_{nm} (for nucleic acids and aromatic amino acids) and 595_{nm} (for proteins). A portion of the extract was added to fresh sterile water in a 20 mL bottle in an amount which would achieve concentration of half, one and double MIC values after addition of 2 mL of the inoculum. Bacterial growth without plant extract and blank without inoculum were used as controls. Then, 4 mL of each test sample were removed immediately after addition of inoculum, at 30 and 60 min of incubation. The samples were filtered through a 0.2 µm membrane filter into a sterile test tube. The absorbance at 260_{nm} (OD_{260}) was measured for nucleic acids placed in quartz cuvettes. The mean OD_{260} was expressed as proportion of the initial OD_{260}. The presence of DNA in the cell free filtrates obtained after 60min was assessed by running 1.0 mL aliquots of the phenol-chloroform-isoamyl alcohol (25:24:1)(v/v) concentrated supernatants on a 0.8% agarose gel. The remaining filterate was used to quantify the amount of proteins by Bradford assay (Bradford, 1976). A hundred micro-litres of each filterate and 5 mL of Bradford reagent was added to a sterile test tube. The mixture was vortexed and incubated for 5 min at room temperature and absorbance measured at 595_{nm}. The standard curve of bovine serum albumin was used to as a reference. The experiment was carried out in triplicates.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The method of Hossain and Rahman, (2011) was used to characterize the chemical composition of extracts using a GC-MS. In this study Schimadzu QP2010PLUS system was used. Six micro-liter of each extract were injected and analysed on a BPX-5 SGE ultra-low-bleed 5% phenyl polydimethylsiloxane capillary column (30m × 0.25mm i.d. × 0.25µm film thickness). The purge time of 1.0min with splitless injection. The helium gas was used as carrier at a flow rate of 1mL min^{-1}. The temperature of the column was adjusted at 50°C for 3min followed by increasing 5°C min^{-1} upto to 80°C and then at 10°C min^{-1} upto 340°C. The temperature of inlet and detector was 250°C and 340°C, respectively and the solvent delay was 4 min. The peaks were identified based on computer matching of the mass spectra with the National Institute of Standards and Technology (NIST 08 and NIST 08s) library and according to the published data.

Gas Chromatography (GC) Analysis

The concentration of acetic acid present in the extracts of different parts was determined by Gas Chromatography (Shimadzu GC-14A). The 20 mM 4–methyl-n-valeric acid was used as an internal standard. A volume of 0.5mL(each extract) was added to 0.5 mL of the internal standard. The column used was packed with 10% (w/v) PEG 600 on Shimalate TPA 60/80. The temperature of oven was maintained at 160°C, the FID at 230°C and the injector port at 230°C. The carrier gas was nitrogen (20 mL min^{-1}). One microlitre of sample was injected into the column. The experiment was carried out in triplicates.

Statistical Analysis

General linear models (GLM) procedure of SAS in a completely randomized design (CRD) was used to analyse the data and the means were compared with Duncan's Multiple Range test. The differences were considered significant when the p value was <0.05.

Results

Cell Viability

Fig. 1 shows the effect of different *J. curcas* plant part extracts at half MIC on the cell viability of *S. aureus* 1434 and *E. coli* E216. In both species, the μ_{24} was significantly ($P<0.05$) reduced when compared to the control. However, more reduction in μ_{24} was observed with *S. aureus* 1434 compared to that of *E. coli* E216. The μ_{24} for *S. aureus* S1434 was 69 and 66% in the presence of kernel meal and leaves extract, respectively, whereas μ_{24} for *E. coli* E216 was 44 and 42% in the presence of kernel meal and leaves extract, respectively.

As observed in Fig. 2, different *J. curcas* plant part extracts at MIC value showed varying effects on the cell viability of *S. aureus* 1434 and *E. coli* E216. With *S. aureus* S1434, each extract, except the root wood showed a decrease in the number of viable cells between 0 and 5h. The viable cells of *E. coli* E216 significantly decreased ($P<0.05$) between 0 and 7.5h in the presence of root wood and kernel meal extracts. However, no decrease in cell viability was observed in the presence of leaves, stem bark and root bark extracts.

The effect of different *J. curcas* plant part extracts at double MIC on the cell viability of *S. aureus* 1434 and *E. coli* E216 is shown in Fig. 3. With *S. aureus* S1434, less than 5% of viable cells were detected in the leaves and root bark extracts after 5h, but after 24 h in the presence of stem bark and kernel meal extract. Conversely, less than 5% of

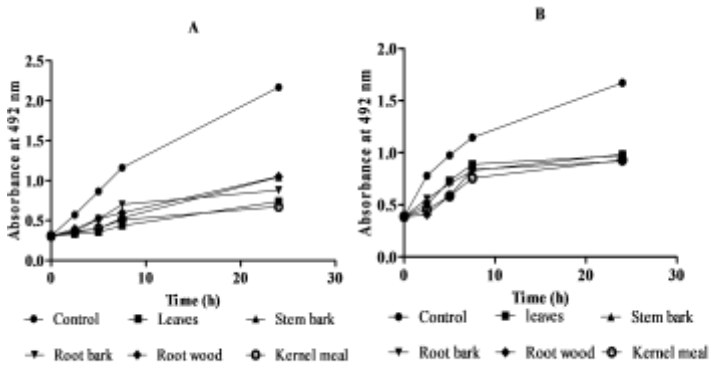

Fig. 1: Cell viability of (A) *S. aureus* S1434 and (B) *E. coli* E216 in broth media supplemented with different plant part extracts at half MIC

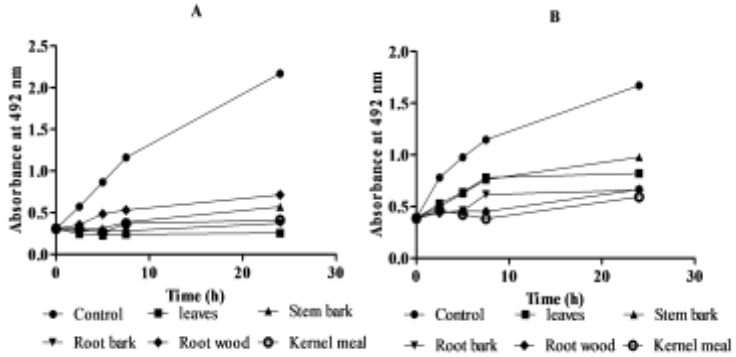

Fig. 2: Cell viability of (A) *S. aureus* S1434 and (B) *E. coli* E216 in broth media supplemented with different plant part extracts at MIC

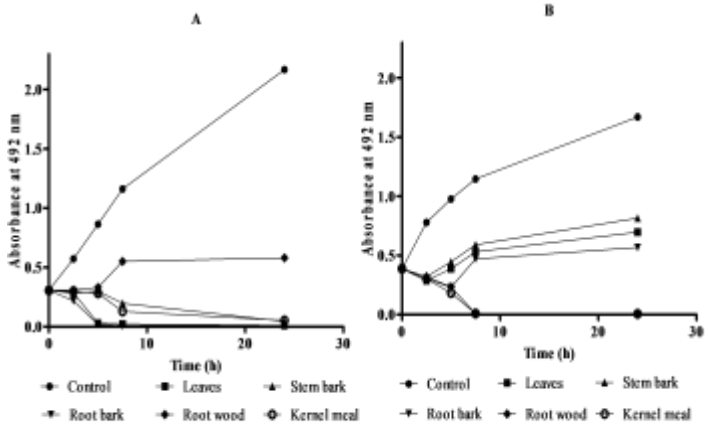

Fig. 3: Cell viability of (A) *S. aureus*S1434 and (B) *E. coli* E216 in broth media supplemented with different plant part extracts at double MIC

the viable cells of *E. coli* E216 were detected in the presence of kernel meal and root wood extract after 7.5 h, whereas increase in cell viability was observed even after 24 h in the presence of stem bark, root bark and leaves extract.

Salt Tolerance

Fig. 4 shows the salt tolerance of *S. aureus* S1434 and *E. coli* E216 cells in PBS containing 75 or 20g/L NaCl,

Table 1: Absorbance at 260_{nm} of cell-free filtrates of *S. aureus* S1434 and *E. coli* E216 cells exposed to different concentrations of various plant part extracts

Concentration (mg/mL)	Bacterial species	Proportion of initial OD_{260} (after 30min)						Proportion of initial OD_{260} (after 60min)					
		L	SB	RB	RW	KM	C	L	SB	RB	RW	KM	C
Half MIC	*S. aureus* S1434	1.05^{a}	1.05^{a}	1.11^{a}	1.03^{a}	1.10^{a}	1.03^{a}	1.16^{ab}	1.09^{b}	1.13^{b}	1.12^{b}	1.28^{a}	1.06^{b}
	E. coli E216	1.06^{a}	1.04^{a}	1.09^{a}	1.02^{a}	1.09^{a}	1.01^{a}	1.11^{bc}	1.04^{c}	1.24^{b}	1.03^{c}	1.40^{a}	1.01^{c}
MIC	*S. aureus* S1434	1.07^{b}	1.06^{b}	1.15^{a}	1.04^{b}	1.17^{a}	1.03^{b}	1.18^{ab}	1.09^{b}	1.33^{a}	1.16^{b}	1.32^{a}	1.06^{b}
	E. coli E216	1.09^{ba}	1.04^{b}	1.11^{ba}	1.06^{b}	1.18^{a}	1.01^{c}	1.13^{c}	1.04^{cd}	1.22^{b}	1.10^{c}	1.44^{a}	1.01^{c}
Double MIC	*S. aureus* S1434	1.08^{b}	1.09^{b}	1.22^{a}	1.10^{b}	1.23^{a}	1.03^{b}	1.19^{b}	1.14^{bc}	1.42^{b}	1.17^{bc}	1.41^{a}	1.06^{c}
	E. coli E216	1.10^{b}	1.04^{bc}	1.11^{b}	1.06^{bc}	1.21^{a}	1.01^{c}	1.50^{a}	1.13^{c}	1.50^{a}	1.25^{b}	1.53^{a}	1.01^{c}

Different superscripts in each row (at the same time) indicate significant difference (P<0.05). L, SB, RB, RW, KM and C represent leaves, stem bark, root bark, root wood, kernel meal and control, respectively

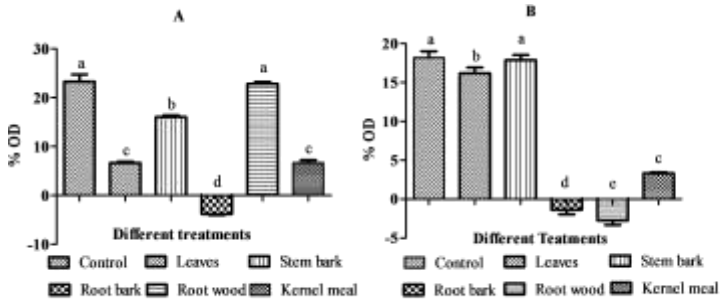

Fig. 4: Salt tolerance of A) *S. aureus* S1434 and B) *E. coli* E216 cells in 75 gL^{-1} and 20 gL^{-1} of NaCl, respectively, following 1 h of treatment with half MIC value of different plant part extracts. Different letters indicate significant difference at P<0.05. Percentage OD was calculated as [Mean OD_{680} of the sample containing bacterial cell + NaCl] – [Mean OD_{680} of the sample containing bacterial cell]/[Mean OD_{680} of the sample containing bacterial cell]*100. Bar indicates SD

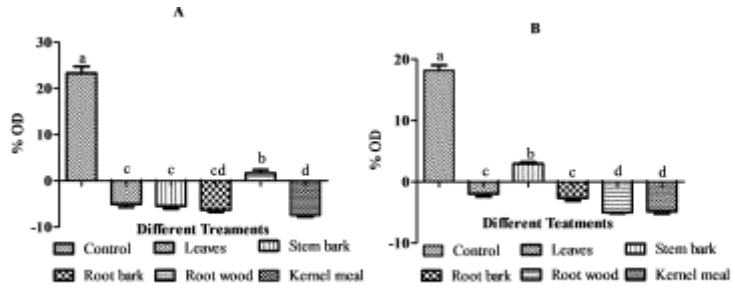

Fig. 5: Salt tolerance of A) *S. aureus*S1434 and B) *E. coli* E216 cells in 75 gL^{-1} and 20 gL^{-1} of NaCl, respectively, following 1 h of treatment with MIC value of different plant part extracts. Different letters indicate significant difference at P<0.05. Percentage OD was calculated as [Mean OD_{680} of the sample containing bacterial cell + NaCl] – [Mean OD_{680} of the sample containing bacterial cell]/[Mean OD_{680} of the sample containing bacterial cell]*100. Bar indicates SD

respectively, following 1h of treatment with different extracts at half MIC value. Both species showed significant differences (P<0.05) in the percent OD except the root wood extract (*S. aureus* S1434) and stem bark (*E. coli* E216) which showed no significant difference (P<0.05) compared to the control. The bacterial cell membrane of *S. aureus* S1434 and *E. coli* E216 cells were more damaged by root bark and root wood extracts, respectively.

The salt tolerance of *S. aureus* S1434 and *E. coli* E216 cells in PBS containing 75 or 20 g L^{-1} NaCl, respectively, following 1h of treatment with different extracts at MIC value is shown in Fig. 5. Both species showed significant differences (P<0.05) in the percent OD. The bacterial cell membrane of *S. aureus* S1434 was more damaged by kernel meal extract and least affected by the root wood extract, whereas *E. coli* E216 cells were more damaged by kernel meal and root wood extracts.

Fig. 6 shows the salt tolerance of *S. aureus* S1434 and *E. coli* E216 cells in PBS containing 75 or 20 g L^{-1} NaCl,

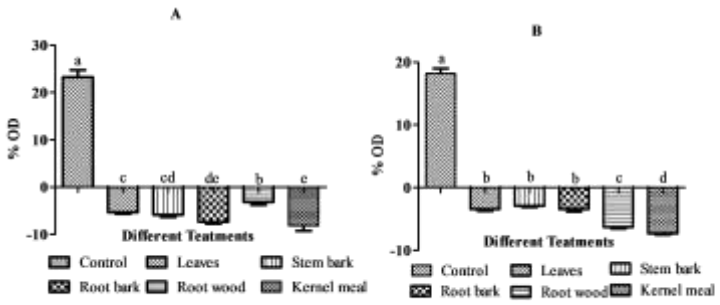

Fig. 6: Salt tolerance of A) *S. aureus* S1434 and B) *E. coli* E216 cells in 75 $gL^{-1}$and 20 gL^{-1} of NaCl, respectively, following 1 h of treatment with double MIC value of different plant part extracts. Different letters indicate significant difference at P< 0.05. Percent OD was calculated as follows; [Mean OD_{680} of the sample containing bacterial cell + NaCl) – [Mean OD_{680} of the sample containing bacterial cell)]/[Mean OD_{680} of the sample containing bacterial cell] *100. Bar indicates SD

Table 2: Proteins concentrations in cell-free filtrates of *S. aureus* S1434 cells exposed to different concentrations of different plant part extracts

Concentration (mg/mL)	Bacterial species	Protein concentration (µgmL^{-1}) (after 30min)						Protein concentration (µgml^{-1}) (after 60min)					
		L	SB	RB	RW	KM	C	L	SB	RB	RW	KM	C
Half MIC	*S. aureus* S1434	148.15^{ab}	148.15^{ab}	174.07^{ab}	131.48^{b}	216.67^{a}	0	338.89^{a}	246.30^{b}	329.63^{a}	318.52^{a}	337.04^{a}	0
	E. coli E216	150.00^{bc}	120.37^{c}	157.41^{b}	114.82^{c}	168.52^{a}	0	227.78^{b}	192.59^{c}	331.48^{a}	155.56^{d}	340.74^{a}	0
MIC	*S. aureus* S1434	174.08^{b}	161.11^{b}	325.93^{b}	148.15^{b}	335.19^{a}	0	342.59^{b}	318.52^{b}	485.18^{a}	331.48^{b}	488.89^{a}	0
	E. coli E216	175.93^{b}	124.07^{b}	162.96^{b}	142.59	194.44	0	340.74^{a}	198.15^{b}	361.11^{a}	270.37^{ab}	355.56^{a}	0
Double MIC	*S. aureus* S1434	172.22^{c}	181.48^{c}	298.15^{b}	244.45^{bc}	457.41^{a}	0	514.82^{b}	477.78^{b}	657.41^{a}	496.29^{b}	694.45^{a}	0
	E. coli E216	335.19^{ab}	162.96^{c}	290.74^{b}	166.67^{c}	372.22^{a}	0	437.04^{a}	429.63^{a}	433.33^{a}	412.96^{a}	440.74^{a}	0

Different superscripts in each row (at the same time) indicate significant difference (P<0.05). L, SB, RB, RW, KM and C represent leaves, stem bark, root bark, root wood, kernel meal and control, respectively

respectively, following 1 h of treatment with different extracts at double MIC value. The leaves, stem bark and root bark extracts showed no significant difference (P<0.05) against *S. aureus* S1434, whereas stem bark and root bark extracts showed a significant difference (P<0.05) against *E. coli* E216. Cell membranes of both *S. aureus* S1434 and *E. coli* E216 cells were most affected by the kernel meal extract.

Cellular Leakage

Table 1 shows the OD_{260} after 30 and 60min of treatmentof *S. aureus* S1434 and *E. coli* E216 cells with different concentrations of various plant part extracts expressed as a proportion of the initial OD_{260}. After 60 min, the OD_{260} of filtrates of cells exposed to double MIC value (of different plant part extracts) was significantly (P<0.05) higher than that for control values. Generally for both species, after 30 and 60 min, all extracts (at half MIC value) except kernel meal showed slight or no significant (P<0.05) difference when compared to the control. Table 2 shows the amount of proteins present in the cell free filtrates after 30 and 60min of treatment of *S. aureus* S1434 and *E. coli* E216 cells with different concentrations of different plant part extracts. For both species, no protein was detected in cell filtrates of the control. Generally, the results showed that increasing the time of exposure and the concentrations of plant part extracts had significant (P<0.05) effect on cell leakage. However, more proteins were released from *S. aureus* S1434 cells compared to *E. coli* E216 cells by all extracts at various times except the leaves extract (after 30 min).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The main organic compounds detected by GC-MS in the methanolic extracts are shown in Table 3. Oxalic and acetic acids were found to be the major compounds detected in the leaves, stem bark, root bark and root wood extracts. In addition, the stem bark also contained citric acid. The fatty acids, which were identified in all the extracts, except root bark were hexadecanoic acid (palmitic acid) and 9-octadecenoic acid (oleic acid) in the kernel meal.

GC Analysis

The concentration of acetic acid in the different part of the plant was analysed using GC and leaves, stem bark, root bark, root wood and kernel meal showed the values of 7.35, 6.11, 5.22, 12.91, 2.74 mg acetic acid g^{-1} dry weight, respectively (Table 4).

Table 3: The GC-MS analysis of methanolic extract of various parts of *J. curcas* plant

Plant part	Main compounds[a]	Area %
Leaves	Oxalic acid	43.2
	Acetic acid	31.5
	Hexadecanoic acid	25.3
Stem bark	Oxalic acid,	28.2
	Acetic acid	25.6
	Citric acid	20.12
	Hexadecanoic acid	18.7
Root bark	Oxalic acid	79.3
	Acetic acid	26.4
Root wood	Acetic acid	55.3
	Oxalic acid	19.8
	Hexadecanoic acid	17.4
Kernel meal	9-octadecenoic acid	37.8
	Hexadecanoic acid	21.2
	Oxalic acid	18.9
	Acetic acid	15.7

[a]Selected compounds showed above 10% area.

Table 4: The acetic acid content of different plant parts analysed by GC

	Leaves	Stem bark	Root bark	Root wood	Kernel meal
Concentration (mg g^{-1} DW)*	7.35	6.11	5.22	12.91	2.74

*Concentration determined by gas chromatography
DW: Dry weight

Discussion

As regards cell viability, the *E.coli* cells were less affected compared to those of *S. aureus* S1434, because *E. coli* species possess effective permeability barriers comprising of the outer membrane, which restrict penetration of antimicrobial compounds and also possess efflux systems that extrude the antibacterial agents out of the cell (Tenover, 2006). Both bacterial species showed bacteriostatic activity when extracts were used at half or MIC and showed bactericidal activity when double MIC value was used.

The results of the salt tolerance test demonstrated that *S. aureus* species could better tolerate higher NaCl concentration (75 g L^{-1}), than that of *E. coli* (20 g L^{-1}). In contrast, the halophilic bacteria such as *S. aureus* can withstand hyper osmotic conditions by increasing the solutes inside their cells like glycine, betaine, ectoine, proline, glutamate and trehalose (Ikeuchi *et al.,* 2003). In fact, the outer membrane determines the bacteria's ability to tolerate any particular change in a set of ionic conditions (Brown and Turner, 1963) thus, loss of tolerance to salts or other potentially toxic compounds may reveal membrane damage or weakening (Iandolo and Ordal, 1966). In the present study, it is proposed that the extracts caused membrane damage during the one hour of incubation by increasing the membrane permeability and this caused the decrease in absorbance since plasmolysis did not occur when the cells were placed in PBS containing NaCl. It has been reported that in a plasmolysed cell protoplast, the scattering of light increases and this is what eventually results in increase in OD (Korber *et al.,* 1996). In the current study, the absorbance (OD_{680}) decreased (interpreted as loss in tolerance to salt) with increase in the concentration of different plant part extracts.

A marked leakage of cellular material indicates irreversible damage to the cytoplasmic membrane (Hugo and Longworth, 1964). Antibacterial agents such as polymyxins which is an antibiotic (Tenover, 2006) and *Psidium guajava* plant extract (Henie *et al.,* 2009) have been reported to disrupt the membrane by increasing bacterial membrane permeability and causing leakage of bacterial contents. The data obtained in the present study suggested that nucleic acids and amino acids leaked out of the bacterial cells. However, the agarose gel electrophoresis experiments did not show any DNA being present in the cell free filtrates. This could be attributed to the fact that the amount of DNA that leaked out of the cell was too little to be detected by agarose gel electrophoresis as it relies on visual detection and the compounds detected at 260_{nm} were not DNA but RNA and other aromatic amino acids that absorb light at 260_{nm} (Cleaves and Miller, 1998). The suggestions were confirmed when the compounds that absorb at 260_{nm} which had leaked out of the *S. aureus* MF 31 cell after heating were RNA (Iandolo and Ordal, 1966).

The bactericidal or bacteriostatic effect observed with different concentrations of various extracts against the tested microorganisms could be attributed to the presence of organic and fatty acids detected in the extracts as shown in Table 3 and 4. The interaction of these hydrocarbons with the hydrophobic structures of bacteria has been reported to result in antimicrobial activity (Sikkema *et al.,* 1995; Cowan, 1999; Vaquero *et al.,* 2007). The antibacterial activity of 9-octadecanoic acid and hexadecanoic acid against *S. aureus* and *E. coli* has been reported by Pu *et al.* (2010). Similarly acetic and hexadecanoic acids as the main antibacterial compounds have been reported in the aqueous extract of pine needles (Feng *et al.,* 2010). The result of Koga *et al.* (1996) indicated that, organic acids have a higher bactericidal effect than fatty acids. According to Ryssel *et al.* (2009) acetic acid possesses excellent bactericidal effects toward *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

Consequently, this study revealed that different *J. curcas* plant extracts especially the root bark, leaves and kernel meal methanolic extracts induce antibacterial action through cell membrane damage and oxalic acid, acetic acid, hexadecanoic acid, citric acid and 9-octadecenoic acid are found to be the active-membrane antibacterial compounds.

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