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Antibacterial activity of *Jatropha tanjorensis* leaf extracts against bacteria associated with wound infections from the clinical setting

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Abstract. *Babayemi OO, Oke EA, Bayode MT. 2021. Antibacterial activity of* Jatropha tanjorensis *leaf extracts against bacteria associated with wound infections from the clinical setting. Nusantara Bioscience 13: 239-246.* The use of microbial agents to treat infectious wounds based on ethnobotanical knowledge is still minimal. Antibacterial activity of *Jatropha tanjorensis* J.L.Ellis & Saroja leaf crude extracts and commercial antibiotics were evaluated against bacterial isolates associated with wound samples using agar well diffusion and disc diffusion techniques, respectively. Phytochemical analysis of the sections was carried out using standard methods. Saponin (58 mg/g) was the highest phytochemical in the methanol extract, while flavonoid (0.1 mg/g) was the lowest percentage in the cold water extract. Methanol extract had the highest Zone of Inhibition (ZOI) of 33 mm against *Staphylococcus aureus* coagulase-positive. In contrast, the lowest ZOI (5 mm) against *S. aureus* coagulase-positive was obtained from cold water extract. Methanol extract resulted in the highest ZOI ranging from 16 mm to 17 mm against *Escherichia coli*, while the lowest ZOI was obtained from coldwater extract (3-4 mm). The highest Minimum Inhibitory Concentration (MIC) value of all extracts (100 mg/mL) was obtained against *P. aeruginosa*, while the lowest MIC value was obtained against coagulase-positive *S. aureus* (12.5 mg/mL). All extracts contain Octadecanoic acid, n-hexadecanoic acid, and phytol. This study revealed that methanol extract had the highest inhibitory activity against bacteria isolated from wound samples compared to other crude extracts and ciprofloxacin. Therefore, *Jatropha tanjorensis* could be used as a potent herbal remedy to reduce the adverse effects of wound infection.

Keywords: Antibacterial, bioactive compounds, extraction, *Jatropha tanjorensis*, phytochemical, wound

INTRODUCTION

A wound may be defined as a disruption in the epithelial integrity of the skin or as a loss or breaking of cellular and anatomic or functional continuity of living tissue (Murti et al. 2011). According to Wong et al. (2013), the human skin is a remarkably plastic organ that sustains insults and injury throughout life. The skin is under constant stress from the sun, smog, friction, tension, temperature, and other external factors. Therefore, under sufficient stress that causes injury, it results in wounds. Moreover, wounds may be classified as; open and closed, acute and chronic, avulsion and degloving, clean and contaminated, infected and colonized, laceration, incision and abrasion, puncture, penetration, and gunshot wounds (Escandon et al. 2011). Nonetheless, they exist in various forms comprising crush injuries, ulcers, skin tears, bruises, and post-operative, which directly or indirectly affect human health conditions. If it is not treated correctly, it may ultimately lead to death (Escandon et al. 2011). Various microorganisms, such as bacteria, fungi, parasites, and viruses, can cause wounds. Some commonly associated organisms include *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella* spp., *Acinetobacter* spp., *Candida albicans*, and *Bacteroides fragilis*. The wound is one of the most common hospital-acquired infections, resulting in sepsis, limb loss, length of hospital stay, and higher costs (Shreshta and Sharma 2013).

Wound treatment is expected to alleviate any associated detrimental effects, as previously mentioned. Several factors affect wound healing, such as; aging, nutrition, obesity, repetitive trauma, skin moisture, chronic conditions, and medications (Guo and Di-Pietro 2010). Antibiotics have been of great value in treatment and prophylaxis for wound infections; however, several factors also affect reducing wound infections, i.e., the timing of administration, choice of antimicrobial agent, and durations of administration (Tatah et al. 2014). In addition, advances in infection control have not completely eradicated the wound infection problem due to drug resistance development (Mohammed et al. 2017).

Several species of plants have a significant therapeutic effect on alleviating the ailments of humankind (Yamac and Bilgili 2006). The genus *Jatropha* belongs to the family Euphorbiaceae. *J. tanjorensis* has been a potent ethnobotanical remedy in wound infection management (Ebana et al. 2019). *Jatropha tanjorensis* J.L. Ellis & Saroja is a perennial herb distributed in rainforest zones of West Africa, including Nigeria (Iwalewa and Agbani 2005). It has several local names, i.e., Chaya leaf (English), Ugo-Oyibo (Igbo)", and is commonly referred to as "Hospital too far" in Pidgin English; Catholic vegetable, and "Iyana-ipaja" (Yoruba). They are predominant in the tropics and subtropics (Swarbrick 1997). This plant is mainly used for fencing but possesses many ethnobotanical

and medicinal benefits in most parts of Southern Nigeria. The juice of Chaya leaf is used as herbal medicine.

Wounds result in the loss of epithelial continuity, and infections pose a significant health problem for patients. Most infectious microorganisms that cause wounds have developed resistance to most commercially available antibiotics. That has necessitated the search for novel sources of anti-infective compounds derived from medicinal plants to inhibit the growth of infectious bacteria (Ebana et al. 2019). This study evaluated the antibacterial activity of *J. tanjorensis* leaf extracts against bacteria associated with wound samples from the University Teaching Hospital, Ado-Ekiti, Nigeria.

MATERIALS AND METHODS

Collection of *Jatropha tanjorensis* leaves

Fresh leaves of *J. tanjorensis* were collected from a local market in Ado-Ekiti, Ekiti State, South-West, Nigeria. The plant was identified and authenticated by a plant scientist in the Department of Crop, Soil, and Pest Management, Federal University of Technology, Akure. The leaves were rinsed using clean water and air-dried for three weeks to a constant weight and then pulverized using an electric blender (Binatone blender/grinder-BLG 450). The pulverized plant material was kept in an air-tight plastic container for further use.

Collection of wound swabs

A total of 259 wound swabs were collected from various types of wound samples ranging from burns, chronic arterial foot ulcers, Diabetes Mellitus foot ulcers, post skin graft wound dehiscence, chronic leg ulcer, and avulsion wound at the wound care unit of the Ekiti State University Teaching Hospital, Ado-Ekiti, Nigeria. The wounds were cleaned with a cotton swab moistened with normal saline. The swab was aseptically obtained using the Levine wound collection method (Levine et al. 1976) by rotating the tip of the cotton swab over at least a 1 cm^2 area of viable wound bed tissue for 5 seconds with sufficient pressure to extract fluid from the wound tissue of each patient without contamination. The wound swab samples were transported to the Microbiology Laboratory of the Federal University Oye Ekiti, Nigeria, for bacteriological analysis.

Isolation of bacteria from wound swabs

The collected swab sticks of wound samples were inoculated on a nutrient agar slant and incubated at 37ºC for 24 to 48 hours (Mulu et al. 2013). Colonies formed after incubation were sub-cultured on blood agar, mannitol salt agar (MSA) (Hi-Media, India), and eosin methylene blue (EMB) agar (Hi-Media, India) to produce pure, distinct colonies. These were further subjected to various biochemical tests for bacterial identification concerning Bergey's manual of determinative Bacteriology (Don et al. 2006).

Preparation of *Jatropha tanjorensis* leaf extracts

The extraction of J. tanjorensis leaf was performed using absolute methanol, ethanol, hot and cold water as solvents. The extraction of the active ingredients of the plant leaf was performed using the method described by Harbone (1994) with slight modification. One hundred grams (100 g) of the pulverized leaves was Soxhlet extracted using 1000 mL of each solvent. The extracts were concentrated using a rotary evaporator (Resona, Germany) and were transferred into sterile bottles. The volatile oil obtained was purified by filtration using Whatman No.1 filter paper (Atata et al. 2003) and further sterilized by filtration through a millipore membrane filter of 0.45 µm pore size (HAWP04700) (Merck, Darmstadt, Germany) (Sule and Agbabiaka 2008) and after that concentrated by evaporation using water bath at 42°C. The sterile extracts were stored in sterile capped bottles and refrigerated at 4°C until use. The recovery rate of the extract was calculated using the formulae below;

$$\% \text{ Recovery of extract} = WA/IW \times 100$$

Where:
IW: Initial weight of extracts
WA: Weight of extracts recovered after extraction

Microbial-free and sterility test of the extracts

The extracts were tested for the presence or absence of turbidity using the Millipore filtration technique by introducing 2 mL of these extracts into 10 mL of sterile Mueller–Hinton broth and incubating at 37°C for 24 h. The microbial-free extract was indicated by the absence of turbidity or clearness of the broth medium after the incubation (Sule and Agbabiaka 2008; Bodunrinde et al. 2020). Extracts were also tested for microbial growth and contaminants: extracts were re-dissolved in absolute ethanol. Then, the appropriate concentrations for bioassay analysis were made using sterile deionized distilled water and sterilized using a 0.45 µm millipore membrane filter. Finally, one mL of each extract was inoculated onto nutrient agar to determine its sterility and incubated at 37°C for 24 hours. The absence of microbial growth in the extracts after incubation indicated that the extracts were sterile (Ashish et al. 2016).

Phytochemical analysis of *Jatropha tanjorensis* crude extracts

The phytochemical screening of the crude extracts was conducted using the slightly modified methods of Douye et al. (2013) and Paul et al. (2013).

Bacterial inoculum standardization

Bayode et al. (2021) modified the method to prepare the McFarland 0.5 turbidity standard to measure bacterial cell density. 0.5 mL of 1% (W/V) Barium chloride ($BaCl_2$) solution was added to 99.5 mL of 1% (vol/vol) sulfuric acid (H_2SO_4). McFarland standard tube was then sealed with Paraffin to prevent evaporation and stored in the dark at room temperature. The prepared McFarland standard's density accuracy was checked using a spectrophotometer

with a 1 cm light path. The 0.5 McFarland standard was vigorously agitated before use.

Antibacterial assay of *Jatropha tanjorensis* crude extracts against bacteria isolated from wounds

The antibacterial assay was determined by the agar diffusion method described by Morales-Cabrera et al. (2013). Bacterial isolates were cultivated in nutrient broth for 18 hours, and the bacterial suspension was prepared and compared to McFarland 0.5 turbidity standard. The bacterial suspension was inoculated onto solidified Mueller-Hinton agar surface by streaking with a sterile cotton-tipped swab to achieve a confluent growth. The inoculated plates were allowed to dry. Each extract (2 g) was dissolved in 10 mL dimethylsulfoxide (DMSO 30%) to obtain a 200 mg/mL concentration of methanol, ethanol, and hot and cold water, respectively. Ciprofloxacin (30µg) was used as a positive control for all the bacterial isolates except *Klebsiella pneumoniae*. The positive control for *K. pneumonia* was chloramphenicol (30µg), while DMSO was the negative control. Next, 0.1 mL of each plant extract was dropped onto a 10 mm filter paper disc and allowed to dry in the incubator at 45 ℃ for 15 minutes. The paper discs were then impregnated on the surface of the inoculated nutrients agar plate using a dispenser and incubated for 24 hrs, 48 hrs, and 72 hrs at 37℃. The zones of inhibition were measured with a vernier caliper (Mitutoyo 530-119) (Cranbury, New Jersey, United States) at every 24 hrs interval. The analytical grade solvents used for the extraction were used as control experiments (CLSI 2014).

Determination of Minimum Inhibitory Concentration (MIC) of *Jatropha tanjorensis* crude extracts against bacteria isolated from wounds

The initial concentration of leaf extracts (200 mg/mL) was diluted using double-fold serial dilution by transferring 5 mL of the sterile leaf extract (stock solution) into 5 mL of sterile nutrient broth to obtain 100 mg/mL concentration. That was repeated to obtain subsequent dilutions such as 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, and 3.125 mg/mL (Ibekwe et al. 2001). Each concentration was inoculated with 0.1 mL of the standardized bacterial cell suspension and incubated at 37℃ for 24 hours. The turbidity or cloudiness of the broth indicated the growth of the bacterial inoculum in the broth medium. The lowest concentration of extract that inhibited bacterial isolates' growth was considered the Minimum Inhibitory Concentration (MIC).

Determination of *Jatropha tanjorensis* crude extracts bioactive compounds

The crude extracts of *J. tanjorensis* were analyzed to determine the chemical constituents using a Varian 4000 GC-MS system equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm) with a running time of 40 minutes. In addition, an Agilent column, HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm) was also utilized (Colombini et al. 2010). The particular compounds present in the extract of the leaves were identified by relating their mass spectral fragmentation patterns of the respective peaks in the chromatogram with those stored in the National Institute of Standards and Technology Mass Spectral database (NIST 1998) library.

Statistical analysis

Data were analyzed using SPSS 16.0 for the windows evaluation version by using analysis of variance (ANOVA) and Duncan's Multiple Range Test to estimate means. $P<0.05$ was considered significantly different.

RESULTS AND DISCUSSION

Percentage of bacterial isolate on wound samples

Coagulase-positive *S. aureus* was present in the highest percentage in all wound samples compared to other isolates. For example, in the crushed leg injury, *S. aureus* was present at 100%, while *S. pyogenes* and *Proteus mirabilis* were found to have the least at 1 (3%) and 2 (4%), correspondingly as shown in Table 1.

The percentage recovery of *Jatropha tanjorensis* crude extracts

Methanol extract of *J. tanjorensis* had the highest percentage recovery (82%), while cold water extract had the lowest percentage (53%), as shown in Table 2.

Qualitative and quantitative phytochemical compounds of *Jatropha tanjorensis* leaf extracts

Bioactive compounds of *J. tanjorensis* leaf extracts in different solvents varied, as presented in Table 3. The results of the quantitative analysis of phytochemical compounds are presented in Table 4. It showed that saponin content in all solvents was high compared to other compounds, and the highest was in the methanol extract (57.72 mg/g). Flavonoid content had the lowest percentage of all extracts, and the highest flavonoid content was obtained in the methanol extract (2 mg/g). There were significant differences between the mean values of quantitative phytochemical analysis of extracts at $p < 0.05$.

Gas Chromatography-Mass Spectrophotometer (GCMS) profile of *Jatropha tanjorensis* crude extracts

The GCMS analysis of *J. tanjorensis* showed that all extracts contained Octadecanoic acid, n-Hexadecanoic acid, and phytol. The methyl ester was obtained in the methanol and ethanol extracts. Eicosane was extracted from methanol extract only, while ethanol extract revealed the presence of 6-Octadecanoic acid. In addition, oleic acid was extracted from hot water and cold water. Hot water also revealed the presence of vitamin E, while hydroquinone was only obtained in the cold-water extract of *J. tanjorensis*, as shown in Table 5.

Antibacterial activities of *Jatropha tanjorensis* leaf crude extract

The antibacterial activities of *J. tanjorensis* leaf extract against Gram-positive and Gram-negative bacteria are shown in Tables 6a and 6b, respectively. The methanol, ethanol, hot water, and cold-water extracts of *J. tanjorensis* leaf inhibited the growth of *S. aureus* coagulase positive from all the wound samples at 200 mg/mL concentration. *Staphylococcus aureus* coagulase positive had the highest Zone of Inhibition (ZOI) of 33 mm, while the lowest ZOI (5 mm) was obtained from cold water extract. Methanol extract had the highest ZOI ranging from 16 mm to 17 mm against *E. coli*, while the lowest inhibitory zone against *E. coli* was obtained from cold water extract (3-4 mm).

Minimum Inhibitory Concentration (mg/mL) of methanol leaf extracts of *Jatropha tanjorensis* against bacterial isolates from wound samples

The MIC of the methanol extracts of *J. tanjorensis* on the isolated bacteria is shown in Table 7, ranging from 12.5 to 100 mg/mL.

Table 2. Percentage of leaf extracts of *Jatropha tanjorensis*

Extracts	Weight of leaves (OW) (g)	Weight of extract (EW) (g)	Percentage of extract (%)
Methanol	100	82.01	82
Ethanol	100	79.50	79.50
Hot water	100	56.36	56.4
Cold water	100	53.04	53

Table 3. The qualitative phytochemical compound of *Jatropha tanjorensis* leaf extracts

Phytochemicals	Methanol	Ethanol	Hot water	Cold water
Saponin	+	+	+	+
Tannin	+	+	+	+
Phlobatannin	-	-	-	-
Flavonoid	+	+	+	+
Steroid	+	+	-	-
Terpenoid	+	+	+	+
Alkaloid	-	-	-	-
Anthraquinone	-	-	-	-
Cardiac glycosides	NA	NA	NA	NA
Legal test	+	+	+	+
Keller kiliani test	+	+	+	+
Salkwoski test	+	+	+	+
Lieberman test	+	+	-	-

Note: + : positive; - : negative NA: Not applicable

Table 4. The quantitative phytochemical compound of *Jatropha tanjorensis* leaf extracts

Phyto-chemical (mg/g)	Methanol	Ethanol	Hot water	Coldwater
Tannin	8.33 ± 0.01^{d}	5.32 ± 0.03^{c}	4.01 ± 0.01^{b}	2.55 ± 0.01^{a}
Saponin	57.72 ± 0.39^{d}	43.91 ± 0.39^{c}	11.36 ± 0.39^{b}	3.55 ± 0.39^{a}
Flavonoid	2.14 ± 0.01^{d}	1.56 ± 0.01^{c}	1.44 ± 0.01^{b}	0.96 ± 0.01^{a}
Terpenoid	24.74 ± 0.07^{d}	19.14 ± 0.06^{c}	15.70 ± 0.06^{b}	12.25 ± 0.06^{a}
Glycosides	23.44 ± 0.06^{d}	18.38 ± 0.07^{c}	15.45 ± 0.07^{b}	12.43 ± 0.07^{a}
Steroid	6.71 ± 0.03^{c}	5.15 ± 0.01^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Note: Values represent the means ± standard deviation of triplicate observations. Superscripts of the same letter in a row are not significantly different at $p \leq 0.05$

Table 1. Percentage occurrence of bacteria from wound samples

Wound types	*S. aureus* Coag +ve	*S. aureus* Coag -ve	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*P. mirabilis*	*S. pyogenes*	SWG
				Number/ (%)				
DMFU	14 (57)	4 (21)	2 (4)	0 (0)	1 (3)	1 (3)	0 (0)	3 (12)
BW	11 (56)	3 (12)	2 (4)	2 (4)	2 (4)	2 (4)	2 (4)	3 (12)
PSD	9 (18)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	41 (82)
AW	31 (76)	6 (12)	2 (4)	2 (4)	2 (4)	0 (0)	0 (0)	0 (0)
CLU	13 (64)	5 (15)	3 (9)	2 (6)	2 (6)	0 (0)	0 (0)	0 (0)
CALU	11 (60)	3 (9)	6 (12)	3 (6)	3 (6)	0 (0)	2 (8)	0 (0)
SCLU	12 (58)	0 (0)	6 (12)	6 (12)	0 (0)	6 (12)	0 (0)	0 (0)
CHI	10 (50)	0 (0)	5 (25)	5 (25)	0 (0)	0 (0)	0 (0)	0 (0)
CLI	10 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Note: DMFU: Diabetes Mellitus foot ulcer wounds; BW: Burn wounds; PSD: Post skin graft wound dehiscence; AW: Avulsion wounds; CLU: Chronic leg ulcer wounds; CALU: Chronic arterial leg ulcer wounds; SCLU: Sickle cell leg ulcer wounds; CHI: Crushed hand injury; CLI: Crushed leg injury; SWG: Samples with no growth; N: Number of bacterial isolates; %: percentage occurrence. 1st: number indicated the number of occurrences. 2nd: number indicated the percentage of occurrence

Table 5. Identified chemical compounds in *Jatropha tanjorensis* extracts by GC-MS analysis

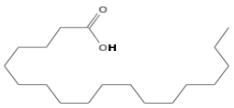
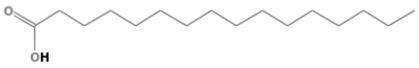
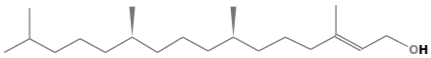
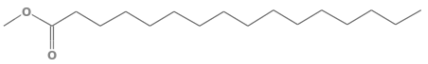
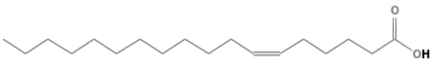
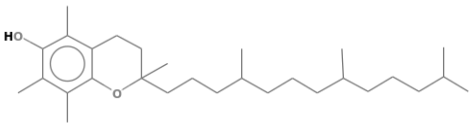
Extracts	Molecular weight	Name	Molecular formula	Structure
Methanol Ethanol	284 284	octadecanoic acid n-	$C_{18}H_{36}O_2$	
Hot water	256	Hexadecanoic acid	$C_{16}H_{32}O_2$	
Coldwater	296	Phytol	$C_{20}H_{40}O$	
Methanol	270	Methyl ester	$C_{17}H_{34}O_2$	
Methanol	282	Eicosane	$C_{20}H_{42}$	
Ethanol	282	6-Octadecenoic acid	$C_{18}H_{34}O_2$	
Hot water Coldwater	282 282	Oleic acid	$C_{18}H_{34}O_2$	
Hot water	430	Vitamin E	$C_{29}H_{50}O_2$	
Coldwater	110	Hydroquinone	$C_6H_6O_2$	

Table 6A. Diameter of inhibitory zone of *Jatropha tanjorensis* leaf extracts against Gram-positive bacteria isolated from wound samples

Isolates	Wound types	Ethanol	Methanol	Hot water	Coldwater	Positive control	Negative control
Streptococcus pyogenes	DMFU	22.67±0.57^{a}	33.00±0.00^{a}	12.67±0.57^{a}	7.67±0.57^{a}	31.67±0.57^{a}	00.00±0.00^{a}
	BW	22.33±0.57^{ab}	32.33±0.57^{b}	12.00±0.00^{b}	7.33±0.57^{ab}	30.33±0.28^{b}	00.00±0.00^{a}
Coagulase positive *Staphylococcus aureus*	DMFU	23.67±0.57^{ab}	32.33±0.57^{a}	14.33±0.57^{ab}	6.00±0.00^{a}	31.50±0.87^{a}	00.00±0.00^{a}
	BW	24.00±0.00^{ac}	27.67±0.57^{ab}	14.67±0.57^{a}	6.33±0.57^{ab}	26.50±1.00^{c}	00.00±0.00^{a}
	PSD	24.67±0.57^{b}	33.00±0.00^{ac}	13.33±0.57^{bc}	6.67±0.57^{b}	32.67±0.29^{b}	00.00±0.00^{a}
	AW	23.00±0.00^{c}	26.67±0.57^{bc}	12.67±0.57^{ab}	5.67±0.57^{a}	25.17±0.76^{ab}	00.00±0.00^{a}
	CLU	23.67±0.57^{a}	33.00±0.00^{ab}	14.67±0.57^{c}	5.00±0.00^{ab}	32.67±0.58^{ac}	00.00±0.00^{a}
	CALU	22.33±0.57^{ab}	27.33±0.57^{a}	14.33±0.57^{b}	6.33±0.57^{ac}	25.17±0.76^{bc}	00.00±0.00^{a}
	SCLU	24.00±0.00^{ab}	26.67±0.57^{ab}	13.67±0.57^{a}	6.33±0.57^{c}	24.50±0.50^{a}	00.00±0.00^{a}
	CHI	23.00±0.00^{a}	32.33±0.57^{a}	14.67±0.57^{ab}	5.33±0.57^{ab}	31.33±1.15^{ab}	00.00±0.00^{a}
	CLI	24.33±0.57^{bc}	30.00±0.00^{ab}	13.33±0.57^{a}	6.33±0.57^{a}	28.17±0.29^{a}	00.00±0.00^{a}
Coagulase negative *Staphylococcus aureus*	DMFU	22.33±0.57^{a}	28.33±0.57^{b}	12.33±0.57^{a}	7.67±0.57^{a}	26.50±0.87^{bc}	00.00±0.00^{a}
	BW	24.00±0.00^{ab}	30.00±0.00^{a}	12.00±0.00^{ab}	7.33±0.57^{ab}	28.83±0.29^{ab}	00.00±0.00^{a}
	PSD	22.33±0.57^{a}	28.33±0.57^{bc}	14.00±0.00^{a}	6.67±0.57^{a}	26.67±0.29^{a}	00.00±0.00^{a}
	AW	22.33±0.57^{bc}	30.00±0.00^{a}	12.67±0.57^{ab}	7.00±0.00^{c}	26.67±0.29^{ab}	00.00±0.00^{a}
	CLU	23.67±0.57^{a}	28.66±0.57^{ab}	12.67±0.57^{ac}	7.67±0.57^{bc}	24.83±0.29^{a}	00.00±0.00^{a}

Note: DMFU: Diabetes Mellitus foot ulcer wounds; BW: Burn wounds; PSD: Post skin graft wound dehiscence; AW: Avulsion wounds; CLU: Chronic leg ulcer wounds; CALU: Chronic arterial leg ulcer wounds; SCLU: Sickle cell leg ulcer wounds; CHI: Crushed hand injury; CLI: Crushed leg injury; Positive control: Ciprofloxacin (30µg), Negative control: Dimethyl sulfoxide (DMSO). The result represents the mean of triplicate values expressed in mean ± standard error with a significant difference at $p < 0.05$.

Table 6B. Diameter of inhibitory zone of *Jatropha tanjorensis* leaf extracts against Gram-negative bacteria isolated wound samples

Isolates	Wound types	Methanol	Ethanol	Hot water	Coldwater	Positive control	Negative control
Escherichia coli	DMFU	16.67±0.17^{a}	9.67±0.33^{ab}	6.33±0.33^{a}	3.83±0.17^{ab}	17.83±0.17^{a}	00.00±0.00^{a}
	BW	16.33±0.33^{ab}	12.00±0.17^{b}	6.50±0.29^{bc}	3.83±0.17^{bc}	17.50±0.33^{ab}	00.00±0.00^{a}
	PSD	17.00±0.00^{a}	10.33±0.17^{ab}	5.67±0.33^{a}	3.67±0.17^{b}	17.83±0.17^{b}	00.00±0.00^{a}
	AW	16.83±0.33^{ab}	11.83±0.17^{a}	5.33±0.33^{b}	3.83±0.17^{a}	17.33±0.33^{ab}	00.00±0.00^{a}
	CLU	17.00±0.00^{b}	10.33±0.00^{bc}	6.33±0.17^{ac}	3.00±0.00^{ac}	17.67±0.17^{ac}	00.00±0.00^{a}
	CALU	17.00±0.29^{ab}	12.00±0.17^{ab}	6.67±0.33^{ab}	4.00±0.00^{a}	17.67±0.29^{b}	00.00±0.00^{a}
	SCLU	16.67±0.17^{a}	10.33±0.17^{ab}	6.00±0.00^{a}	3.83±0.17^{ab}	17.67±0.17^{a}	00.00±0.00^{a}
Pseudomonas aeruginosa	DMFU	20.33±0.33^{ab}	15.83±0.17^{ac}	9.67±0.33^{bc}	4.00±0.00^{bc}	30.33±0.33^{ab}	00.00±0.00^{a}
	BW	22.00±0.00^{bc}	15.67±0.17^{ab}	8.33±0.33^{a}	4.83±0.17^{a}	31.33±0.33^{bc}	00.00±0.00^{a}
	PSD	20.67±0.33^{b}	15.00±0.00^{b}	8.00±0.00^{ab}	4.17±0.17^{ab}	30.00±0.00^{ac}	00.00±0.00^{a}
	AW	21.67±0.33^{ac}	14.67±0.33^{a}	9.00±0.00^{a}	5.00±0.17^{a}	31.33±0.33^{a}	00.00±0.00^{a}
	CLU	20.00±0.00^{a}	16.00±0.00^{ab}	9.67±0.33^{b}	4.33±0.17^{b}	30.33±0.33^{a}	00.00±0.00^{a}
	CALU	20.67±0.33^{ab}	15.33±0.33^{b}	8.00±0.00^{ab}	5.00±0.00^{ab}	30.67±0.33^{b}	00.00±0.00^{a}
Proteus mirabilis	DMFU	24.00±0.00^{b}	15.83±0.17^{a}	12.17±0.17^{a}	6.33±0.33^{a}	27.00±0.00^{bc}	00.00±0.00^{a}
	BW	28.17±0.17^{ab}	15.67±0.17^{ab}	12.00±0.00^{ab}	6.50±0.29^{bc}	30.83±0.17^{ab}	00.00±0.00^{a}
	PSD	28.17±0.17^{a}	15.00±0.00^{ac}	12.67±0.33^{ac}	5.67±0.33^{ab}	31.33±0.17^{a}	00.00±0.00^{a}
	AW	28.33±0.33^{b}	14.67±0.33^{a}	12.00±0.00^{bc}	5.33±0.17^{a}	30.33±0.17^{c}	00.00±0.00^{a}
	CLU	24.00±0.00^{c}	16.00±0.00^{bc}	10.00±0.00^{a}	6.33±0.00^{ab}	27.33±0.33^{b}	00.00±0.00^{a}
Klebsiella pneumoniae	DMFU	22.00±0.00^{a}	14.00±0.00^{ab}	8.00±0.00^{a}	4.00±0.00^{ab}	24.00±0.00^{a}	00.00±0.00^{a}
	BW	24.83±0.17^{ab}	12.67±0.33^{bc}	7.33±0.33^{ab}	3.67±0.17^{a}	27.33±0.17^{ab}	00.00±0.00^{a}
	PSD	22.00±0.00^{bc}	12.67±0.33^{a}	7.00±0.00^{a}	3.67±0.17^{c}	24.33±0.33^{bc}	00.00±0.00^{a}

Note: DMFU: Diabetes Mellitus foot ulcer wounds); BW: Burn wounds; PSD: Post skin graft wound dehiscence; AW: Avulsion wounds; CLU: Chronic leg ulcer wounds; CALU: Chronic arterial leg ulcer wounds; SCLU: Sickle cell leg ulcer wounds; CHI: Crushed hand injury; CLI: Crushed leg injury; Positive control: Ciprofloxacin (30µg), Negative control: Dimethyl sulfoxide (DMSO). The result represents the mean of triplicate values expressed in mean ± standard error with a significant difference at $p < 0.05$.

Table 7. Minimum Inhibitory Concentration (mg/mL) of methanol leaf extracts of *Jatropha tanjorensis* against bacteria isolated from wounds

Wounds	*S. aureus* Coag. +ve (mg/mL)	*S. aureus* Coag. –ve (mg/mL)	*E. coli* (mg/mL)	*P. aeruginosa* (mg/mL)	*P. mirabilis* (mg/mL)	*K. pneumoniae* (mg/mL)	*S. pyogenes* (mg/mL)
DMFU	12.5	25	50	NA	100	50	NA
BW	25	12.5	100	100	50	50	12.5
PSD	12.5	NA	NA	NA	NA	NA	NA
AW	25	25	100	100	50	NA	NA
CLU	12.5	12.5	50	100	50	NA	NA
CALU	25	25	100	100	100	NA	NA
SCLU	25	NA	100	100	NA	50	12.5
CHI	12.5	NA	50	100	NA	NA	NA
CLI	12.5	NA	NA	NA	NA	NA	NA

Note: DMFU: Diabetes Mellitus foot ulcer wounds; BW: Burn wounds; PSD: Post skin graft wound dehiscence; AW: Avulsion wounds; CLU: Chronic leg ulcer wounds; CALU: Chronic arterial leg ulcer wounds; SCLU: Sickle cell leg ulcer wounds; CHI: Crushed hand injury; CLI: Crushed leg injury; Positive control: Ciprofloxacin (30µg), Negative control: Dimethyl sulfoxide (DMSO). NA: Not available, Coag. +ve: Coagulase positive, Coag. –ve: Coagulase-negative, Values presented in the table were the average of triplicate experiments

Discussions

This study showed that bacteria isolates were present in all wound samples. A previous study by Gautam et al. (2013) reported that 60% of pus culture was positive for bacterial growth, including; *S. aureus*, *P. aeruginosa*, *S. pyogenes*, *K. pneumoniae*, and *Proteus* species. A study by Meenakshi et al. (2015) showed the presence of several bacteria, i.e., *S. aureus* 38 (29.6%), *S. pyogenes* 8 (6.2%), *P. aeruginosa* 28 (21.8%), *E. coli* 26 (20.3%), and *K. pneumonia*e 2 (1.5%). Thomsen et al. (2010) observed the presence of a high percentage of coagulase-positive *S. aureus* from chronic venous leg ulcers, similar to the results of this study. Ogba et al. (2014) reported that burn wound samples collected from public hospitals in Calabar, Nigeria, infected by *S. aureus* as the most prevalent bacteria (30.8%), followed by *P. aeruginosa* (17.3%) and *Streptococcus* spp. were being the lowest (1.3%), which was in agreement with the findings of this study.

The high yield percentage of *J. tanjorensis* methanol extract is similar to the findings of Beni et al. (2014) on

methanol leaf extracts of *J. curcas*. The high yield of *J. tanjorensis* methanol extract could be due to its polarity as an extraction solvent. Methanol is a universal solvent that dissolves all compounds, i.e., polar, semi-polar, or non-polar. In the extraction process, the composition, color, aroma, and extract yield are influenced by the raw material's type, size, maturity level, type of solvent, temperature, extraction time, and extraction method (Farrel 1990). In addition, the percentage of secondary metabolites in the methanol extract might be strongly influenced by the plant leaves used for extraction, which is generally less than 10% (Van Beek 1999).

A study by Omoregie and Osagie (2007) on the phytochemical analysis of *J. tanjorensis* revealed that it contains biochemical compounds such as alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones, and saponins which are similar to the findings in this study. The results of GC-MS analysis of *J. tanjorensis* crude extracts in this study were in line with the results of Ebana et al. (2019), which identified the presence of a total of 13 compounds, specifically octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)-ethyl ester, n-hexadecanoic acid, octadecanoic acid, and propanoic acid. Ebana et al. (2019) also reported the presence of three flavonoids, three amines, two associated hydrocarbons, two alkanes, and two alkaloids. Flavonoids and alkaloids were also detected in the results of GC-MS analysis.

The high inhibitory effect of *J. tanjorensis* leaf extracts on bacterial isolates could be due to the chemical compounds with wound-healing potency in the plant, as Hartwell reported earlier (1969). Furthermore, Viswanathan et al. (2012) reported that *J. tanjorensis* extracts exhibit potent anti-inflammatory and antioxidant activities contributing to its wound healing properties, which could also be a reason for the high activity index of *J. tanjorensis* crude extract observed in this study. So, antioxidant properties in *J. tanjorensis* crude extracts might play a role in reducing complicated/adverse effects. Therefore, antioxidant properties seem crucial in successfully treating wounds (Houghton et al. 2005).

This study revealed that the methanol extract of *J. tanjorensis* had the highest inhibitory activity against bacteria-infected wounds compared to other crude extracts and commercial antibiotics. It might be due to several bioactive chemical compounds that are synergistically active and increase the antibacterial activity of *J. tanjorensis* crude extract in vitro. This study's findings suggest using *J. tanjorensis* as an herbal remedy to reduce the adverse effects of wound infection.

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