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Full Length Research Paper

Phytochemical compounds and antibacterial activity of *Jatropha curcas* Linn. extracts

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The present study was conducted to determine the phytochemical compounds in different solvent extracts of *Jatropha curcas* Linn. plant and antibacterial activity of crude extracts. Aqueous, methanolic and hexane extracts of various plant parts were analysed for phytochemical compounds by spectrophotometry, high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry analysis (GC-MS). Antibacterial activity was studied by paper disc diffusion assay against Gram positive and Gram negative bacteria. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by micro-broth dilution. The root bark methanolic extract contained high phenolics (11.51 mg gallic acid equivalents/g DW) and flavonoids (0.94 mg rutin equivalents/g DW). Kernel meal aqueous extract contained high saponins (0.65 mg diosgenin equivalents/g DW) and the methanolic extract contained 1.13 mg/g DW phorbol esters. Phytochemicals detected by RP-HPLC were pyrogallol, gallic acid, naringin, rutin and vanillic acid. The main compounds detected by GC-MS were oxalic acid (root bark), acetic acid and oleic acid (stem bark). Inhibition zones ranged from 8.0 to 17.7 mm. Low MIC (1.2 to 2.3 mg/ml) and MBC (0.4 to 6.3 mg/ml) values were observed in methanolic extract of all plant parts. The present study showed that stem bark, root bark and kernel meal of *J. curcas* contained compounds with antibacterial activities. The results indicate the potential of *J. curcas* as a source of antibacterial compounds.

Key words: *Jatropha curcas* Linn., phorbol esters, phytochemicals, antibacterial activity.

INTRODUCTION

Antibiotic resistance that develop from prolonged usage of certain drugs has led to continuous efforts in searching for metabolites that possess antimicrobial activities. Plant secondary metabolites (alkaloids, terpenoids and phenolic compounds) are potential antimicrobial agents that can help to alleviate problem of antibiotic resistance (Krishnaiah et al., 2009). *Jatropha curcas* Linn. (family Euphorbiaceae) is a drought resistant shrub which is widely grown in Central and South America, South-east Asia, India and Africa. *J. curcas* plant has been initially considered a traditional herb in many parts of the world (Gubitz et al., 1999). It has gained importance in Malaysia, but as a source of seed oil for biofuel production. Traditionally, different parts of *J. curcas* have been used in treatment of different forms of infection. The leaves decoction is used as antiseptic substance during birth, the root decoction is used to treat sexually transmitted diseases and the seed is used to treat skin diseases (Gubitz et al., 1999; Joubert et al., 1984). However, its application as a remedy for many of these ailments has not been fully substantiated by the actual bioactive compounds responsible for the various effects. Hence, this study was conducted to relate the phyto-chemicals present to the antimicrobial activity of plant extracts.

MATERIALS AND METHODS

Collection of plant materials

J. curcas Linn. seeds were obtained from the Malaysian Agricultural

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Research and Development Institute (MARDI), whereas the whole plant was freshly collected from Universiti Putra Malaysia farm. The plants were about four years old. 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 to 60°C) for 16 h at 40°C (AOAC, 1990). The residue called kernel meal was dried in the oven at 50°C to remove the petroleum ether. Thirty two grams of each sample were placed in 800 ml of solvent (hexane, water and methanol) and refluxed at 50°C for 60 min (Chen et al., 2007). The extracts were filtered through Whatman filter paper No. 1 and both hexane and methanol extracts were evaporated to dryness using a rotary evaporator (Buchi) at 40°C, while the aqueous extracts were freeze dried. The residues obtained were dissolved in methanol (for spectrophotometric analysis) or 1% DMSO (for antimicrobial assay).

Spectrophotometric analyses of total phenolic, total flavonoid and total saponin content

Total phenolic content was determined by using Folin-Ciocalteu reagent (Slinkard and Singleton, 1977) and expressed as gallic acid equivalents (GAE) per gram of dry weight (DW), while total flavonoid content was determined using aluminium chloride colorimetric assay (Zhisen et al., 1999) and expressed as rutin equivalents (RE) per gram DW, and total saponin content was determined by using vanillin-sulphuric acid calorimetric method (Makkar and Becker, 1997) and expressed as diosgenin equivalents (DE) per gram DW.

Analyses of phenolic and flavonoid compounds by high performance liquid chromatography (HPLC)

Eight grams of each plant sample was extracted with 200 ml of high performance liquid chromatography (HPLC) grade solvents (hexane, water and methanol). The methanol and hexane samples were concentrated by evaporation using the rotary evaporator. The aqueous samples were partially freeze dried. The exact volume of each sample was measured.

The sample was then filtered through a 0.2 µm syringe filter. One millilitre of each sample was placed in the HPLC (Agilent-1200) vials in triplicates. The analysis was carried out according to the method described by Crozier et al. (1997) with slight modifications. Solvents comprising deionized water (solvent A) and acetonitrile (solvent B) were used.

The pH of solvent A was adjusted to 2.5 with concentrated trifluoroacetic acid. The column (Intersil ODS-3 (5 µm 4.6×150 mm, GI Science Inc) was equilibrated by 85% solvent A and 15% solvent B for 15 min before injection. Then the ratio of solvent B was increased to 85% after 50 min. After 5 min, the ratio of solvent B was reduced to 15%. This ratio was maintained for 60 min for the next analysis with a flow rate of 0.6 ml/min. Phenolic compounds were detected at 280 nm while isoflavonoids and flavonoids were detected at 350 nm. Phenolic standards used in this study were gallic acid, syringic acid, vanillic acid, caffeic acid and pyrogallol; while flavonoid standards were quercetin, rutin, myricetin, kaempferol and naringin; and isoflavonoid standards were genistein and daidzein. Analysis for phorbol esters was carried out by HPLC using phorbol-12-myristate-13-acetate (Calbiochem) as the standard. Deionized water (solvent A), acetonitrile (solvent B) and tetrahydro furan (solvent C) were used. The column [(LiChroCart® 250-4,6 Purospher® Star RP-18e (5 µm)] was equilibrated by 40% solvent A and 60% solvent B for 10 min before injection. Solvent B was increased to 100% in 30 min and was held for 5 min. Then solvent C was increased to 100% in the next 5 min and was held for 10 min. Finally the ratio of solvent A and B was adjusted to 40 and 60% in 5 min and was held for 10 min before the next injection. The flow rate was 1.3 ml/min and phorbol ester was detected at 280 nm.

Gas chromatography- mass spectrometry analysis (GC-MS)

The compounds in different extracts of different *J. curcas* plant parts were quantitatively measured by GC-MS based on the method described by Hossain and Rahman (2011) with some modification. The GC-MS used was a Schimadzu QP2010PLUS system. Six microliters were analysed on a BPX-5 SGE ultra-low-bleed 5% phenyl polydimethylsiloxane capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). Splitless injection was performed with a purge time of 1.0 min. The carrier gas was helium at a flow rate of 1 ml min^{-1}. The column temperature was maintained at 50°C for 3 min, then programmed at 5°C min^{-1} to 80°C and then at 10°C min^{-1} to 340°C. The inlet and detector temperatures were 250 and 340°C, respectively, and the solvent delay was 4 min.

The identification of the peaks was based on computer matching of the mass spectra with the National Institute of Standards and Technology (NIST 08 and NIST 08s) library and by direct comparison with published data.

Bacterial strains

Five Gram positive bacteria (*Staphylococcus aureus* S1434, methicillin resistant *Staphylococcus aureus* S1434 (MRSA), *Bacillus subtilis* B145, *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* B43) and four Gram negative species (*Escherichia coli* E253, *Escherichia coli* E274, *Pseudomonas aeruginos*a P196 and *Klebsiella pneumonia* K36) were used. *Staphylococcus aureus* ATCC 25923 was obtained from the stock culture maintained in the Halal Institute, Universiti Putra Malaysia whereas other species were obtained from the Institute for Medical Research (IMR), Malaysia.

Antimicrobial assay

The antibacterial assay of different crude extracts was carried out by the disc diffusion method as described by Chandrasekaran and Venkatesalu (2004). An inoculum OD (optical density) of 0.1 (approximately about 1.7×10^8 CFU/ml) was used. Each Mueller Hinton agar (Oxoid, Germany) Petri dish contained a positive control (antibiotic) and negative control (1% DMSO). The reference controls were ampicillin (10 µg), penicillin (10 µg) and tetracycline (30 µg). Each test was done in triplicate. The inhibition zone was measured to the nearest millimeter. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by micro-broth dilution as described by Sarker et al. (2007) with some modification. Powdered resazurin was used instead of resazurin crystals. The lowest concentration at which no colour change occurred was taken as the MIC value. To determine the minimum MBC, 100 µl from each of the blue/black colour wells were plated out on nutrient agar. The plates were allowed to solidify and were incubated at 37°C for 48 h. The lowest concentration of the crude extract that did not show any growth on the nutrient agar was taken as the MBC.

Table 1. Total phenolics, flavonoids and saponins content in different *J. curcas* plant part extracts.

Phytochemical	Solvent	Leaves	Stem bark	Root bark	Root wood	Kernel meal	SEM
Total phenolics (mg gallic acid equivalents/g DW	Hexane	0.34^{b}	0.26^{c}	0.37^{a}	0.20^{d}	0.15^{e}	0.001
	Aqueous	4.20^{a}	1.13^{d}	3.19^{b}	0.40^{e}	1.51^{c}	0.011
	Methanol	9.29^{b}	3.09^{d}	11.51^{b}	1.05^{e}	3.95^{c}	0.252
Total flavonoids (mg rutin equivalents/g DW)	Hexane	0.33^{a}	0.19^{b}	0.36^{a}	0.16^{bc}	0.14^{c}	0.01
	Aqueous	0.86^{a}	0.23^{b}	0.89^{a}	0.08^{d}	0.10^{c}	0.005
	Methanol	0.91^{a}	0.38^{b}	0.94^{a}	0.26^{c}	0.19^{d}	0.017
Total saponins (mg diosgenin equivalents/g DW)	Hexane	ND	ND	ND	ND	ND	ND
	Aqueous	0.64^{a}	0.40^{ba}	0.28^{b}	0.21^{b}	0.65^{a}	0.076
	Methanol	0.37^{a}	0.28^{ab}	0.11^{bc}	0.06^{c}	0.38^{a}	0.06

Each value is a mean of 3 replicates. Different superscripts in the same row indicate significant difference ($p<0.05$) between extracts of different parts. SEM, standard error mean. ND - Not detected.

Statistical analysis

The data were subjected to the general linear models (GLM) procedure of SAS in a completely randomized design (CRD) and the means were compared with Duncan's multiple range test. The difference was considered significant when $P<0.05$.

RESULTS AND DISCUSSION

Phytochemical analyses

Table 1 shows the total phenolic (TP), flavonoid (TF) and saponin (TS) content of different extracts of various plant parts. The methanol extracts showed the highest TP and TF followed by aqueous and hexane extracts. This is as expected because methanol has a high polarity index (Cowan, 1999) than the other solvents and thus it is able to extract more phenolic and flavonoid compounds. In the present study, spectrophotometric analysis using aluminium chloride method was employed to quantify the TF. The TF values obtained ranged from 0.19 to 0.95 mg RE/g DW. According to Chang et al. (2002), spectrophotometric analysis using aluminium chloride may under estimate the content of flavonoids as it is specific to flavones and flavonols and not to flavanones, hence the amount of TF detected could be underestimated. The saponin content was the highest in the aqueous extracts followed by the methanol extracts and none was detected in the hexane extracts. This is attributed to the fact that saponins are composed of one or more hydrophilic glycoside moieties hence are soluble in water and a few are soluble in methanol and none in non polar solvents such as hexane (Cowan, 1999).

Using qualitative analysis, Igbinosa et al. (2009) and Akinpelu et al. (2009) observed the presence of saponins and flavonoids in *J. curcas* stem bark and leaves extract, respectively. Table 2 shows the main compounds detected by HPLC that were present in the various extracts. The compounds listed were those that showed a concentration of greater than 0.10 mg/g DW. The phyto-chemicals detected were pyrogallol, gallic acid, vanillic acid, rutin and naringin, The presence of gallic acid has been reported in the leaves extract (Manpong et al., 2009) and in the stem bark and root extracts (Diwani et al., 2009) of *J. curcas* plant. The presence of benzoic acid in the residues of nodes, leaves, stem and root of Egyptian *J. curcas* has been reported by Diwani et al. (2009). In this study, vanillic acid, a form of benzoic acid, was observed to be present in the methanol extracts of the leaves and root bark. However, vanillic acid was not observed in the stem bark extracts but has been reported to be present in the stem (Vaithanomsat and Apiwatanapiwat, 2009). Pyrogallol was abundant but this was not surprising since pyrogallol can be formed by heating garlic acid and in this experiment, samples were heated during the extraction process. This explains why the amount of garlic acid was lower than the pyrogallol content. Phorbol esters were not detected in different plant part extracts except in methanol and hexane kernel meal extracts. The presence of these compounds in kernel meal has been reported by Gubitz (1999) and recently by Oskoueian et al. (2011). Phorbol esters are lipophilic in nature, hence they are soluble in organic solvents only.

Other compounds in different parts of *J. curcas* plant reported by other authors but were not observed or analysed in this study include curcin, lectin, vitexine and isovitexine in the seed (Gubitz, 1999; Makkar and Becker, 1997), phenolic compounds such as salicyclic acid and ellagic acid in the stem and root, corilagin and ellagic acid in the leaves (Diwani et al., 2009; Manpong et al., 2009). Flavonoid compounds not detected in the present study but have been reported include; apigenin, vitex and isovitexin in leaves (Subramanian et al., 1971). The types of compounds detected may vary according to the MS in the three solvent extracts of different plant parts and the extraction method, the solvents used, as

Table 2. Main compounds in different solvent extracts of various parts of *J. curcas* plant analysed by HPLC.

Solvent	Plant part	Main compounds detected at 280 nm[a]	Concentration (mg/g DW)	Main compounds detected at 350 nm[b]	Concentration (mg/g DW)
Hexane	Kernel meal	Pyrogallol	0.12		
		Phorbol esters	0.75		
Aqueous	Leaves	Pyrogallol	0.11	Rutin	0.34
		Gallic acid	0.27		
	Stem bark	Pyrogallol	1.60	Naringin	0.15
		Gallic acid	0.24		
	Root bark	Pyrogallol	0.25		
	Root wood	Pyrogallol	0.31	Naringin	0.73
		Gallic acid	0.13		
	Kernel meal	Pyrogallol	0.27		
		Gallic acid	0.11		
Methanol	Leaves	Gallic acid	0.22	Rutin	0.66
		Vanillic acid	0.46	Naringin	0.30
	Stem bark	Pyrogallol	0.55	Naringin	0.12
	Root bark	Vanillic acid	2.87		
	Root wood			Naringin	0.41
	Kernel meal	Pyrogallol	0.82		
		Gallic acid	0.32		
		Phorbol esters	1.13		

[a]-Phorbol esters, phenolic and isoflavonoid compounds; [b] Flavonoid compounds.

well as the type of standards in the HPLC analysis. Table 3 shows the main compounds detected by GC-proportion of each compounds was expressed as a percentage of the total peak area. Acetic acid and furfural were the main compounds observed in the aqueous extracts. Acetic acid is a short chain carboxylic acid and furfural is an aldehyde, thus, the two can easily form hydrogen bond with water to produce acetate and a hydrate compound, respectively. Oxalic acid was observed to be present in the methanolic extract of all plant parts, in particular, the root bark. Various esters including hexa-decanoic acid, methyl ester and oxalic acid, and dimethyl ester were detected. The esters dissolve by forming hydrogen bond with the oxygen atom in the ester moiety (Stoker, 2009). Oleic acid and β-sitosterol were the main components in the hexane extracts of stem bark and leaves.

Antibacterial activities

Table 4 shows the susceptibility of clinical isolates to aqueous extracts of different parts of *J. curcas* plant. Aqueous extracts were effective against Gram positive species. Weak or slight activity was observed with the Gram negative species except with the kernel meal extract. Generally, the aqueous extracts contained mainly acetic acid, furfural, gallic acid, pyrogallol naringin and saponins. Pyrogallol has been reported to be an effective antimicrobial agent and its toxicity is attributed to the three hydroxyl groups present in its structure (Kocacaliskan et al., 2006; Cowan (1999). Acetic acid is a well known antimicrobial agent used in food industry. Haesebrouck et al. (2009) observed that acetic acid solution (0.5%) exerted bactericidal effect against *Staphylococcus pseudintermedius*. Similarly, root wood extract which contained high percentage of acetic acid showed strong antibacterial activity against *K. pneumonia* K36. However, Huang et al. (2010) observed that acetic acid exhibited no antimicrobial activity against various oral microorganisms.

Table 5 shows the susceptibility of clinical isolates to methanol extracts of different parts of *J. curcas*. The methanol extracts were effective against both Gram positive and Gram negative bacteria (especially to *E. coli* E274 and *K. pneumonia* K36). Generally, the kernel meal

Table 3. Main compounds in different solvent extracts of various parts of *J. curcas* plant analysed by GC-MS.

Plant part	Aqueous extract	Area %	Methanol extract	Area %	Hexane extract	Area %
	Main compound		Main compound		Main compound	
Leaves	Acetic acid	37.5	Oxalic acid, dimethyl ester Hexadecanoic acid, methyl ester	39.9 20.3	β-Sitosterol	16.5
Stem bark	Acetic acid Furfural Acetic acid, anhydride with formic acid	51.7 14.4 38.6	Oxalic acid, dimethyl ester Hexadecanoic acid, methyl ester Citric acid, trimethyl ester 10-Octadecenoic acid, methyl ester	20.2 12.5 15.5 10.2	Oleic acid	41.6
Root bark	Furfural	15.9	Oxalic acid, dimethyl ester	70.9	Spiro[cyclobutane-1.1'(2H) phenanthrene], 3',4'a,9',10',10'a-hexahydro-4'a-methyl-trans	16.4
Root wood	Acetic acid, anhydride with formic acid Furfural Acetic acid	59.7 20.4 16.2	Oxalic acid, dimethyl ester Hexadecanoic acid, methyl ester	15.4 16.2	β -sitosterol 1,4-Epoxynaphthalene-1(2H)-methanol, 4,5,7-tris(1,1-dimethylethyl)-3,4-dihydro	14.1 12.0
Kernel meal	Furfural 2-furancarboxaldehyde,5(hydroxymethy)	14.6 29.7	Oxalic acid, dimethyl ester Hexadecanoic acid, methyl ester 9-octadecenoic acid, methyl ester 2-Furancarboxaldehyde,5-(hydroxymethy) 9,12- octadecenoic acid, methyl ester	12.3 15.2 29.9 11.2 10.5	β-Sitosterol	3.8

exhibited good activity as eight of the species were inhibited. Methanol extracts contained high TP and TF and most of the compounds detected in these extracts have been reported to be effective antimicrobials such as pyrogallol (Cowan, 1999), vanillic acid (Vaquero et al., 2007), gallic acid (Vaquero et al., 2007; Cowan, 1999); saponins (Mandal et al., 2005), fatty acid alkyl esters such as fumaric acid, dimethyl ester (Wang et al., 2001), linoleic acid ethyl ester and oleic acid methyl ester (Huang et al., 2010). Phytochemicals present in the leaves extracts were high but low antimicrobial activity was observed, probably due to the fact that different phytochemicals exert their effects differently. It is also possible that potent antibacterial flavonoids may not diffuse through the paper disc due to their low rate of diffusion (Cushnie and Lamb, 2005). The kernel meal methanol extract showed good antibacterial activity, although it contained low amounts of terpenes, flavonoids and phenolics. Its activity may have come from other antimicrobial agents such as saponins or phorbol esters that were observed to be present in the kernel meal. Table 6 shows the susceptibility of clinical isolates to hexane extracts of different parts of *J. curcas*. The hexane extracts of the root wood and root bark were active against Gram negative species (*E. coli* E274 and *K. pneumonia* K36) and Gram positive species (*B. cereus* B43 and *B. subtilis* B145), respectively. The kernel meal extract inhibited only Gram negative species. Generally, the leaves and stem bark hexane extracts showed no activity or slight antimicrobial activity. The nature of phytochemicals in the extract affects the antimicrobial activity. The main constituent in the hexane extract was oleic acid which has been reported to have low antimicrobial activity (Huang et al., 2010). The leaves hexane extract contained

Table 4. Susceptibility of human pathogens to aqueous extracts of different parts of *J. curcas.*

Bacterial spp.	Inhibition zone diameter (mm)							SEM
	L	SB	RB	RW	KM	A	DMSO	
B. cereus B43	NI	NI	12.0^{a}	9.0^{b}	11.3^{ab}	15.2	NI	0.83
B. subtilis B145	13.3^{b}	11.3^{b}	15.0^{a}	12.3^{bc}	13.3^{b}	17.5	NI	0.47
E. coli E253	8.0^{b}	10.0^{a}	10.3^{a}	10.3^{a}	10.7^{a}	NI	NI	0.45
E. coli E274	8.0^{c}	8.3^{bc}	9.7^{b}	8.3^{bc}	11.7^{a}	NI	NI	0.47
K. pneumonia K36	8.3^{b}	9.3^{b}	9.7^{b}	10.3^{b}	14.3^{a}	NI	NI	0.80
P. aeruginosa P196	10.0^{c}	10.7^{bc}	11.3^{b}	11.3^{b}	12.3^{a}	NI	NI	0.00
S. aureus ATCC 25923	10.0^{bc}	13.3^{a}	12.3^{a}	11.7^{ba}	9.0^{c}	28.0	NI	0.58
MRSA S1274	9.0^{b}	11.3^{a}	11.3^{a}	12.3^{a}	11.0^{a}	11.0	NI	0.58
S. aureus S1434	10.7^{b}	13.7^{a}	15.0^{a}	10.0^{b}	10.3^{b}	11.0	NI	0.68

Data represent means of 3 replicates (diameter of paper disc inclusive). A, Ampicillin (10 µg/disc) and DMSO (dimethyl sulphoxide, 1 %). NI, no inhibition (equivalent to 7.0 and 6.0 mm for the plant extract and antibiotic disc respectively). L, SB, RB, RW and KM represent respectively leaves, stem bark, root bark, root wood and kernel meal extracts at a concentration of 1000 µg/disc. SEM, standard error means. No inhibition (NI), 8.0 to 8.9, 9.0 to 10.9, 11.0 to 12.9, 13.0 to 16.0, > 16.0 mm were interpreted respectively as no activity, weak, slight, moderate, high and strong antimicrobial activity (Vaquero et al., 2007), respectively. Mean values in the same row bearing different superscripts are significantly different ($p<0.05$).

Table 5. Susceptibility of human pathogens to methanol extracts of different parts of *J. curcas.*

Bacterial spp.	Inhibition zone diameter (mm)							SEM
	L	SB	RB	RW	KM	P	DMSO	
B. cereus B43	NI	8.7^{d}	12.7^{b}	10.7^{c}	15.0^{a}	18.4	NI	0.65
B. subtilis B145	12.3^{bac}	11.3^{c}	13.7^{ba}	12.0^{c}	14.3^{a}	23.2	NI	0.65
E. coli E253	8.0^{c}	9.7^{b}	9.0^{bc}	9.7^{b}	13.0^{a}	NI	NI	0.33
E. coli E274	9.7^{dc}	8.7^{d}	10.0^{c}	14.7^{a}	12.3^{b}	NI	NI	0.30
K. pneumonia K36	8.3^{e}	14.3^{b}	9.7^{d}	17.7^{a}	12.3^{c}	NI	NI	0.42
P. aeruginosa P196	10.3^{b}	8.7^{c}	9.3^{bc}	12.3^{a}	12.3^{a}	NI	NI	0.49
S. aureus ATCC 25923	12.7^{b}	11.3^{c}	14.0^{a}	10.0^{d}	14.7^{a}	31.3	NI	0.37
MRSA S1274	11.7^{bc}	10.3^{c}	13.0^{ba}	10.7^{c}	14.0^{a}	10.8	NI	0.68
S. aureus S1434	12.0^{b}	10.3^{b}	14.3^{a}	11.7^{b}	14.0^{a}	14.7	NI	0.63

Data represent means of 3 replicates (diameter of paper disc inclusive). P, Penicillin (10 units/disc) and DMSO (dimethyl sulphoxide, 1%). NI, no inhibition (equivalent to 7.0 and 6.0 mm for the plant extract and antibiotic disc respectively). L, SB, RB, RW and KM represent respectively leaves, stem bark, root bark, root wood and kernel meal extracts at a concentration of 1000 µg/disc. SEM, standard error mean. No inhibition (NI), 8.0 to 8.9, 9.0 to 10.9, 11.0 to 12.9, 13.0 to 16.0, >16.0 mm were interpreted as no activity, weak, slight, moderate, high and strong antimicrobial activity (Vaquero et al., 2007), respectively. Mean values in the same row bearing different superscripts are significantly different ($p<0.05$).

β-sitosterol as the main constituent and the extract only inhibited *B. subtilis* B145. The results were similar to those reported by Beltrame et al. (2002), who observed that β-sitosterol isolated from the aerial parts of *Cissus Sicyoides* was inactive to *S. aureus, E. coli* and *P. aeruginosa* but showed inhibitory activity against *B. subtilis*.

The lowest value of MIC and MBC values for each solvent extract of different plant parts that had inhibited bacterial species are shown in Table 7. The values of MIC/MBC of the methanolic extracts and aqueous extracts were lower compared to those of the hexane extracts. Water and methanol extract more polar compounds than hexane and many of these compounds (such as acetic acid, pyrogallol, gallic acid and vanillic acid) at lower concentrations have been reported to be effective antibacterial agents (Vaquero et al., 2007; Cowan, 1999).

In conclusion, methanolic extract of root bark showed the highest phenolic and flavonoid compounds, while aqueous extract of both leaves and kernel meal showed the highest saponin content. The main phenolic compound detected in the methanolic extract of root bark was vanillic acid. The main compounds detected by GC-MS analysis were oxalic acid in the methanolic extract of root bark, acetic acid in the aqueous extract of stem bark and oleic acid in the hexane extract of stem bark. Generally low MIC (1.2 to 2.3 mg/ml) and MBC (0.4 to 6.3 mg/ml) values were observed when the methanolic extract of all plant parts were tested against both the Gram positive

Table 6. Susceptibility of human pathogens to hexane extracts of different parts of *J. curcas.*

Bacterial spp.	Inhibition zone diameter (mm)							SEM
	L	SB	RB	RW	KM	T	DMSO	
B. cereus B4	NI	NI	16.0[a]	NI	NI	35.7	NI	0.45
B. subtilis B145	8.0[b]	9.3[b]	17.7[a]	8.0[b]	NI	27.3	NI	0.67
E. coli E253	NI	NI	NI	10.3[a]	8.3[b]	8.7	NI	0.21
E. coli E274	NI	NI	NI	13.7[a]	10.7[b]	8.0	NI	0.33
K. pneumonia K36	NI	NI	NI	16.3[a]	12.3[b]	19.3	NI	0.59
P. aeruginosa P196	NI	NI	NI	NI	NI	NI	NI	0.00
S. aureus ATCC 25923	NI	NI	14.7[a]	8.0[b]	NI	24.3	NI	0.39
MRSA S1274	NI	NI	12.7[a]	8.0[b]	NI	23.3	NI	0.15
S. aureus S1434	NI	NI	12.3[a]	NI	NI	23.7	NI	0.15

Data represent means of 3 replicates (diameter of paper disc inclusive). T, Tetracycline (30 µg/disc) and DMSO (dimethyl sulphoxide, 1 %). NI, no inhibition (equivalent to 7.0 and 6.0 mm for the plant extract and antibiotic disc respectively). L, SB, RB, RW and KM represent leaves, stem bark, root bark, root wood and kernel meal extracts at a concentration of 1000 µg/disc, respectively. SEM, standard error means. No inhibition (NI), 8.0 to 8.9, 9.0 to 10.9, 11.0 to 12.9, 13.0 to 16.0, >16.0 mm were interpreted as no activity, weak, slight, moderate, high and strong antimicrobial activity (Vaquero et al., 2007), respectively. Mean values in the same row bearing different superscripts are significantly different ($p<0.05$).

Table 7. The lowest value of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of different plant part extracts tested against bacterial species.

Bacterial spp.	Aqueous extract		Methanol extract		Hexane extract	
	Lowest value of		Lowest value of:		Lowest value of:	
	MIC (mg/ml) (plant part)[a]	MBC (mg/ml) (plant part)[a]	MIC (mg/ml) (plant part)[a]	MBC (mg/ml) (plant part)[a]	MIC (mg/ml) (plant part)[a]	MBC (mg/ml) (plant part)[a]
B. cereus B43	4.3 (root bark and root wood)	8.6 (root wood)	2.3 (root bark)	4.7 (root bark)	15.6 (root bark)	31.3 (root bark)
B. subtilis B145	4.7 (kernel meal)	9.4 (root bark and kernel meal)	2.3 (kernel meal and leaves)	4.7 (kernel meal and leaves)	15.6 (leaves)	25.0 (stem bark and root bark)
E. coli E253	3.1 (root wood)	6.3 (root wood)	2.3 (leaves, root wood and kernel meal)	6.3 (leaves, root wood, root bark and kernel meal)	14.1 (root wood)	25.0 (kernel meal)
E. coli E274	6.3 (root wood, root bark, kernel meal)	12.5 (root wood, root bark, kernel meal)	3.1 (leaves, root bark and kernel meal)	6.3 (root bark and kernel meal)	18.8 (kernel meal)	25.0 (root wood)

Table 7. Contd.

K. pneumonia K36	4.7 (kernel meal)	15.6 (root bark)	3.1 (leaves and kernel meal)	6.3 (leaves and kernel meal)	18.8 (kernel meal)	50.0 (Root wood and kernel meal)
P. aeruginosa P196	3.1 (root wood and leaves)	6.3 (root wood)	1.2 (root wood)	4.7 (root wood and leaves)	ND	ND
S.aureus ATCC 25923	6.3 (all plant parts)	12.3 (all plant parts)	2.3 (stem bark, root bark, kernel meal)	4.7 (stem bark, root bark, kernel meal)	10.2 (root wood)	20.3 (root wood)
MRSA S1274	2.7 (root wood)	10.9 (root wood)	2.3 (root bark)	5.5 (kernel meal)	15.6 (root wood)	50.0 (root bark)
S.aureus S1434	1.6 (root wood and root bark)	3.1 (root wood and root bark)	0.4 (stem bark)	0.4 (stem bark)	9.4 (leaves)	18.8 (leaves)

[a]- Represent the lowest of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the various extracts of different parts of *J. curcas* to susceptible bacterial species. ND-not done

and Gram negative bacterial species. However, susceptibility of each bacterial species differs according to the plant parts. The present study showed that stem bark, root bark and kernel meal of *J. curcas* contained com-pounds with antibacterial activities. The results indicate the potential of *J. curcas* as a source of antibacterial compounds.

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