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ANTIBACTERIAL POTENTIAL OF *JATROPHA CURCAS* LEAF EXTRACTS AGAINST PATHOGENS ASSOCIATED WITH SICKLE CELL DISEASE IN BENIN

Armelle Sabine Yélignan Hounkpatin[1,2]*, Dossou Armel Géraldo Houndeton[1,3], Amoussatou Sakirigui[4], Mamatchi Mélina[5], Franck Yovo[4]

[1]Pluridisciplinary Research Laboratory for Technical Education (LARPET), University of Sciences, Technologies, Engineering and Mathematics of Abomey (UNSTIM), Benin.
[2]Laboratory of Hygiene, Sanitation, Toxicology and Environmental Health (HECOTES), Interfaculty Center for
[3]Training and Research in the Environment for Sustainable Development (CIFRED), University of Abomey-Calavi, Benin.
[4]Multidisciplinary Laboratory for Practical Work of the Faculty of Health Sciences (FSS), University of Abomey-Calavi, Benin.
[5]Kaba Research Laboratory in Chemistry and Applications (LaKReCA), University of Technical Sciences, Engineering and Mathematics (UNSTIM), PO Box 266 Natitingou, Bénin.

***Corresponding Author: Armelle Sabine Yélignan Hounkpatin**
Pluridisciplinary Research Laboratory for Technical Education (LARPET), University of Sciences, Technologies, Engineering and Mathematics of Abomey (UNSTIM), Benin.

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ABSTRACT
Sickle cell disease patients are highly susceptible to bacterial infections, and therapeutic options are often limited by antimicrobial resistance. This study evaluated the antibacterial potential of hydroethanolic (50 :50 v/v) leaf extracts of *Jatropha curcas* and their ability to reverse bacterial resistance to common antibiotics. The extract was tested against Gram-positive (*Staphylococcus aureus*, *S. epidermidis*) and Gram-negative bacteria (*Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa*) using broth microdilution to determine minimum inhibitory (MIC) and bactericidal (MBC) concentrations. Synergy assays with amoxicillin and ciprofloxacin were performed using the fractional inhibitory concentration (FIC). The extract exhibited strong activity against Gram-positive strains and several Gram-negative isolates, with MICs of 6.25–12.5 mg/mL and MBC/MIC ratios of 4, indicating bactericidal effects. *P. aeruginosa* was less sensitive (MIC 25 mg/mL; MBC >200 mg/mL). When combined with amoxicillin or ciprofloxacin, the extract produced synergistic effects (FIC ≤0.125) across all tested strains, including resistant *P. aeruginosa*, leading to marked reductions in antibiotic MICs. Hydroethanolic extracts of *Jatropha curcas* leaves demonstrate promising bactericidal activity against pathogens relevant to sickle cell disease in Benin. Beyond direct antibacterial effects, the extract significantly enhances the activity of standard antibiotics, supporting its potential as a source of adjuvants to combat antimicrobial resistance. Further studies should aim to identify active compounds and validate these findings in vivo.

KEYWORDS: *Jatropha curcas*; Antibacterial potential; hydroethanolic extract; sickle cell disease.

1 INTRODUCTION
Sickle cell disease (SCD) affects millions of people worldwide and remains a serious inherited haemoglobin disorder. Globally, It is estimated that in 2021, approximately 7.74 million people were living with sickle cell disease, with more than 300,000 severe births each year, the majority in low- and middle-income countries (GBD 2021 Sickle Cell Disease Collaborators, 2023). In sub-Saharan Africa, the situation is even more worrying: more than 70% of global SCD cases are found

there, and mortality among children under 5 with the disease remains very high, often due to bacterial infections, anemic complications, and a lack of adequate healthcare infrastructure(Adigwe et al., 2023); (Elendu et al., 2023). In terms of prevalence, sickle cell trait (heterozygous S) is estimated to affect approximately 20% of the Beninese population, with double heterozygosity SC affecting around 4-5%, while homozygotes SS are less common but highly susceptible to infectious complications (Dodo et al., 2018). Sickle cell patients are highly vulnerable to bacterial infections, mainly due to impaired spleen function, anemia, and weakened immunity. A study conducted in Cotonou at the National University Hospital Center (CNHU-Hubert Koutoukou Maga) showed that among sickle cell emergencies, non-localized bacterial infections accounted for 27.9% of cases, pneumonia for 18.6%, and osteomyelitis and pyelonephritis for the same percentage (Dodo et al., 2018). Traditional medicine plays an important role in the management of SCD in Benin. Among medicinal plants used, *Jatropha curcas* L. (Euphorbiaceae) is commonly employed, notably its leaves, for treating infections and symptoms related to SCD such as fever, wounds, and perhaps indirectly to relieve oxidative stress. Previous studies have demonstrated that *Jatropha curcas* leaves contain a variety of bioactive phytochemicals including phenols, flavonoids, tannins, saponins, steroids, alkaloids and terpenoids (Yakubu & Yebpella, 2024). Several of these secondary metabolites are known for their antimicrobial activity. In fact, previous research has reported antibacterial effects of *J. curcas* extracts against a range of pathogenic bacteria (Rahu et al., 2021); (Ikoyi et al., 2023). However, little information is available on the antibacterial properties of the leaves specifically against bacteria commonly associated with sickle cell infections in Benin. Based on this background, the present study aimed to evaluate the antibacterial activity of *J. curcas* leaf extracts against selected pathogenic bacteria of clinical relevance to SCD.

2. MATERIALS AND METHODS

2.1. Leaves collection and extraction

Fresh leaves of *Jatropha curcas* were collected at Adjagbo in the commune of Abomey - Calavi. Identification was carried out at the Benin National Herbarium under number HY 843/HNB. After being washed, cut and dried at a temperature of 20 °C±2 under air conditioning for two weeks, the leaves were pulverized using an electric grinder (MARLEX Electronic Excella). The resulting powders were stored in plant packaging bags (double PM kraft bags) prior to extraction. The hydroethanolic extract was prepared according to the method described previously by (Kpètèhoto et al., 2019). The powdered plant material (200 g) was extracted by maceration with stirring for 48 h at room temperature (20 °C±2) with 2000 ml of the ethanol-water mixture (50:50) using a mechanical shaker (IKA KS 260 basic). After the first maceration, the plant residue was re-extracted under the same conditions to ensure maximum recovery of bioactive compounds. The two macerates obtained were pooled, filtered first through cotton and then through Whatman filter paper (N°1001-150, Grade 1, 150 mm Ø, batch of 100). The combined filtrates were concentrated under reduced pressure using a rotary evaporator (BUCHI Rotavapor R-100) and then dried to obtain the crude extract. The resulting extracts were stored at 4 °C until further antibacterial analyses.

2.2. Bacterial strains

Hydroethanolic extracts of *Jatropha curcas* leaves were tested against a panel of bacteria including three Gram-positive*: Staphylococcus aureus* (*ATCC 6538*), *Staphylococcus aureus* (clinical), *Staphylococcus epidermidis* (*CIP8039)*, and seven Gram-negative : *Escherichia coli (CIP 53126), Escherichia coli* (clinical), and *Pseudomonas aeruginosa* (CIP82118) obtained from Laboratory of Biochemistry and Bioactive Natural Substances (LBSNB), University of Abomey Calavi.

2.3. Nutrient media

Müller Hinton Agar (MHA) was obtained by dissolving 38 g of the agar medium in 1 L of distilled water (pH = 7.5 ± 0.2). Müller Hinton broth was obtained by dissolving 21 g in 1 L of distilled water. Each medium was sterilized in an autoc lave at a temperature of 121˚C for 15 min.

2.4. Antibacterial activity

2.4.1. Direct Antimicrobial Activity Determination of leaves extract

Antimicrobial activity was evaluated using Mueller-Hinton agar culture plates and the agar well diffusion method (Balouiri et al., 2016). All tested strains were first suspended and seeded according to the French Society of Microbiology Antibiogram Comity guidelines (CA-SFM/EUCAST, 2017). Plant extracts were prepared at 100mg/mL in DMSO (Sigma Aldrich, Saint-Louis, USA) and then filtered using 0.4μm multipore membranes (Millex, Merck Millipore, Darmstadt, Germany) in order to be sterile. 50μl of each plant extract was placed in 6mm wellsdug in the agar plates as previously described (Agbankpe & Dougnon, 2016). DMSO was also tested as a negative control. Gentamycine was used as positive contol. Culture plates were left at room temperature for one hour for prediffusion and then incubate at 37°C during 18 hours as previously described (Tsirinirindravo & Andrianarisoa, 2010). The average of each inhibition diameter obtained was compared with the mean diameters obtained for references antibiotics discs. Graph pad software version 6.00 at the significance level of 5% has been used.

2.4.2. MIC, MBC, and Antibacterial Activity Measurements

The Minimum Inhibitory Concentration (MIC) was measured according to a previously reported method performed in microwells with several lines per plate

(Koudokpon et al., 2018). Each culture plate (Thermo Fisher, California, United States) included 8 lines. Each line included 12 wells. Line 1 was the positive control : bacterial suspension in Mueller-Hinton broth. Line 8 was the negative control : DMSO in Mueller-Hinton broth. Lines 2 to 7 were dedicated to different bacteria strains tested as follows. Well 1 contained 180μL of 50mg/mL plant extract. Wells number 2 to 10 were filled with 180μL of plant extract successively diluted into Mueller-Hinton broth (two per two). Wells 11 and 12 were filled with 180 μL of Mueller-Hinton broth. Wells 1 to 11 were spiked with 20μL ofMueller-Hinton broth containing 10% of bacteria measured at 0.5 McFarland. Well 12 did not contain any bacteria in order to check broth sterility. Culture plates were then agitated during 5 minutes and incubated at 37∘C for 18 hours. Each well received 40μL of 0.2% p-iodonitrotetrazolium (INT) aqueous solution (Sigma-Aldrich, Missouri, United States). Plates were stored 20 minutes and protected from light. Red colored wells indicated the viability of bacteria as described previously (Eloff, 1998). Thus, MIC was defined as the lowest concentration corresponding to viable bacteria. Wells without red color were cultivated on new Muller-Hinton culture plates. Minimum Bactericidal Concentration (MBC) was defined as the lowest concentration corresponding to the presence of colonies after culture. Antibacterial power (AP) was defined as the MBC/MIC ratio.

2.4.3. Reversion of bacterial resistance

The main objective of this test is to determine the synergy of action between the extract and the conventional antibiotics Amoxicillin (AMX) and Ciprofloxacin (CIP). It consisted in finding the MICs of conventional antibiotics and those of the combination of antibiotics and extract in order to calculate the fractional inhibitory concentration (CFI). Accord ing to the Antibiotic Committee of the French Microbiology Society reported by (Yuan et al., 2024), there are four levels of interpretation, namely:

- Synergy (CFI $\leq$ 0.5)
- Addition (0.5 < CFI $\leq$ 1)
- Indifference (1 < CFI $\leq$ 4)
- Antagonism (CFI > 4).

Principle

50 µL of the 1 mg antibiotic solution was dispensed into the first and second wells of the microplate. 50 µL of the HD solution was distributed into the wells from the second line. Then a cascade dilution was made. Then, each well receives 50 µL of extract and 100 µL of inoculum. After 24 hours of incubation at 37˚C, the reading was made by adding 40 µL of 0.01% INT. A second incubation was carried out for 30 minutes. The presence of germs is reflected by the red coloring of the medium. Controls without plant extract, without bacterial suspension and without antibiotics were used.

3. RESULT

3.1. Sensitivity testing for bacterial strains

The inhibition diameters obtained during sensitivity testing of the hydroethanolic extracts in contact with bacterial strains are shown in the table below.

Table 1: Sensitivity Test Result.

Bacterial strains	ID (200 mg/ml) ± SD	ID (400 mg/ml) ± SD	DMSO	% Increase 200→400	Gentamicin (mm)	% activity vs Gentamicin
S. aureus ATCC 6538	15.33 ± 0.57	20.33 ± 0.57	0	32.6 %	24.6 ± 0.5	82.7 %
S. aureus (clinique)	14.33 ± 0.57	18.33 ± 0.57	0	27.9 %	23.8 ± 0.6	77.0 %
S. epidermidis CIP 8039	13.66 ± 0.57	17.66 ± 0.57	0	29.3 %	22.5 ± 0.6	78.4 %
E. coli CIP 53126	11.66 ± 0.57	14.33 ± 0.57	0	22.9 %	22.4 ± 0.5	64.0 %
E. coli isolé	12.66 ± 0.57	15.33 ± 0.57	0	21.1 %	21.9 ± 0.5	70.0 %
P. aeruginosa CIP 82118	10.66 ± 0.57	13.33 ± 0.57	0	25.0 %	21.5 ± 0.6	62.1 %
P. aeruginosa isolée	9.66 ± 0.57	12.66 ± 0.57	0	31.1 %	20.8 ± 0.5	60.8 %
Salmonella spp	11.33 ± 0.57	14.66 ± 0.57	0	29.4 %	22.1 ± 0.6	66.3 %
Salmonella paratyphi A	12.33 ±0.57	15.33 ± 0.57	0	24.3 %	22.7 ± 0.5	67.5 %

Hydroethanolic extracts from *Jatropha curcas* leaves showed no antibacterial activity at 100 mg/mL. Moderate inhibition appeared at 200 mg/mL (zones of 9.66 ± 0.57 to 15.33 ± 0.57 mm), then increased at 400 mg/mL (12.66 ± 0.57 mm for *P. aeruginosa* to 20.33 ± 0.57 mm for *S. aureus ATCC 6538*), reflecting a dose-dependent effect. Compared to gentamicin, the extracts showed weaker activity, but reached 78–83% of that of the antibiotic on Gram-positive bacteria (*S. aureus, S. epidermidis*) and only 60–67% on Gram-negative bacteria, particularly *P. aeruginosa*.

3.2. Antimicrobial activity of hydroethanolic extract

Table 2: Antimicrobial affect of the hydroethanolic mixture of *Jatropha curcas* at 200mg/ml.

Bacterial strains	MIC	MBC	MBC/MIC	Antibacterial effects
	(200mg/ml)			
S.aureus ATCC 6538	6,25	25	4	Bactericide
S.aureus	6,25	25	4	Bactericide
S.épidermidis CIP 8039	6,25	25	4	Bactericide
E.coli CIP 53126	12,5	50	4	Bactericide
E.coli isolé	12,5	50	4	Bactericide
P.aeruginosa CIP 82118	25	>200	8	Undetermined
P.aeruginosa isolée	25	>200	8	Undetermined
Salmonella spp	12,5	50	4	Bactericide
Salmonella paratyphi A	12,5	50	4	Bactericide

The hydroethanolic extract (50:50) from *Jatropha curcas* leaves showed significant activity, with MICs ranging from 6.25 to 25 mg/mL. Strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Salmonella spp*. were inhibited at low concentrations (MIC ≤ 12.5 mg/mL), indicating marked sensitivity. For these strains, the MBCs were on average four times higher than the ICMs (MBC/ICM = 4), suggesting a bactericidal effect. In contrast, *Pseudomonas aeruginosa* had an MIC of 25 mg/ml and an MBC > 200 mg/mL (MBC/IMIC ≥ 8), indicating low sensitivity to the extract. These results confirm the bactericidal potential of *Jatropha curcas* against Gram-positive and certain Gram-negative bacteria, but highlight its limited efficacy against *Pseudomonas aeruginosa*.

3.3. Reversal of bacterial resistance with the extract combined with antibiotics

To test the reversal of bacterial resistance, we investigated the sensitivity of the bacteria to antibiotics and then to the combination of extract and conventional antibiotic.

3.3.1. Reversal of bacterial resistance with Jatropha curcas and Amoxicillin

The table 3 shows the results of the reversal of resistance in bacterial strains with the aqueous mixture of *Jatropha curcas* and the antibiotic amoxicillin.

Table 3: Reversal of bacterial resistance with Jatropha curcas and Amoxicillin.

Bacterial strains	*Jatropha curcas*	AMX	AMX + *Jatropha curcas*	FIC	Antibacterial effects
	CMI (mg/ml)				
S.aureus ATCC 6538	6,25	0,0625	0,0078	0,1248	Synergistic
S.aureus	6,25	0,0625	0,0078	0,1248	Synergistic
S.épidermidis CIP 8039	6,25	0,0625	0,0078	0,1248	Synergistic
E.coli CIP 53126	12,5	0,125	0,0156	0,1248	Synergistic
E.coli isolé	12,5	0,125	0,0156	0,1248	Synergistic
P.aeruginosa CIP 82118	25	0,5	0,0625	0,125	Synergistic
P.aeruginosa isolée	25	0,5	0,0625	0,125	Synergistic
Salmonella spp	12,5	0,125	0,0156	0,1248	Synergistic
Salmonella paratyphi A	12,5	0,125	0,0156	0,1248	Synergistic

The combination of hydroethanolic extract from *Jatropha curcas* leaves with amoxicillin resulted in a marked reduction in the minimum inhibitory concentrations (MIC) of the antibiotic across all strains tested. In *Staphylococcus aureus*, the MIC of amoxicillin decreased from 0.0625 mg/mL to 0.0078 mg/mL in the presence of the extract, a reduction by a factor of eight. Similar reductions were observed for *Escherichia coli* and Salmonella, with a decrease in MIC ranging from four to eight times depending on the strain. The calculated combination indices (CFI) were all below 0.5, indicating a synergistic interaction between the extract and the antibiotic.

3.3.2. Reversal of bacterial resistance with *Jatropha curcas* and Ciprofloxacin

The table 4 shows the results of the reversal of bacterial strain resistance with the aqueous mixture of Jatropha curcas and the antibiotic Ciprofloxacin.

Table 4.

Bacterial strains	*Jatropha curcas*	CIP	CIP + *Jatropha curcas*	FIC	Antibacterial effects
	CMI (mg/ml)				
S.aureus ATCC 6538	6,25	0,03125	0,00195	0,0624	Synergistic
S.aureus	6,25	0,03125	0,00195	0,0624	Synergistic
S.épidermidis CIP 8039	6,25	0,03125	0,0039	0,1248	Synergistic
E.coli CIP 53126	12,5	0,0625	0,0078	0,1248	Synergistic
E.coli isolé	12,5	0,0625	0,0078	0,1248	Synergistic
P.aeruginosa CIP 82118	25	0,25	0,03125	0,125	Synergistic
P.aeruginosa isolée	25	0,25	0,03125	0,125	Synergistic
Salmonella spp	12,5	0,03125	0,0078	0,1248	Synergistic
Salmonella paratyphi A	12,5	0,0625	0,0078	0,1248	Synergistic

The effect of Jatropha curcas extract was also very pronounced when combined with ciprofloxacin. The results show an even greater decrease in MICs compared to amoxicillin. For Staphylococcus aureus, the MIC of ciprofloxacin decreased from 0.03125 mg/mL to 0.00195 mg/mL in the presence of the extract, a sixteen-fold reduction. For other strains such as Escherichia coli and Salmonella, the reductions generally ranged from a factor of eight to sixteen. All combination indices obtained were also below 0.5, confirming the presence of synergy.

4. DISCUSSION

Hydroethanolic extracts of *Jatropha curcas* leaves showed no activity at 100 mg/mL, moderate inhibition at 200 mg/mL (9.66–15.33 mm), and increased at 400 mg/mL (12.66 mm for *P. aeruginosa* to 20.33 mm for *S. aureus*), indicating a dose-dependent effect. While weaker than gentamicin, the extracts reached 78–83% of its activity on Gram-positive bacteria and 60–67% on Gram-negative bacteria. These results are consistent with those of recent studies showing that antibacterial activity increases with the concentration of the extract, often weak or absent at low doses but optimal at high doses (Rahu et al., 2021); (Kamaruddin et al., 2024). At 200mg/ml, (Ikoyi et al., 2023) obtained inhibition zones of 5 mm and 10 mm for Staphylococcus aureus with aqueous and ethanolic extracts of Jatropha curcas leaves, respectively. At the same concentration, only *Pseudomonas aeruginosa* showed an inhibition zone of 6 mm with the ethanolic extract. These values remain lower than those obtained in our study with the hydroethanolic extract. This difference can be explained by the choice of solvent. Indeed, the water-ethanol mixture allows for the extraction of a greater diversity of bioactive metabolites, which improves antibacterial efficacy (Rafiq et al., 2025). This type of mixed solvent has intermediate polarity capable of solubilizing both highly polar and moderately polar compounds, thus offering better chemical coverage and enhanced biological activities, both antibacterial and antioxidant (Jam et al., 2021; Palos-Hernández et al., 2024). The results showed that the hydroethanolic extract of *Jatropha curcas* leaves has marked bactericidal activity against Gram-positive and certain Gram-negative bacteria, with low MICs (6.25-12.5 mg/mL) and MBCs approximately 4 times higher, while Pseudomonas aeruginosa requires much higher concentrations and has an MBC/MIC ratio ≥ 8. These results are similar to those of (Ado et al., 2025) and (Ezekwe, 2025). The weaker activity observed on P. aeruginosa is consistent with the characteristics of this bacterium. It has a thick outer membrane and powerful efflux systems, which limit the entry of active compounds often found in plant extracts(Giovagnorio et al., 2023; Kavanaugh et al., 2025; Lorusso et al., 2022). Furthermore, combining the extract with standard antibiotics (amoxicillin and ciprofloxacin) showed a marked synergistic effect against all strains tested, including P. aeruginosa. FIC indices (< 0.5) confirm this synergy, reflecting a significant reduction in antibiotic MICs in the presence of the extract. Similar studies have shown that plant extracts can potentiate the action of antibiotics by increasing membrane permeability or inhibiting bacterial efflux pumps. Overall, these results suggest that *J. curcas* could represent a promising source of antibacterial molecules, capable not only of directly inhibiting certain pathogenic bacteria, but also of improving the efficacy of existing antibiotics. However, further studies are needed to isolate the active ingredients responsible and confirm these effects in vivo.

5. CONCLUSION

In conclusion, the hydroethanolic extract of Jatropha curcas leaves shows promising bactericidal activity against pathogens frequently found in sickle cell patients, with low MICs for Gram-positive and certain Gram-negative bacteria, but reduced efficacy against Pseudomonas aeruginosa. The hydroethanolic solvent allows for the extraction of a broad spectrum of active compounds, thereby optimizing the observed antibacterial effect. These results suggest that J. curcas could be an interesting source of therapeutic alternatives or adjuvants, provided that its efficacy and safety are validated in vivo.

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