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Full Length Research Paper

Research on Antibacterial profile of *Jatropha curcas* latex extracts against selected human pathogenic bacteria

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***Jatropha curcas* is a drought-resistant tree belonging to the family *Euphorbiaceae*. The latex of *J. curcas* has been traditionally used to heal wounds and has reported anti-coagulant and coagulant properties. In this study, fractions of the methanol, aqueous, ethyl acetate and hexane extracts from the *Jatropha* latex were tested for antibacterial properties against nine different human pathogenic bacteria, namely three different isolates of *Staphylococcus aureus* (one methicillin-resistant strain MRSA ATCC 33591, and two methicillin-sensitive strains MSSA ATCC 29213 and ATCC 25174), *Salmonella enterica* serovar *typhi*, *Streptococcus agalactiae*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Enterococcus faecalis* (E248), *Escherichia coli* (ATCC 35218), *Listeria monocytogenes* (IMR L10), and *Morganella morgenii* (IMR MM99). The zones of inhibition produced by the extracts using an agar well diffusion method against the test microorganisms were found to range from 2.7 to 29 mm. The methanol extract was found to be the most effective extract with MIC values ranging from 0.39 mg/ml for methicillin-sensitive *S. aureus* and 6.25 mg/ml for *S. marcescens*. Hexane extracts did not show any antibacterial activity. Phytochemical screening of the *J. curcas* latex indicated the presence of saponins and tannins which are known to have antibacterial properties.**

Key words: *Jatropha curcas* latex, antibacterial properties and human pathogenic bacteria.

INTRODUCTION

The extensive use of antibiotics has led to the rapid emergence of multi-drug resistance among pathogenic bacteria; for example, penicillin which was introduced in the 1930's was initially hyped to be the "magic bullet" against pathogenic bacteria is now much less effective as bacterial strains develop resistance against the antibiotic (Shamweel and Dar, 2011). The pharmaceutical industry is struggling to keep up with the production of new synthetic antibiotics as more and more bacterial strains develop resistance against existing drugs. The con-sumption of synthetic antimicrobials are known to affect the human body because of the disruption to the natural human microbiota which are known to play key roles in nutrition, development, metabolism, pathogen resistance and regulation of the human immune responses (Dethlefsen et al., 2008). There are many side effects of synthetic antibiotics towards the human body such as diarrhea which is caused by the disruption of the normal intestinal floral which induces the growth of *Clostridium difficile* (anaerobic bacteria) (Pirotta and Garland, 2006). Therefore, there is a pressing need for the development of new antimicrobial drugs particularly from natural sources as these may reduce the risk of toxicity observed in synthetic antibiotic.

Parts of *Jatropha curcas* have been used in traditional medicine and for veterinary purposes in regions where the plant is grown (Gübitz et al., 1999). The latex is used to treat fungal infections in the mouth, bee and wasp stings and digestive problems of children in Mexico (Schmook and Serralta-Peraza, 1997). Nevertheless, no

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thorough studies have been carried out to determine if the latex of *J. curcas* contain any potential antibacterial agents. Through this study, it is hoped that new information will be made available regarding the presence or absence of antibacterial agents from the latex of *J. curcas*. Previous research carried out by Igbinosa et al. (2009) investigated the antimicrobial properties of the crude extracts from the stem bark of *J. curcas* against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*. Thus, the objective of the present study was to determine the antimicrobial activity of crude extract and various fractions of crude latex of *J. curcas* against nine selected human pathogenic bacteria.

MATERIALS AND METHODS

Plant materials

The latex of the *J. curcas* was collected from an estate in Setiu Kuala Terengganu. The stem/branch of the tree was cut and a bottle was hanged to collect the fresh latex. The plants used for latex collection were four years old, having been planted in 2006. Latex was collected into vials containing a few drops of 95% ethanol to prevent browning and oxidation (Osoniyi and Onajobi, 2003). The materials used to cut the stem/branch are secateur, wire, sterilized bottles, ice box, ice, gloves and dropper. Full bottles were placed in the ice box to maintain samples at the appropriate temperature. The samples collected were directly brought to the chemistry lab for the extraction process.

Bacterial strains

Nine human pathogenic bacteria, namely *Enterococcus faecalis* (E248), *Listeria monocytogenes* (IMR L10), *Serratia marcescens* (ATCC 8100), *Streptococcus agalactiae* (Isolation from UMT), *Salmonella enterica* serovar *typhii* (Isolation from UPM), *E. coli* (ATCC 35218), *Klebsiella pneumonia* (ATCC 10273), *Morganella morganii* (IMR MM99), one ATCC strain of methicillin-resistant *S. aureus* (MRSA) (ATCC 33591) and two ATCC strain of methicillin-sensitive *S. aureus* (MSSA) designated MSSA 1 (ATCC 29213) and MSSA 2 (ATCC 25174), were used in this study to test for antimicrobial properties of the *J. curcas* latex extracts. The bacteria were selected based on their pathogenicity onto human body. The bacteria were cultured in nutrient agar, NA (Difco) to obtain pure single colony by appropriate four quadrants streaking on the solid media and incubating the agar plates overnight at 37°C.

Preparation of plant extracts

The fresh latex collected in the bottle was directly soaked in methanol. It was left for three days in the dark, placed on a rack. After three days, it was filtered using Whatmann paper directly into a conical flask. The filtrate in the conical flask was extracted by using a rotavapor machine (BUCHI R-210). The temperature was maintained by using waterbath (BUCHI B-491) at below 50°C to ensure that the bioactive compounds are not destroyed by high temperatures. The dried compound is known as crude extract methanol before partition. This extract will be tested to see the differences with the crude methanol after partition. The partition process was done to get four different fractions which are ethyl acetate, aqueous, hexane and methanol. The crude extract methanol before partition was divided into two portions: to the first portion, methanol and hexane at a volume-to-volume ratio of 1:1 was added, and to the second portion, water and ethyl acetate at a volume-to-volume ratio of 1:1 was added. These were left to stand until two layers formed. The layers were separated using a Funnel tube. The separated layers were dried using the rotavapor machine and weighed in different bottles. The crude extracts obtained were kept at 4°C.

Preparation of bacterial strains

There were nine bacteria used in this study; *E. faecalis* (E248), *L. monocytogenes* (IMR L10), *S. marcescens* (ATCC 8100), *S. agalactiae* (Isolation from UMT), *S. enterica* serovar *typhii* (Isolation from UPM), *E. coli* (ATCC 35218), *K. pneumonia* (ATCC 10273), *M. morganii* (IMR MM99), one ATCC strain of methicillin-resistant *S. aureus* (MRSA) (ATCC 33591) and two ATCC strain of methicillin-sensitive *S. aureus* (MSSA) designated MSSA 1 (ATCC 29213) and MSSA 2 (ATCC 25174). Those bacteria were cultured from the freeze stock available in UniSZA. The bacteria were first revived before used because they were kept in -80°C and do not activated. The reviving process begins by thawing the bacteria in the room temperature. Followed by transfer into broth solution (Nutrient Broth) and leave it for overnight in the wisecube (Fuzzy Control System) in 37°C. After an overnight the bacteria were striking on Mueller-Hinton agar, MHA (Difco). The single colony obtain was kept in the beads. The bacteria can be kept in the beads for a long time. Before the bacteria can be used from the bead it was revived first in the NB and in the following day the bacteria was strike on the agar media for single colony formation. Single colony without contamination will be used in the antibacterial activity test and it was kept in 4°C.

Antibacterial activity

The antibacterial activities of the crude extracts were determined by using an agar well diffusion method. The bacterial isolates were first grown in Mueller-Hinton broth MHB (Difco) overnight at 37°C. The bacterial suspensions were then standardized to an OD_{600} of 0.5 using spectrophotometer machine (SHIMAZU/UV-Mini 1240). The wells were punched on the Mueller-Hinton agar using a Graham tube which has a standard diameter of 6 mm. The bacterial suspensions were spread onto the agar by using sterile cotton buds. The crude extracts were diluted two-fold with 20% DMSO as diluents (Aiyelaagbe et al., 2007). 20 µl of the crude extract was pipetted into the wells and allowed to dry before leaving overnight in an incubator at 37°C. The test was carried out in three replicates.

Minimum inhibitory concentration (MIC) determination

The test was performed in 96 well plates according to the method of Aiyelaagbe et al. (2007). Two-fold dilutions of the crude extracts were prepared. The concentration of each extract for each bacterial strain is different as it depends on the results of the antibacterial activity. A 200 µl aliquot of bacterial culture standardized to OD_{600} = 0.5 was added to each well. This was followed by 200 µl of the crude extracts. With each subsequent well, the concentration of the crude extract was diluted. The 96 well plates were then kept in a 37°C incubator overnight. To determine the viability of the bacterial cultures in the wells, a colorimetric assay was utilized whereby 20 µl MTT diluted in 20% DMSO (it was chosen because it has no effect to the bacterial growth) was added to each well and left to incubate at 37°C for 20 min. The appearance of a yellow colour indicates that the bacterial culture is not viable and has been killed by the presence of the crude extract. Viable cultures will appear blue.

Table 1. *In vitro* antimicrobial activity of the methanol extracts of *J. curcas* latex before partition against human pathogenic bacteria.

Conc. of extracts (mg/ml)	Microorganisms / zone of inhibition (mm)										
	EF	L	S	SAGA	ST	EC	K	MM	MRSA	MSSA1	MSSA2
100	22±1.2	12.3±2.6	11±1.2	-	12.3±0.9	11.7±0.7	17±0.9	16.3±1.7	25.7±0.4	24.3±0.7	17.7±1.5
50	16.3±3.7	9.3±2.8	5.7±2.8	-	10±0	6.3±3.2	10±0	11.7±0.7	18.7±1.3	20.3±0.3	4.7±4
25	9.7±4.8	3±3	3±3	-	8.3±0.7	-	2.7±2.7	8±0.6	13.3±1.2	7.3±3.7	-
12.5	2.7±2.7	-	-	-	-	-	-	-	8.7±1.2	3±3	-
DMSO	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: EF = *E. faecalis*; L = *L. monocytogenes*; S = *S. marcescens*; SAGA = *S. agalactiae*; ST = *S. enterica serovar typhi*; EC = *E. coli*; K = *K. pneumoniae a*; MM = *M. morganii*; MRSA = methicillin-resistant *Staphylococcus aureus* ATCC 33591; MSSA1 = methicillin-sensitive *S. aureus* ATCC 29213; MSSA2 = methicillin-sensitive *S. aureus* ATCC 25174; - = No zone of inhibition

Phytochemical screening

Tests were carried out to determine the presence of tannins and saponins in the latex extracts. The tests were adapted from standard procedures to identify the constituents as reported by Sofowora (1993), Trease and Evans (1989) and Harborne (1973). To test for the presence of saponins, 2 g of the samples was boiled in 20 ml distilled water on a hot water plate and then filtered. 10 ml of the filtrate was mixed with 5 ml distilled water and shaken vigorously for a stable persistent froth. To the frothing mixture, 3 drops of olive oil were added and the mixture was again shaken vigorously. The frothing was then observed for the formation of an emulsion which is indicative of the presence of saponins (Sofowora, 1993; Trease and Evans, 1989; Harborne, 1973).

The test for tannins was performed by using 0.5 g of the samples which were boiled in 20 ml distilled water and filtered. A few drops of 0.1% ferric chloride was added to the filtrate and then observed for brownish-green or blue-black colouration indicative of the presence of tannins (Sofowora, 1993; Trease and Evans, 1989; Harborne, 1973).

RESULTS AND DISCUSSIONS

Jatropha has traditionally been used for the treatment of various ailments such as skin infections, sexually transmitted diseases like gonorrhoea, as well as for jaundice and fever (Dalziel, 1937; Burkill, 1994; Chopra et al., 1956; Martinez, 1959). Scientific studies carried out have reported that extracts from *Jatropha* species have anti-bacterial, antitumor as well as anti-insect activities (Aiyelaagbe et al., 1998, 2000; Akinpelu et al., 2009). Antimicrobial activity of *J. curcas* latex against *E. coli* and *S. aureus* have previously been reported (Thomas et al., 2008) and validated by the results obtained in this study.

The antibacterial activity of the various extracts of *J. curcas* latex was tested using agar well diffusion method against nine human pathogenic bacteria. The crude extracts obtained from the four different extracts (methanol, ethyl acetate, water and hexane) were diluted ranging from 12.5, 25, 50 and 100 mg/ml. The methanol crude extracts before and after partition was tested (Table 1 and 2). The extracts, at a concentration of 100 mg/ml, showed activity against all nine pathogenic bacteria except for the Gram positive *S. agalactiae*. Interestingly, the diameters of the zone of inhibition at a concentration of 100 mg/ml were larger than methanol extract after partition as compared to before partition (Figure 1). Thus, the partitioning process did not appear to affect the antibacterial activity of the latex extract and in fact, it appears to lead to a higher activity level when compared to the extract before partition. At a concentration of 100 mg/ml, the ATCC type strain of MRSA ATCC 33591 showed the largest zone of inhibition diameter at (25.7 ± 0.4) mm for the methanol extract before partition and (30.0 ± 0.0) mm for the extract after partition. Meanwhile, the MSSA ATCC 29213 (MSSA 1) showed similar zones of inhibition but the other MSSA ATCC 25174 (MSSA 2) displayed smaller zones of inhibition at (17.7 ± 1.5) mm for the methanol extract before partition and (29.0 ± 0.0) mm for the extract after partition. The methanol extract before partition was not effective against MSSA 2 at concentrations lower than 50 mg/ml but was still effective against all three *S. aureus* strains at a concentration of 12.5 mg/ml for the methanol extract after partition. *E. coli* showed the smallest zone of inhibition for 100 mg/ml of the *Jatropha* methanol extract: (12.3 ± 0.9) mm for the extract after partition and (11.7 ± 0.7) mm for the extract before partition. No zone of inhibition was observed for *E. coli* at a concentration of 50 mg/ml of the methanol extract after partition but for the extract before partition, a smaller zone of inhibition (6.3±3.2 mm) was observed at this concentration but not for lower concentrations. At a concentration of 25 mg/ml of the methanol

Table 2. *In vitro* antimicrobial activity of the methanol extracts of *J. curcas* latex after partition against human pathogenic bacteria.

Conc. of extracts (mg/ml)	Microorganisms / zone of inhibition (mm)										
	EF	L	S	SAGA	ST	EC	K	MM	MRSA	MSSA1	MSSA2
100	26.7±1.2	15.3±1.3	13±0.9	-	15±0.9	12.3±0.9	20.3±0.3	19.3±0.3	30±0	29±0	20.7±0.3
50	21.7±0.9	10±0	3.7±3.7	-	4±3.9	-	17.7±0.3	16.3±0.7	23.3±1.7	21.3±1.3	17.7±1.8
25	18.7±0.3	-	-	-	4.7±4.7	-	16±0.6	4.7±4.7	13.7±2.8	12.3±3.4	9.3±4.1
12.5	15.7±1.2	-	-	-	-	-	13.7±0.9	-	18.7±0.3	12.3±1.8	10.7±0.3
DMSO	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: EF = *E. faecalis*; L = *L. monocytogenes*; S = *S. marcescens*; SAGA = *S. agalactiae*; ST = *S. enterica* serovar *typhi*; EC = *E. coli*; K = *K. pneumoniae*; MM = *M. morganii*; MRSA = methicillin-resistant *Staphylococcus aureus* ATCC 33591; MSSA1 = methicillin-sensitive *Staphylococcus aureus* ATCC 29213; MSSA2 = methicillin-sensitive *S. aureus* ATCC 25174; - = No zone of inhibition.

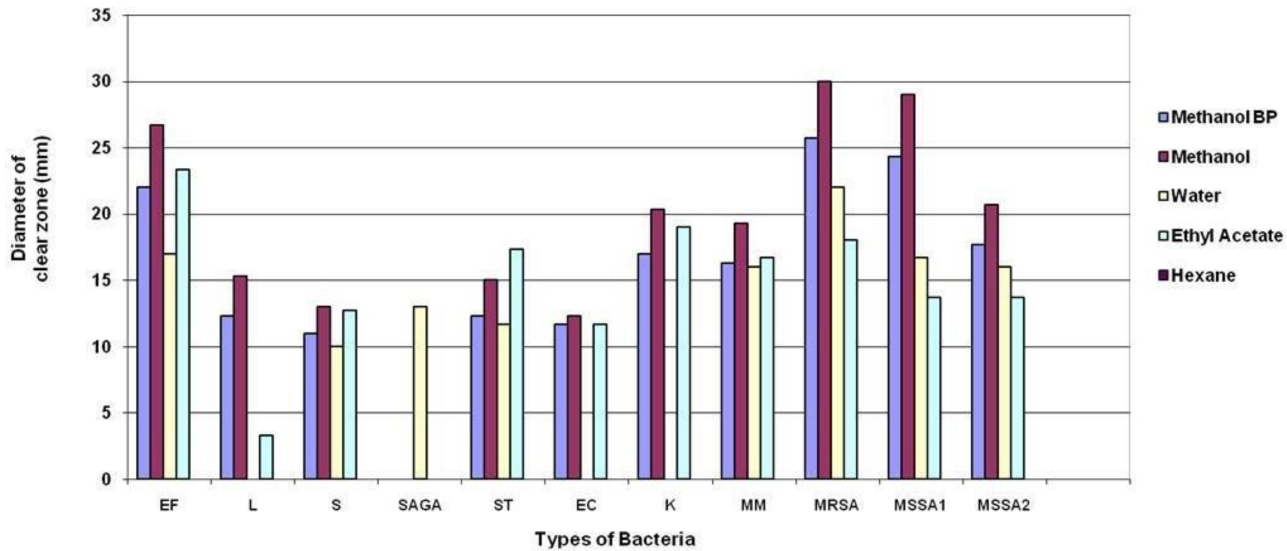

Figure 1. Overall diameter of inhibitory zone of different *J. curcas* fractions against various types of bacteria. Abbreviations: EF = *E. faecalis*; L = *Listeria monocytogenes*; S = *S. marcescens*; SAGA = *S. agalactiae*; ST = *S. enterica* serovar *typhii*; EC = *E.coli*; K = *K. pneumoniae*; MM = *M. morganii*; MRSA = methicillin-resistant *S. aureus* ATCC 33591; MSSA1 = methicillin-sensitive *S. aureus* ATCC 2921; MSSA2 = methicillin-sensitive *S. aureus* ATCC 25174; Methanol BP = methanol extract before partition.

extract after partition, only 7 bacterial strains were inhibited. Besides *E. coli*, other bacterial strains that were not inhibited by the extract at 25 mg/ml were *L. monocytogenes* and *S. marcescens.* At a lower concentration of 12.5 mg/ml only 5 strains were inhibited: the three isolates of *S. aureus*, *K. pneumoniae* and *E. faecium*. The methanol extract of *J. curcas* latex showed the highest antibacterial activity when compared to the other

Table 3. *In vitro* antimicrobial activity of the water extracts of *J. curcas* latex against human pathogenic bacteria.

Conc. of extracts (mg/ml)	Microorganisms / zone of inhibition (mm)										
	EF	**L**	**S**	**SAGA**	**ST**	**EC**	**K**	**MM**	**MRSA**	**MSSA1**	**MSSA2**
100	17±0.9	-	10±5	13±0	11.7±0.7	-	-	16±0.6	22±1.5	16.7±0.3	16±0
50	14.7±1.2	-	7.7±3.9	10.3±0.9	11±0.7	-	-	16±0	20±1.2	15.3±0.3	14.3±0.7
25	-	-	7±3.5	10.3±0.9	5.7±3.6	-	-	14±0	21±0.9	14.7±0.6	9.7±0.3
12.5	-	-	6.7±3.3	-	-	-	-	12±0.9	16.3±2.2	10.7±0.3	9.3±0.7
DMSO	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: EF = *E. faecalis*; L = *L. monocytogenes*; S = *S. marcescens*; SAGA = *S. agalactiae*; ST = *S. enterica* serovar *typhi*; EC = *E. coli*; K = *K. pneumoniae*; MM = *M. morganii*; MRSA = methicillin-resistant *S. aureus* ATCC 33591; MSSA1 = methicillin-sensitive *S. aureus* ATCC 29213; MSSA2 = methicillin-sensitive *S. aureus* ATCC 25174; - = No zone of inhibition

extracts that were tested in this study. This could be observed from the number of bacterial strains that were inhibited by the extract as well as the size of the zones of inhibition (Figure 1). The methanol extract of *J. curcas* root bark was also reported to display potent broad spectrum *in vitro* antimicrobial activity (Aiyelaagbe et al., 2007). In addition, a study on the stem bark of *J. curcas* also indicated that the methanol extract was a more effective antimicrobial when compared to the ethanol extract (Igbinosa et al., 2009). Kowalski and Kedzia (2007) had described the methanol extract as more active than the other extracts and attributed it to the presence of phenolic and polyphenolic compounds that are more soluble in methanol compared to other solvents. In a study of *Psidium guajava* L., it was found that the methanol extract contained tannins and was very effective against *E. coli* (strain isolated from the urinary tract), followed by *K. pneumoniae* (also isolated from the urinary tract) and *S. aureus* (isolated from the urinary tract, abcess, ear and wound) (Abdelrahim et al., 2002). The water extract of *J. curcas* latex was not effective against *L. monocytogenes*, *E. coli* and *K. pneumoniae* up to a concentration of 100 mg/ml (Table 3). The extract was not effective against *E. faecalis* at a concentration of 25 mg/ml and below whereas at a concentration of 12.5 mg/ml, the extract was ineffective against *S. agalactiae* and *S. enterica* serovar *typhi*. Methicillin-resistant *S. aureus* ATCC 33591 (MRSA) showed the largest zone of inhibition diameter, (22 ± 1.5) mm, at a concentration of 100 mg/ml extract, while *S. marcescens* displayed the smallest zone of inhibition, that is (10 ± 5) mm, at the same extract concentration. The water extract of the *J. curcas* latex only affected eight out of the elevan bacterial strains tested and was not effective against *Listeria*, *E. coli* and *K. pneumoniae*. This is similar to other studies carried out using the water extracts of other parts of *J. curcas* in which it was reported to have very little or no antibacterial activity (Koduru et al., 2006; Aliero et al., 2006; Ashafa et al., 2008; Aiyegoro et al., 2008). In a study on the antimicrobial activity of *P. guajava* L., Abdelrahim et al. (2002) found that the aqueous extract contained saponins and tanins based on the phytochemical screening tests. The aqueous extract was found to inhibit *E. coli*, *K. pneumoniae* and *S. aureus* as well as *Proteus vulgaris* and *P. aeruginosa* (Abdelrahim et al., 2002). However, the water extract of *Jatropha* latex was the only extract that was able to inhibit the growth of *S. agalactiae*.

The ethyl acetate crude extract of *J. curcas* did not show any antibacterial activity against *S. agalactiae* (Table 4), similar to the methanol and hexane extracts. *Listeria monocytogenes* was least affected by the ethyl acetate extract as it only showed a zone of inhibition at an extract concentration of 100 mg/ml. The ethyl acetate extract was most effective against *E. faecalis* at a concentration of 100 mg/ml where it displayed the largest zone of inhibition at (23.3 ± 1.7) mm. However, at a concentration of 12.5 mg/ml, the ethyl acetate extract was less effective against *E. faecalis* as it gave the smallest zone of inhibition when compared to the other pathogenic bacteria tested. The ethyl acetate extract was only effective against both methicillin-resistant and methicillin-sensitive *S. aureus* strains at concentrations of 50 mg/ml and higher, except for *S. aureus* ATCC 2921 (MSSA1) which showed a small zone of inhibition at a concentration of 25 mg/ml extract. The hexane extracts of *J. curcas* latex did not show any antimicrobial activity against all the pathogenic bacteria tested at concentrations between 12.5 to 100 mg/ml (Table 5). This indicated that the ethyl acetate extracts do not have a good potency level and the active

Table 4. *In vitro* antimicrobial activity of the ethyl acetate extracts of *J. curcas* latex against human pathogenic bacteria.

Conc. of extracts (mg/ml)	Microorganisms / zone of inhibition (mm)										
	EF	L	S	SAGA	ST	EC	K	MM	MRSA	MSSA1	MSSA2
100	23.3±1.7	3.3±3.3	12.7±1.2	-	17.3±0.9	11.7±1.2	19±4.5	16.7±0.7	18±2	13.7±2.3	13.7±0.7
50	20.7±0.3	-	12.3±1.2	-	15±0	10.7±0.7	20.3±0.9	14.3±0.3	8±4.3	10.7±0.3	8.7±4.3
25	16±1.2	-	6.3±3.3	-	12.3±0.3	5.7±2.8	11.3±0.7	10.3±0.3	-	4.7±2.3	-
12.5	3.3±3.3	-	4.7±4	-	9±0.5	-	7.7±4.1	7±0	-	-	-
DMSO	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: EF = *E. faecalis*; L = *L. monocytogenes*; S = *Serratia marcescens*; SAGA = *S. agalactiae*; ST = *S. enterica* serovar *typhi*; EC = *E. coli*; K = *K. pneumoniae*; MM = *M. morganii*; MRSA = methicillin-resistant *S. aureus* ATCC 33591; MSSA1 = methicillin-sensitive *S. aureus* ATCC 29213; MSSA2 = methicillin-sensitive *S. aureus* ATCC 25174; - = No zone of inhibition

Table 5. *In vitro* antimicrobial activity of the hexane extracts of *J.curcas* latex against human pathogenic bacteria.

Conc. of extracts(mg/ml)	Microorganisms / zone of inhibition (mm)										
	EF	L	S	SAGA	ST	EC	K	MM	MRSA	MSSA1	MSSA2
100	-	-	-	-	-	-	-	-	-	-	-
50	-	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-	-
12.5	-	-	-	-	-	-	-	-	-	-	-
DMSO	-	-	-	-	-	-	-	-	-	-	-

Abbreviations: EF = *E. faecalis*; L = *L. monocytogenes*; S = *S. marcescens*; SAGA = *S. agalactiae*; ST = *S. enterica* serovar *typhi*; EC = *E. coli*; K = *K. pneumoniae*; MM = *M. morganii*; MRSA = methicillin-resistant *S. aureus* ATCC 33591; MSSA1 = methicillin-sensitive *S. aureus* ATCC 29213; MSSA2 = methicillin-sensitive *S. aureus* ATCC 25174; - = No zone of inhibition

compounds in the extract are probably not pharmaceutically useful (Rios et al., 1988). High MIC values may be due to the impurity of the bio-active compound in the extracts or the bioactive compounds are present in low concentrations (Tonia and van Staden, 1997). In a study carried out to test the activity of several concentrations of tannins against a panel of bacterial strains, high concentrations of tannins gave low MIC values while low concentrations of tannins yielded high MIC values. Thus, the antibacterial effectiveness of tannins increased with increasing concentration (Kurosaki and Nishi, 1983; Banso and Adeyemo, 2007).

When comparing the antimicrobial activities of the various fractions of the *J. curcas* latex crude extract at a concentration of 100 mg/ml, the methanol fraction appeared to be the most effective against the range of pathogenic bacteria tested. The methanol extract before partition showed the same antimicrobial pattern as the extract after partition but with smaller zones of inhibition. *Streptococcus agalactiae* was however, inhibited only by the aqueous extract of the *J. curcas* latex which was inhibitory to three other pathogenic bacteria, *E. faecalis*, *S. enterica* serovar *typhi* and *S. aureus* but with smaller zones of inhibition when compared to the methanol extracts. The hexane extract was not inhibitory at a concentration of 100 mg/ml against the bacterial strains tested.

The MIC assay was carried out to determine the lowest concentration of the crude extract that would be inhibitory to the growth of the bacterial strain (Table 6). This was performed starting with the lowest concentration of the crude extract that was inhibitory to the bacterial growth and was successively diluted 2-fold. The MIC value was taken at the lowest concentration in which inhibition to bacterial growth was observed. The MIC assay was carried out only on the methanol, ethyl acetate and water fractions as the hexane

Table 6. Minimum inhibitory concentration (MIC) values of the various fractions of the crude extract of *J.curcas* latex against human pathogenic bacteria.

Extracts	Microorganisms / MIC (mg/ml)										
	EF	L	S	SAGA	ST	EC	K	MM	MRSA	MSSA1	MSSA2
Methanol	0.78	0.78	6.25	-	1.56	1.56	6.25	1.56	1.56	0.39	3.13
Water	3.13	-	3.13	1.56	1.56	-	-	1.56	1.56	1.56	1.56
Ethyl acetate	6.25	1.56	12.5	-	12.5	25	12.5	6.25	6.25	12.5	6.25

Abbreviations: EF = *E. faecalis*; L = *L. monocytogenes*; S = *S. marcescens*; SAGA = *S. agalactiae*; ST = *S. enterica* serovar *typhi*; EC = *E. coli*; K = *K. pneumoniae*; MM = *M. morganii*; MRSA = methicillin-resistant *S. aureus* ATCC 33591; MSSA1 = methicillin-sensitive *S. aureus* ATCC 29213; MSSA2 = methicillin-sensitive *S. aureus* ATCC 25174; - = No zone of inhibition

Table 7. Results of phytochemical tests on the extracts of *J. curcas* latex.

Secondary metabolites	Methanol	Picture	Aqueous	Picture	Ethyl acetate	Picture
Saponins	+	Emulsion formation	+	Emulsion formation	+	Emulsion formation
Tannins	+	Extract colour changes (left) after ferric chloride drops into the tube. Right without ferric chloride.	+	Extract colour changes (left) after ferric chloride drops into the tube. Right without ferric chloride.	+	Extract colour changes (left) after ferric chloride drops into the tube. Right without ferric chloride.

fraction did not show any antimicrobial activity against the nine pathogenic bacteria tested. The methanol extract appeared to be most effective against *S. aureus* ATCC 29213 (MSSA1) as it required only 0.39 mg/ml to inhibit its growth whereas a higher concentration of the extract is required to inhibit the growth of the other two *S. aureus* strains – 1.56 mg/ml for *S. aureus* ATCC 33591 (MRSA1) and 3.13 mg/ml for *S. aureus*

ATCC 25174 (MSSA2). In comparison, the MIC value for the aqueous extract was 1.56 mg/ml for all three *S. aureus* strains whereas for the ethyl acetate extract, a much higher concentration is required for growth inhibition – 6.25 mg/ml forMRSA and MSSA2 and 12.5 mg/ml for MSSA1. With the exception of *S. marcescens* and *S. agalactiae*, the methanol extract appeared to be more effective (that is, inhibitory at lower concentrations) against the range of pathogenic bacteria tested. For *S. marcescens*, the aqueous extract showed the lowest MIC value at 3.13 mg/ml as compared to 6.25 mg/ml for the methanol extract and 12.5 mg/ml for the ethyl acetate extract. In the case of *E. coli*, the MIC value for the methanol extract was 1.56 mg/ml whereas for the ethyl acetate extract, the MIC value was much higher at 25 mg/ml. The aqueous extract was not inhibitory against *E. coli*. The methanol and aqueous extracts appeared to be equally effective against *S. enterica* serovar *typhi* and *M. morganii* as both organisms had identical MIC values for both extracts, 1.56 mg/ml (Table 6).

Phytochemical screening carried out on the crude latex extract revealed the presence of saponins and tannins as had previously been reported on other parts of the plant (Igbinosa et al., 2009; Mishra et al., 2010; Oskoueian et al., 2011) (Table 7). Saponins and tannins, in particular, have been reported to possess antimicrobial activity (Diaz et al., 1988; Ogunleye and Ibitoye, 2003; Pretorius et al., 2003; Zakaria et al., 2010). In conclusion, the present study revealed the potential antimicrobial activity of the *J. curcas* latex extract, which could be attributed to the presence of saponins and tannins.

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