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IN VITRO KINETICS OF STAPHYLOCOCCAL DEATH IN THE STEM BARK EXTRACTS OF *JATROPHA CURCAS* LINN (EUPHORBIACEAE)

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ABSTRACT

Medicinal plants present a promising option in the treatment of infectious diseases. Although literature is replete of the traditional utilization of *Jatropha curcas* for healing various ailments, scientific studies validating its phytochemotherapeutic properties are limited. In this study, both aqueous and methanol extracts of *Jatropha curcas* were tested against thirteen clinical staphylococcal isolates including Methicillin Resistant *Staphylococcus aureus* (MRSA) using agar diffusion and microdilution techniques. Nineteen commonly used antibiotics were employed as control drugs and to determine the resistance profiles of the organisms. Bactericidal activity was evaluated by the time-kill assay. The susceptibility patterns of all the staphylococcal isolates tested were similar in aqueous and methanol extracts. The methanol extract (100mg/ml) demonstrated inhibitory activity against *Staphylococcus haemolyticus*, which was resistant to ciprofloxacin, tetracycline and erythromycin. The MIC and MBC of the methanol extract ranged between 0.5 and 8mg/ml and between 4 and 128mg/ml respectively while that of aqueous extract ranged between 1 and 8mg/ml and between 4 and 128mg/ml respectively. The MRSA strain was sensitive to the methanol extract but resistant to twelve of the 20 antibiotics studied. Within the first 2.5h and 5h of incubation with the methanol extract, the MRSA isolate declined by $1.7\log_{10}$ and $2.3\log_{10}$ respectively while incubation with ciprofloxacin declined the growth by $3\log_{10}$ and $4\log_{10}$ respectively. The methanol extract exhibited the highest killing rates of 13.88×10^5cell/h against *S. haemolyticus*. The lowest killing rate, 1.86×10^5 cell/h, was against MRSA. 1×10^6 of the resistant staphylococcal cells was completely inhibited within 8hrs and there was no growth after 7 days. The methanol stem extract of *Jatropha curcas* exhibited enhanced bactericidal activity against staphylococcal strains and could be indicated for the empiric treatment of staphylococcal infections. Toxicity studies may need to be carried out to authenticate its use.

Keywords: *In-vitro* kinetics, *Jatropha curcas*, *Staphylococci*, stem bark, resistance profile, time-kill assay.

INTRODUCTION

Resistance to commonly available and affordable antibiotics is particularly a major concern in the management of bacterial infections, especially in resource poor countries (Morton, 1980). Among the numerous bacterial infections, those caused by *Staphylococcus* are of great public health importance. These gram-positive spherical bacteria have been described as one of the most versatile human pathogens due to their ability of acquiring resistance to virtually all known antibiotics. The genus is divided into two broad groups; known as coagulase positive staphylococci and mainly represented by *S. aureus* which has emerged over the past several decades as a leading cause of hospital – and community – acquired infections. The second group, coagulase negative staphylococci are very diverse and are implicated in various infectious processes especially in immuno-compromised individuals and those with implant devices such as shunts and catheters (Akinkunmi and Lamikanra, 2010). The emergence of multi-drug resistance in *staphylococci* has led to substantial morbidity and mortality rates all over the world.

One of the important approaches to solving the problem of drug resistance has been to seek structurally novel antibiotics that have entirely different mechanisms of action from the currently used agents. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from plants used in the practices of traditional medicine (Gragg and Newman, 2001). Plant-based therapy plays an essential role in healthcare and it has been estimated by WHO that 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care (Farnsworth *et al*., 1985). In the US alone, use of herbal products increased 380% between 1990 and 1997 (Eisenberg *et al*., 1998). In other parts of the world

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traditional utilization of medicinal plants for healing is extremely frequent. The primary benefits of using plant-derived medicines include their desirable safety profile and a more affordable acquisition cost (Iwu, 1993; Nebedum *et al*., 2009).

Several ethnopharmacological claims have been made regarding *Jatropha curcas* (physic nut), a member of the family Euphorbiaceae. The extracts of *Jatropha curcas* have wide traditional uses for treating inflammatory conditions, toothaches, warts, gum-bleeding, piles, rashes, wounds, ulcers, allergies, burns, leucodema, scabies, tumors and small-pox (Morton, 1980; Elewude, 1986). The chemical constituents of the bark and leaves of *Jatropha curcas* are glycosides, phytostrerols, flavonoids, steroidal saponins, and tannins while the latex portion of the plant contains proteolytic enzymes and essential oils (Morton, 1980)

Although, traditional utilization of *Jatropha curcas* for healing various ailments is evident in Nigeria, well designed studies validating its phytochemotherapeutic properties are limited. Nevertheless, previous studies in our laboratory have shown that the latex of the plant has potent hemostatic, antimicrobial and wound healing effects (Odusote *et al*., 1996, 1999). In addition, preliminary studies using the stem bark extract of the plant show potent antimicrobial activity against different species of staphylococci including methicillin-resistant strains. Consequently, we present this detailed study to investigate the kinetics of antistaphylococcal activities of the bark extract. Our emphasis is on staphylococci because of their high infection rate, great capability for developing resistance and the recent increased recognition of their community acquired infections.

MATERIALS AND METHODS

Plant collection and identification

Jatropha curcas plant materials (stem bark) were collected from a sub-tropical forest reserve in Ogbomoso, Oyo State, Southwest, Nigeria. The plant was identified and authenticated by Professor Olowokudejo, Department of Botany and Microbiology, University of Lagos, Akoka and a voucher specimen was deposited in the departmental herbarium for reference.

Preparation of Plant Extract

Fresh stem bark samples were thoroughly rinsed with purified water and dried in a hot-air oven (Vindon Ltd, England) at 40^{o}C for 6 days (to avoid thermal degradation of the active principle at higher temperatures) and crushed into powder using a Tornado Mill (Pennwalt Corporation, Pennsylvania, US). Water extract was prepared by soaking 500g of the bark powder in 1L sterile de-ionized distilled water for 24hrs at 4^{o}C. The mixture was then centrifuged at 2000rpm for 10min at 4^{o}C. The supernatant was then filtered through a 0.45µm membrane with the aid of a suction pressure and freeze-dried (Modulyo Freeze Dryer, England). The methanol extract was prepared by Soxhlet extraction of 500g of the bark powder with methanol for 10hrs. The solvent was removed under reduced pressure at 45^{o}C to give a crude extract. The crude extract was further dried in a vacuum dessicator over anhydrous copper sulphate. Our preliminary study has shown that the maximum solubility of either extract is about 400mg/mL at room temperature (26 ± 2.0ºC). Concentrations (400, 200, 100, 50, 25, 12.5, 6.25, 3.125mg/ml) of aqueous and methanol extracts were serially prepared for immediate use in the susceptibility screening.

Bacterial Isolates

Staphylococcal isolates were obtained from the Microbiology Laboratory of Lagos University Teaching Hospital (LUTH), Idi-Araba, Lagos, and the Nigerian Institute of Medical Research (NIMR), Lagos. The isolates consisted of a control strain methicillin sensitive *S. aureus* ATCC 25923), two clinical isolates of methicillin sensitive *S. aureus (w-388 and w-415),* a clinical isolate of methicillin resistant *S. aureus (GS4*) and nine clinical isolates of coagulase-negative staphylococci *(S. haemolyticus, S. sciuri, S. warneri, S. chromogens, S. cohnii* (strains SU-1 and SC-2), *S. epidermidis, S. lugdunensis* and *S. xylosus).* Preliminary identification was achieved by colony morphology, Gram-staining, catalase and tube coagulase tests and speciation was done using API kit (BioMerieux, France) according to manufacturers' recommendations.

Preliminary Phytochemical Screening

Solvent extracts were tested for phytochemical components by the simple experiments described by Sofowora (1984). 0.5g of each extract was stirred with 5ml of 1% hydrochloric acid on a steam bath. 1ml of the filtrate was treated with a few drops of Mayer's reagent and Dragendorff's reagent respectively to detect the presence of alkaloids. Other qualitative assay methods were used to detect the presence of tannins, phlobatannins, cardiac glycosides, saponins, anthraquinones, flavonoids, saponins and reducing sugars (Sofowora, 1984).

Antimicrobial Susceptibility Testing

The identified staphylococcal isolates were subjected to susceptibility testing using the disk diffusion technique as recommended by CLSI (2006a) guidelines. All isolates were grown on Mueller-Hinton (MH) agar plates (Hi-Media, India) and suspended in MH broth prior to use for the antimicrobial susceptibility test and adjusted to 0.5 MacFarland. The following antimicrobial agents were tested: amoxicillin (25µg); cloxacillin (5µg); tetracycline (30µg); erythromycin (15µg); cotrimoxazole (25µg); cefuroxime (30µg); cefotaxime (30µg); ceftazidime

(30µg); chloramphenicol (30µg); amoxicillin/clavulanic acid (30µg); teicoplanin (30µg); ciprofloxacin (5µg); ofloxacillin (5µg); gentamycin (10µg); penicillin (10 units); amikacin (30µg); vancomycin (30µg); piperacillin-tazobactam (110µg). Resistance to methicillin was determined by placing a 1µg oxacillin disk (oxoid) on Muller Hinton agar without NaCl and incubated for 24hrs at 35°C. Methicillin Resistance was confirmed using CLSI (2006a) published guidelines for the agar screen test.

Screening of extract for antibacterial activities

Eight concentrations of each extract (400, 200, 100, 50, 25, 12.5, 6.25, 3.125 mg/ml) were prepared and antimicrobial effects of each concentration determined using both the agar-well diffusion and calibrated paper disk technique (Perez *et al*., 1990; Abioye *et al*., 2004). The plates were incubated at 37°C for 18 – 24hrs in Isotemp incubator (Fisher Scientific Co., USA). Respective proper controls of solvent extracts, and control antibiotic – (ciprofloxacin) were maintained. All assays were performed in triplicate and the antimicrobial activity of each plant extract was recorded as the mean diameter of resulting inhibition zones of growth measured in millimeters.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

The MIC and MBC of the extracts against the *S. aureus* strains were determined by the micro-broth dilution method according to the CLSI (2006b) guidelines, using Muller-Hinton broth. The inoculum suspension was prepared from 8h broth cultures and adjusted to obtain 0.5 McFarland turbidity standards. Two fold serial dilutions of the extract were tested to obtain the MIC. The bacterial suspensions were incubated for 24hrs at 37°C. The uninoculated tubes containing growth medium or growth medium and extract were maintained as controls.

Time-Kill Analysis

In vitro killing rates of the *Jatropha curcas* methanol and aqueous extracts and ciprofloxacin were investigated respectively against two highly resistant staphylococcal isolates at a concentration twice the MIC. Inocula were standardized and diluted to 10^6 cfu/ml in fresh Mueller Hinton broth. 1ml volume of freshly prepared extract (methanol or aqueous) or ciprofloxacin at 20 times the final concentration were added to 19ml of broth in 50ml reaction vials while one vial contained only 20ml fresh broth and the organism without the extract or antibiotic (control). The vials were shaken properly and incubated at 37^0C. Viable counts at time 0, 0.5, 1.0, 2.5, 5.0, and 10 hours were determined using 6% Tween 80 as the quenching system (Miles *et al*., 1938). The growth inhibition percentage (1%) of the bacterial strains was calculated according to the following formula:

$$I\% = \frac{N_0 - N_t}{N_0} \times 100$$

where N_0 is the initial number of organisms and N_t is the number of organisms remaining at time t. The extract concentration required to produce 50% (EC_{50}) of the maximal zone of inhibition was obtained by interpolation of concentration-inhibition curves while the time required to reduce the microbial population to 50% (T_{50}) was determined from the survivor – time curves by regression analysis.

Statistical Analysis

All parameters measured were expressed as mean ± standard deviation. Differences between mean values were analyzed by a student t-test at 5% level of significance. $P < 0.05$ was considered to be statistically significant. Data on viable cell count were log-transformed and presented graphically using Microsoft Excel 2003 statistical software.

RESULTS

Phytochemical characteristics of the stem bark extracts of *Jatropha curcas* indicated the presence of soluble carbohydrates, cardiac glycosides, saponins, tannins and free flavonoids while starch, alkaloids and anthraquinones were not detected. The result of the antimicrobial resistance profile of the staphylococcal strains determined with nineteen antibiotics is presented on table 1. Methicillin resistant *S. aureus* (MRSA G-4) was resistant to all the antibiotics tested. *Staphylococcus scuiri* was resistant to 11 of the 19 antibiotics. All the strains studied were also susceptible to ciprofloxacin except MRSA-GS4, *S. cohnii* (strain SU-1) and *S. haemolyticus*. *Staphylococcus aureus* (ATCC 25923) was sensitive to all antibiotics and extracts except amoxicillin, penicillin, ceftazidine and cefotaxime, while *S. warneri* was resistant to cloxacillin only. Two clinical strains of *S. aureus* were resistant to penicillin, amoxicillin, cloxacillin, cotrimoxazole, ceftazidime, cefotaxime, tetracycline and chloramphenicol. The result of antibacterial susceptibility patterns of the stem bark extracts on various strains of staphylococci used in this study is presented in table 2.

10 of the 13 (76.92%) staphylococcal strains studied were susceptible to ciprofloxacin, the control drug. While 4 out of the 13 (30.77%) strains used in this study were resistant to ciprofloxacin, both methanol and aqueous extracts of the stem bark of *Jatropha curcas* inhibited all the tested strains with measurable zones of inhibition. The inhibitory activity of *Jatropha curcas* extracts was more pronounced against *S. haemolyticus* (zone diameter: 27.9 ± 1.14mm). MRSA-GS4 was more sensitive to both the aqeous and methanolic extracts (zone diameter: 13.3 ± 0.82 and 14.5 ± 0.98mm respectively) than ciprofloxacin (10.0 ± 0.58mm). The activity of aqueous extract was not

Table 1. Antibiotic Resistance Profiles of *Staphylococcus* strains used in this study.

Staphylococcus Strains	Antibiotic Resistance
Staphylococcus chromogens	None
Staphylococcus aureus (ATCC 25923)	AMX, PEN, CAZ, CTZ
Staphylococcus aureus (MSSA W-388)	AMX, PEN, CXC, COT, CAZ, CTZ
Staphylococcus aureus (MSSA W-415)	AMX, PEN, TET, COT, CHL, CAZ
Staphylococcus aureus (MRSA G4)	OXA, AMX, PEN, CXC, CAZ, CTZ, TET, ERY, CIP, COT, CHL, AMC, CXM, OFX, GEN, TZP, VAN, AMK, TEC
Staphylococcus warneri	CXC
Staphylococcus haemolyticus	TET, ERY, CIP, CAZ, CTZ
Staphylococcus scuiri	OXA, AMX, CXC, TET, ERY, COT, CHL, AUG, CXM, CAZ, CTZ
Staphylococcus cohnii (SC-2)	AMX, CAZ, TET, PEN
Staphylococcus cohnii (SU-1)	AMX, PEN
Staphylococcus epidermidis	AMX, PEN
Staphylococcus lugdunensis	CAZ, CTZ, AMX, AMC, TET, OFX, PEN, COT, CXC, ERY
Staphylococcus xylosus	CAZ, AMX, CIP, PEN

AMX = Amoxicillin; CXC = Cloxacillin; TET = Tetracycline; ERY = Erythromycin; COT = Cotrimoxazole; CXM = Cefuroxime; CHL = Chloramphenicol; AMC = Amoxicillin/Clavulanic acid; CIP = Ciprofloxacin; OFX = Ofloxacin; GEN = Gentamicin; OXA = Oxacillin; PEN = Penicillin; TZP = Piperazilin/Tazobactam; VAN= Vancomycin; AMK = Amikacin; TEC = Teicoplanin; CAZ = Ceftazidime; CTZ = Cefotaxime

Table 2. Antistapylococcal Susceptibility Pattern of Plant Extract of *Jatropha curcas*.

Microorganisms	Mean Diameter of zone of inhibition in mm (mean ± s.d.)				
	Methanol Extract		Aqueous Extract		***Ciprofloxacin Standard (5µg/ml)***
					Disc Diffusion
S. aureus ATCC25923	21.6 ± 1.19*	22.4 ± 1.23*	18.1 ± 0.71*	18.2 ± 1.01*	33.9 ± 0.81
*S. aureus (*MSSA-W388)	21.8 ± 1.12	22.6 ± 1.15	21.1 ± 0.67	21.2 ± 1.00	20.4 ± 0.55
*S. aureus (*MSSA-W415)	19.1 ± 1.11**	19.2 ± 0.94**	19.8 ± 0.62**	19.1 ± 0.78**	25.5 ± 1.32
*S. aureus (*MRSA-GS4)	14.3 ± 1.21**	14.5 ± 0.98**	13.1 ± 0.67**	13.3 ± 0.82**	10.0 ± 0.58
S. haemolyticus	27.5 ± 1.26*	27.9 ± 1.14*	25.7 ± 0.98*	25.0 ± 0.83*	10.0 ± 1.02
S. sciuri	19.5 ± 0.66**	19.8 ± 0.86**	17.0 ± 0.54**	17 4 ± 0.70**	16.6 ± 0.60
S. warneri	22.1 ± 0.30*	22.6 ± 0.44*	20.2 ± 0.36*	20.5 ± 0.40*	32.4 ± 0.90
S. chromogens	19.5 ± 0.66*	19.8 ± 0.86*	16.0 ± 0.54*	16 4 ± 0.70*	30.6 ± 0.60
S. cohnii (SC-2)	19.6 ± 1.03**	20.1 ± 1.28**	18.2 ± 0.81**	18.5 ± 1.37**	24.0 ± 2.08
S. cohnii (SU-1)	23.1 ± 0.96*	24.0 ± 1.04*	22.0 ± 0.77*	22.8 ± 0.84*	12.0 ± 1.12
S. epidermidis	18.7 ± 2.02*	19.6 ± 0.99*	17.9 ± 0.66*	18 2 ± 0.58*	27.6 ± 2.67
S. lugdunensis	20.5 ± 1.98	21.2 ± 0.88	18.0 ± 1.29	19.0 ± 1.06	21.5 ± 1.59
S. xylosus	22.0 ± 0.77	22.7 ± 1.35	20.2 ± 1.09	21 6 ± 1.28	23.0 ± 0.89

Values of inhibitory zone diameter were expressed as mean ± sd for *Jatropha curcas* extract (100mg/ml) and compared to ciprofloxacin (5 µg/ml). *Statistically significant ($p < 0.05$), ** ($p< 0.01$) compared to the value of ciprofloxacin

statistically different from the methanol extract (P>0.05, n=13) (Table 2). The 100mg/ml concentrations of both extracts have similar activity to 5µg/ml ciprofloxacin against methicillin sensitive strains of *S. aureus* (W-388 and W-415).

Table 3 shows the MICs and MBCs of *Jatropha curcas* extracts. The mean MIC of the extracts ranged from 0.5mg/ml for *S. haemolyticus* to 8.0mg/ml for MRSA-GS4 compared to a range of 0.125µg/ml (*S. aureus* ATCC25923, *S. warneri*, *S. chromogens*) to 8.0µg/ml (MRSA, *S. haemolyticus*) for ciprofloxacin. The MBC of the extracts also ranged from 4.0 to 128mg/ml while that of ciprofloxacin ranged from 0.125 to 16.0µg/ml.

The extracts exhibited lower killing rates compared to the control drug - ciprofloxacin (P>0.05, n=13) (Table 4). Methanol extract exhibited the highest killing rate of 13.88 x 10^5 cfu/h against *S. haemolyticus* and the least rate of 1.86 X 10^5 cfu/h against MRSA. Within the first 2.5 and 5.0hrs of incubation with the extract most strains declined by 1.7 to 5$\log_{10}$ (methanol extract) and 1.0 to 3.8$\log_{10}$ (aqueous extract) while ciprofloxacin declined by 3 to 6$\log_{10}$. The antistaphylococcal activities of the extracts and ciprofloxacin are concentration dependent (Table 5). The yield value of each antimicrobial agent on the concentration axis indicates the tolerability potentials of the representative strains (Figs. 1-4). The activity of

Table 3. Minimum Inhibitory and Bactericidal Concentration of Extract of *Jatropha curcas* against Staphylococcal Isolates.

Microorganisms	Mean MIC and MBC of *Jatropha curcas* (mg/ml) and Ciprofloxacin (µg/ml) (mean ± s.d)					
	Methanol Extract		*Aqueous Extract*		*Ciprofloxacin*	
	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus ATCC25923	1.0 ± 0.09	4.0 ± 0.03	2.00 ± 0.06	16.0 ± 0.58	0.125 ± 0.001	0.125± 0.010
*S. aureus (*MSSA-W388)	1.0 ± 0.12	8.0 ± 0.25	2.0 ± 0.07	16.0 ± 0.80	0.5± 0.003	0.5± 0.002
*S. aureus (*MSSA-W415)	1.0 ± 1.21	8.0 ± 3.94	2.0 ± 3.02	16.0 ± 4.73	0.5± 0.009	1.0± 0.006
*S. aureus (*MRSA-GS4)	8.0 ± 1.28	128.0 ± 1.92	4.0 ± 1.97	16.0 ± 4.82	8.0± 0.003	16.0± 0.007
S. haemolyticus	0.5 ± 0.26	2.0 ± 1.04	1.0 ± .2.98	4.0 ± 3.03	8.0± 0.004	16.0± 0.004
S. sciuri	1.0 ± 2.60	16 ± 3.85	4.0 ± 1.89	128 ± 5.38	2.0± 0.001	4.0± 0.005
S. warneri	1.0 ± 3.30	8.0 ± 3.44	8.0 ±2.36	8.0 ± 2.40	0.125± 0.003	0.125± 0.006
S. chromogens	2.0 ± 0.64	16.0 ± 0.46	8.0 ± 3.04	16.0 ± 4.70	0.125 ± 0.001	0.125 ± 0.001
S. cohnii (SC-2)	2.0 ± 0.28	16.0 ± 1.05	8.0 ± 2.17	16.0 ± 2.61	4.0± 0.007	4.0± 0.002
S. cohnii (SU-1)	1.0 ± 0.19	8.0 ± 1.67	8.0 ± 0.96	8.0 ± 1.29	0.5± 0.003	2.0± 0.006
S. epidermidis	1.0 ± 0.90	8.0 ± 2.24	8.0 ± 2.32	8.0 ± 1.96	0.125± 0.001	0.5± 0.005
S. lugdunensis	2.0 ± 0.63	32.0 ± 2.60	8.0 ±2.82	64.0 ± 4.36	0.125± 0.002	0.5± 0.001
S. xylosus	2.0 ± 0.39	16.0 ± 1.88	8.0 ± 2.08	16.0 ± 1.09	0.5 ± 0.006	2.0 ± 0.007

Table 4. Rate of Killing / Log Decrease in Colony Forming Units (cfu) At 2 x MIC of *Jatropha curcas* against Representative Staphylococcal strains.

Microorganism	*J. curcas* Extract		Ciprofloxacin
	Aqueous	Methanolic	
S. aureus (ATCC 25923)			
Killing Rate x10^5 cfu/h	4.74 ± 0.62*	5.79 ± 0.39*	13.80 ± 0.
Log_{10} decrease (cfu/ml)			
2.5h	3.0 ± 0.16*	4.0 ± 0.21*	5.8 ± 0.32
5.0h	3.8 ± 0.19**	5.0 ± 0.24*	6.4 ± 0.51
10.0h	4.6 ± 0.40**	5.6 ± 0.36*	7.1 ± 0.48
S. aureus (MSSA-W388)			
Killing Rate x10^5 cfu/h	2.20 ± 0.09***	2.76 ± 0.16***	6.60 ± 0.20
Log_{10} decrease (cfu/ml)			
2.5h	1.2 ± 0.10***	2.2 ± 0.22**	4.0 ± 0.25
5.0h	1.6 ± 0.20***	2.8 ± 0.31**	4.6 ± 0.17
10.0h	2.0 ± 0.17***	3.2 ± 0.26**	6.0 ± 0.21
*S. aureus (*MRSA-GS4)			
Killing Rate x10^5 cfu/h	1.78 ± 0.20**	1.86 ± 0.21**	1.38 ± 0.29
Log_{10} decrease (cfu/ml			
2.5h	1.0 ± 0.09**	1.7 ± 0.24**	3.0 ± 0.30
5.0h	1.5 ± 0.26**	2.3 ± 0.19**	4.1 ± 0.24
10.0h	1.9 ± 0.18***	3.0 ± 0.17**	6.0 ± 0.48
S. haemolyticus			
Killing Rate x10^5 cfu/h	2.34 ± 0.40**	13.88 ± 0.41**	1.51 ± 0.28
Log_{10} decrease (cfu/ml)			
2.5h	2.6 ± 0.22**	3.0 ± 0.26*	5.0 ± 0.23
5.0h	3.4 ± 0.32*	4.2 ± 0.14*	5.8 ± 0.30
10.0h	4.0 ± 0.24*	5.3 ± 0.32*	6.4 ± 0.22

p-Value is *statistically significant ($p < 0.05$), ** ($p< 0.01$) and *** ($p < 0.001$) compared to the value of ciprofloxacin

both extracts against *S. haemolyticus* was greater than ciprofloxacin by a factor of 1.5 (Figs. 1 and 2).

DISCUSSION

In this study, we investigated the antistaphylococcal activity of *Jatropha curcas* bark extracts in order to provide an *in vitro* basis for its use as natural antimicrobial agents. The sensitivity of all staphylococcal strains to *Jatropha curcas* extracts implies that the intrinsic bio-substances in the extract are naïve to the various drug resistance potential of the isolates. The MIC and the MBC results of the extracts against staphylococcal strains suggest that the activity of the

Table 5. Comparative Effect o*f Jatropha curcas* extract on the growth of *Staphylococcus* strains at 2 x MIC.

Microorganism	Control	*Jatropha curcas* Extract						Ciprofloxacin		
		Aqueous			Methanol					
	Rate of Growth ($x10^6$cfu/ h)	Rate of Kill ($x10^6$cfu / h)	T_{50} (hr)	EC_{50} (mg/ ml)	Rate of Kill ($x10^6$cfu/ h)	T_{50} (hr)	EC_{50} (mg/ ml)	Rate of Kill ($x10^6$cfu/ h)	T_{50} (hr)	EC_{50} (µg/ ml)
Staphylococcus aureus (ATCC 25923)	2.75 ± 0.10	4.74 ± 0.57	2.00 ±0.15	1.59 ± 0.09	3.79± 1.00	1.76 ± 0.10	0.32 ± 0.06	13.80 ± 1.96	1.20 ± 0.07	0.05 ± 0.01
Staphylococcus aureus (MSSA W-388)	2.09 ± 0.24	2.04 ± 0.36	2.22 ±0.34	0.75 ± 0.22	2.19± 0.66	2.13 ± 0.19	0.60 ± 0.08	2.82 ± 0.16	1.80 ± 0.20	0.39 ± 0.13
Staphylococcus aureus (MRSA G-4)	2.69 ± 0.29	1.78 ± 0.30	2.62 ±0.33	0.54 ± 0.07	1.86 ±0.09	2.34 ± 0.56	0.14 ± 0.02	1.38 ± 0.05	3.19 ± 0.27	7.55 ± 1.05
Staphylococcus haemolyticus	2.88 ± 0.17	2.34 ± 0.66	1.77 ±0.09	0.40 ± 0.06	13.88 ± 1.16	1.19 ± 0.17	0.15 ± 0.04	1.51 ± 0.23	2.56 ± 0.31	7.20 ± 0.71

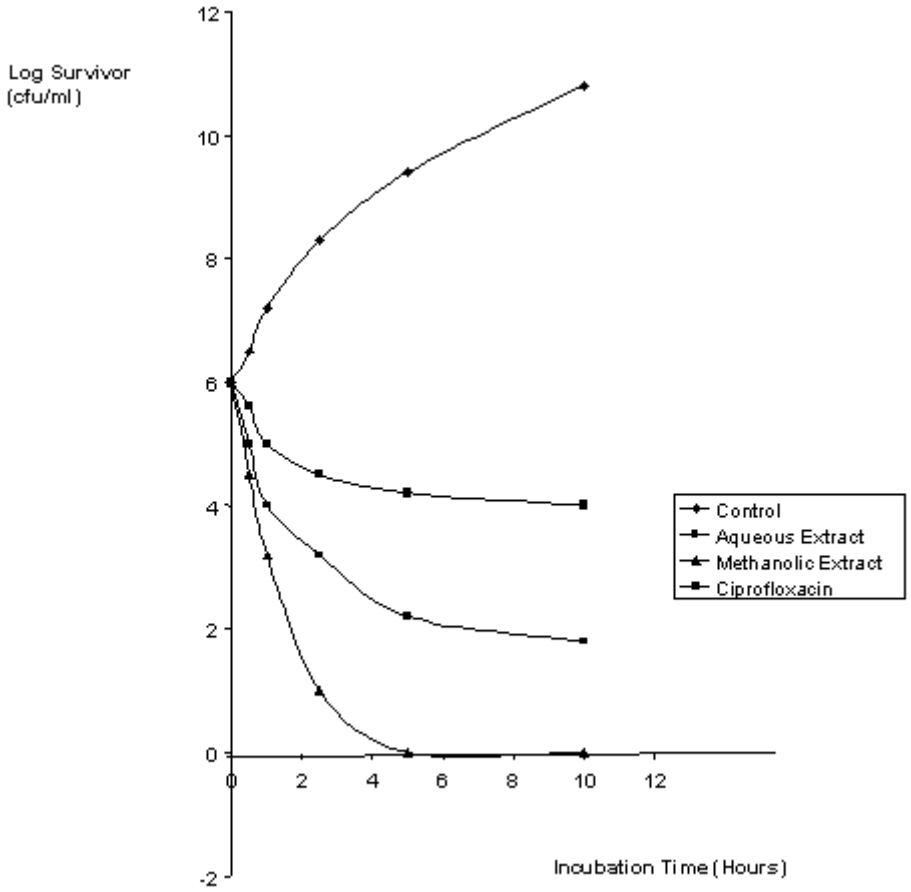

Fig. 1. Comparative Antistaphylococcal activity of *J. curcas* extract at 2 x MIC against *S. haemolyticus*.

extracts is mainly bacteriostatic at lower concentrations as there were no statistically significant differences between the MICs and respective MBCs ($P>0.05$; $n=13$). The presence of organic compounds such as tannin and saponin were revealed by qualitative phytochemical screening of the extracts. These phytochemical agents are familiar for their antimicrobial activity (Abioye *et al*., 2004). Tannin, which was detected in the extract, has been shown to form irreversible complexes with proline-rich protein (Hagerman and Butler, 1987) which could result in the inhibition of cell wall protein synthesis. This property may explain the mechanism of action of the *J.*

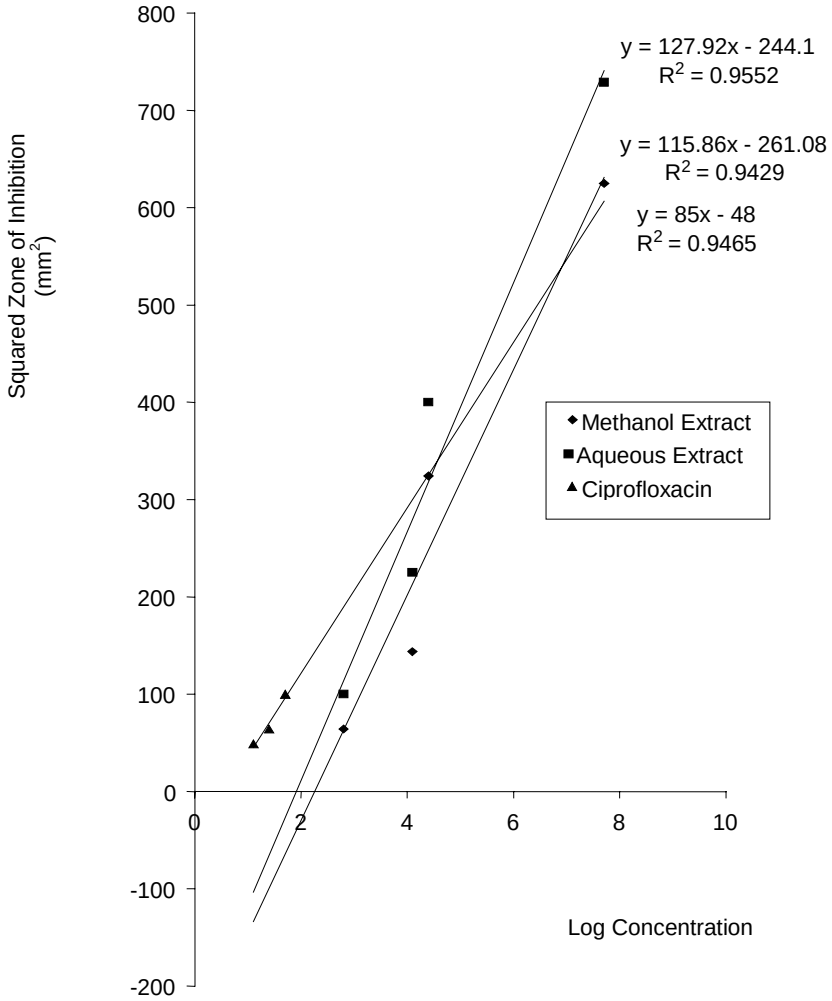

Fig. 2. Graded Concentration effect of *J. curcas* extract on *S. haemolyticus*.

curcas bark extract. Similarly, ciprofloxacin has also been shown to be bactericidal by inhibiting protein synthesis (Sanders *et al*., 1987).

The results of antibacterial susceptibility pattern of *J. curcas* extracts exhibited a concentration – dependent inhibitory activity (bioactivity) on the growth of staphylococcal strains studied. Reactive oxygen species (ROS) has also been implicated in the pathogenesis of staphylococcal infections (Halliwell and Gulteridge, 1990). Elevated synthesis of enzyme methionine sulfoxide reductase (*msr*A) has also contributed significantly to the virulence of *Staphylococcus aureus* (Singh *et al*., 2000). Therefore, the presence of flavonoids in the extract may serve as antioxidant defence mechanisms to ensure removal of reactive oxygen species (ROS) produced by the microorganism, thereby facilitating their rapid death.

The antibiotic resistance profile of the MRSA strain used in this current study indicates resistance to both ciprofloxacin and vancomycin. While ciprofloxacin could be an alternative, vancomycin has been regarded as the "drug of last resort", the most effective in the treatment of MRSA infections. Development of resistance to these antibiotics implies that MRSA infections will soon become untreatable. In a study of 83 MRSA isolates obtained from various sources, ciprofloxacin resistance was detected in 69 isolates (83%) (Raviglione *et al*., 1990). The time-kill analysis of the extracts performed on four strains revealed that the methanol extract exhibited the highest killing rate of 5.79 x 10^5 cfu/h against *S. aureus* (ATTC 25923). This finding is in fair correlation with the study carried out by Igbinosa *et al*. (2009) who found that the antibacterial activities of the ethanol and methanol extracts of the bark of Jatropha curcas compared favourably with the two standard antibiotics used in their investigation. The low activity (1.78 x 10^6 cfu/hr) against

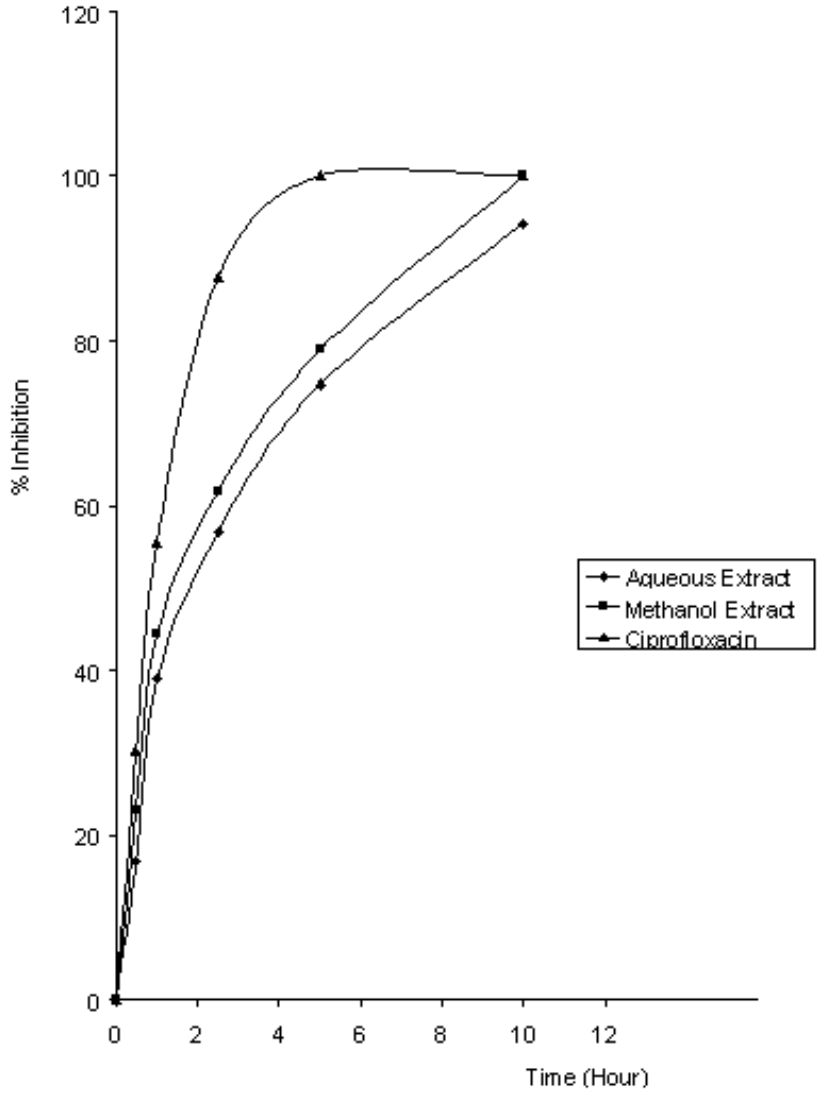

Fig. 3. Effect of *J. curcas* extract on the growth of *S. aureus* (ATCC 25923).

MRSA-G4 could be due to the presence of other compounds, which antagonize the antimicrobial actions of the active principle (Esimone *et al*., 1998). Even so, it is evident that the extract of *J. curcas* has a potential potent bactericidal activity against staphylococcal strains.

The time required to kill 50% population of the microorganism (T_{50}) and the extract concentration that produced 50% (EC_{50}) of the maximal zone of inhibition decreased in the order Aqueous extract > Methanol extract > Ciprofloxacin in most of the strains studied except MRSA-G4 and *S. haemolyticus*. Both extracts and ciprofloxacin did not achieve 100% inhibition of MRSA and *S. aureus* clinical strain (W-388) within 10h of incubation. However, *S. aureus* (ATCC 25923) was completely inhibited (100%) within the first 5hrs and 10hrs by ciprofloxacin and the extracts respectively. We deduce that MRSA-G4 and *S. haemolyticus* strains were more resistant to ciprofloxacin than the extracts. *Staphylococcus haemolyticus* is one of the main coagulase negative staphylococcal species associated with multidrug resistance, a trend that is also tremendously associated with MRSA (Shittu *et al*., 2005; Akinkunmi and Lamikanra, 2010; Amsterdam *et al*., 2010). Apparently, *J. curcas*' extracts could effectively be employed for the treatments of infections caused by these organisms including MRSA.

CONCLUSION

This study revealed that the aqueous and methanol extracts of the stem bark of *Jatropha curcas* Linn (Euphorbiaceae) are effective antistaphylococcal agents *in vitro*. Further research is consequently required to isolate

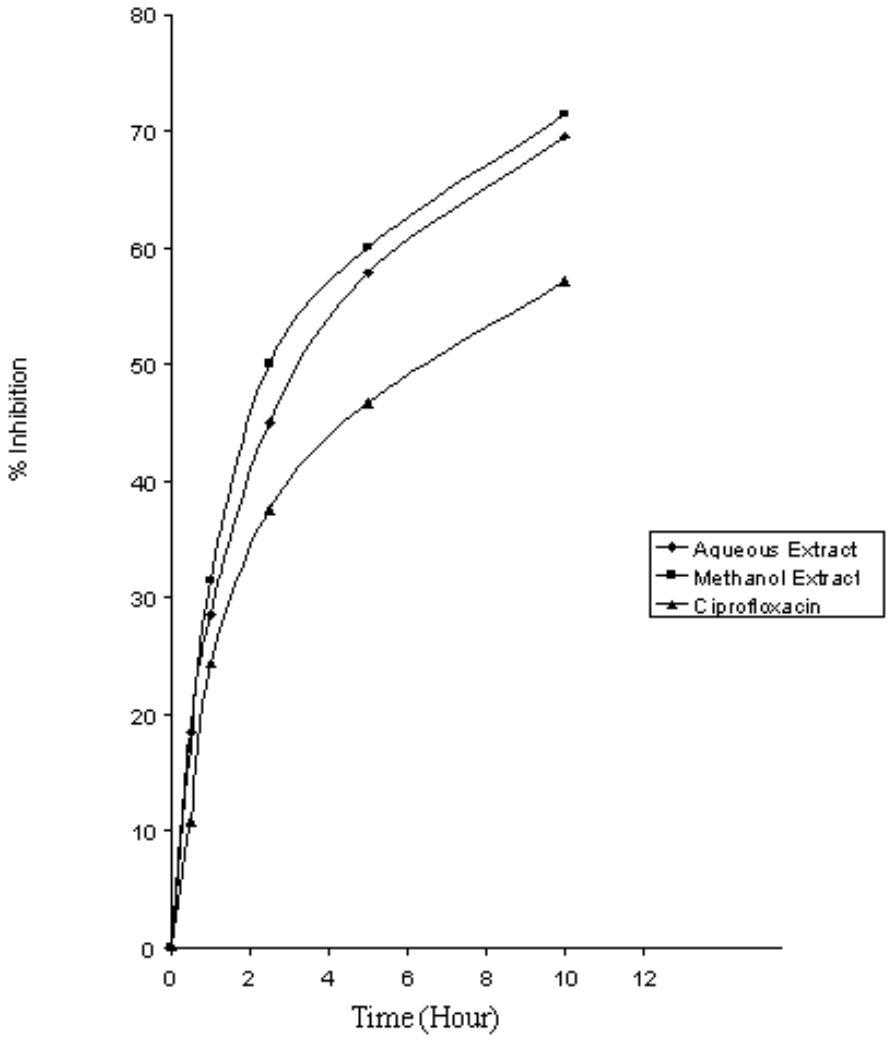

Fig. 4. Effect of *J. curcas* extract on *S. aureus* (MRSA – G4).

the pure active principle responsible for the antimicrobial activity, determine the safety profile and elucidate the molecular basis and mechanism of its activity.

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