Original research Paper

Insights on the Infrared Spectrum, Phytochemical and antibacterial activities of *Calotropis procera* Leaf extracts against vancomycin and methicllin resistant bacterial isolates

Abstract: The Phytochemical and antibacterial activities of *Calotropis procera* Leaf Organic Fractions were tested against vancomycin and methicliin resistant bacteria isolated from wound patients in Ondo State Specialist Hospital. The bacterial isolates are; *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Streptococcus pyogenes*. Agar well diffusion method was used to determine the antibacterial activities of the extracts on resistant bacterial isolates. Purification of the extracts were carried out using column chromatographic techniques, and the fractions obtained were spotted on the precoated TLC plates to obtain the active fractions by calculating the retention factor (Rf). The antibacterial potency of the active purified fractions were investigated against the resistant bacterial isolates. Ethanol extract had the highest zones of inhibition of 24.01 mm against *Staphylococcus aureus* while a lowest zone of inhibition of 10.19 was against *Klebsiella pnuemoniae.* Cold water extract highest zones of inhibition was against *Escherichia coli* at 15.00 mm and lowest inhibition against *Pseudomonas aeruginosa* at 7.20 mm. Phytochemical analysis of the extracts revealed the presence of alkaloids, flavonoids, tannin, saponin, terpenoids, cardic glycoside and phenols. The Fourier Transform Infrared (FT-IR) spectroscopic analysis of the active fractions showed five important functional groups namely; phenols, hydroxyl, carbon-hydrogen, carbonyl and aromatic. Findings from this research indicate that the leaf extracts of *Calotropis procera* posses antibacterial potency which will assist in the preliminary treatment of wound infections, most especially because of its high inhibitory effect against *Staphylococcus aureus.*

Keyword: phytochemical, ft-ir, purification, antibacterial

1.0 INTRODUCTION

Nosocomial infections is a major problem in many health care systems. It has been reported that 10% of hospital patients will acquire an infection while in hospital [1]. Infections can complicate illness, cause distress to patients and family and can lead to death. Among nosocomial infections, there are main infections that has been reported such as blood stream infections (28%), ventilator-associated pneumonia (21%), lower respiratory infection (12%), urinary tract infection (12%), gastrointestinal, skin, soft tissue and cardiovascular infection (10%), surgical-site infection (7%) and ear, nose and throat infection (7%) (Asefzadeh, 2005). Despite the extensive use of antibiotics and vaccine programs, infectious disease continue to be a leading cause of morbidity and mortality worldwide (Bloom, 2000). Emergence of resistant strains of pathogenic microorganism such as Methicillin - Resistant *Staphyloccocus aureus* (MRSA) and Vancomycin- Resistant *Staphyloccocus aureus* (VRSA) are very virulent in humans and are referred as professional pathogens. Which has also

continued to pose a major health concern about the efficacy of several drugs, most importantly antibiotics in current use [2].

Methicillin resistant *S. aureus* (MRSA) is responsible for hospital-acquired (HA) infections [3, 4] and presently community acquired (CA) infections [5, 6, 7, 8]. Hospital associated (nosocomial) staphylococcal infections have been reported to be resistant to as many as 20 antimicrobial compounds, including antiseptics and disinfectants. Resistance to penicillin among *S. aureus* strains appeared a few years after the introduction of penicillin therapy. Introduction of other antibiotics such as streptomycin, tetracycline and chloramphenicol, and the macrolides were similarly followed by emergence of resistant organisms. Resistant organisms that had acquired resistance to these antibiotics were reported to be usually resistant to penicillin through the production of penicillinase [9]. This resulted in the evolution of organisms with a wide spectrum of resistance and a marked ability to survive and spread in the hospital environment. Such multiple resistant *S. aureus* strains were of global significance as early as 1950s [10].

Paucible observations regarding drug resitance are available revealing the increased frequency of methicillin resistant *S. aureus* (MRSA) and VRSA by passage of time [11]. Pathogen developed differing mechanism and means to resist against different antibiotics depending on their mechanism of action. Enzymatic degradation of drug, structural modification of target and antibiotic efflux are mere strategies of resistance followed by the bacteria [12].

The development of drug resistance as well as appearance of undesirable side effects of certain drugs has led to the search of new antimicrobial agents in particular from medicinal plants [13]. Plant extracts, and pure compounds isolated from natural sources have formed the bedrock of modern chemotherapy [14]. Indigeous plants are reservour of various metabolites and provide unlimited source of important chemicals that have diverse biological properties [15]. Over 25% of prescribed medicine in industralized countries are derived directly from plant [16]. Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional medicines, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Plants are reported to have anticancer, antimicrobial, antidiabetic, antiinflammation and antioxidant properties [17].

Recently, attention has been directed towards extracts and biological compounds isolated from medicinal plants. More so, the use of medicinal plants play a vital role in covering the basic health needs in developing countries and these plants may offer new sources of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms [18].

Description of *Calotropis procera*

Calotropis procera is member of plant family Asclepiadaceae, a shrub of about 6m high and is widely distributed in the tropics. The plant is erect, tall, large, much branched and perennial with milky latex throughout. It is found in most parts of the world in dry, sandy and alkaline soils and warm climate and is more common in south western and central India and western Himalayas. It is found in waste lands and grows as a weed in agricultural lands. In ancient Ayurvedic medicines, the plant *Calotropis procera* was known as “Rakta arka” [19]. It is a common plant in Nigeria but it is more abundant in the northern part of the country [20].

For many years now, the normadic Fulani women of the Northern Nigeria use the part of the plant in the production of warankasi (a local soft cheese); the practice is still popular today even in almost all the parts of the country where fluid milk is abundant. The importance of this plant locally called “bomu bomu” in South-West of Nigeria for use in the

country as a cuddling agent in local cheese production came to limelight many decades ago in countries like India where the latex was used in Indian medicine as a blistering agent.

All parts of plant exude white latex when cut or broken, which act as a defence strategy against insects, viruses and fungi. A large number of secondary metabolites have been isolated from this plant that include many flavonoids, cardiac glycosides, Triterpenes and sterols [21].

Calotropis procera is a well known plant and has been traditionally used for the treatment of a wide range of infections globally such as wound, otitis media, rheumatism, epilepsy, hemiplegia, sores, ulcers, spleen complaints, gumboils, smallpox, protracted labor and skin infections [22, 23, 24]. The antimicrobial activity of *C. procera* plant extracts against bacteria and fungi is well documented [25, 26, 27, 28, 29]. Pharmacological studies of *Calotropis* species showed anti-inflammatory, anti-tumoral [30], antioxidant [31, 32], antibacterial [33], antidiarrheal [34], antifungal [35] and Nanoparticles Synthesize [36]activities.

Table 1: Scientistific classification of *Calotropis procera*

Kingdom	**Plantae – Plants**
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Asteridae
Order	Gentianales
Family	Asclepiadaceae – Milkweed family
Genus	Calotropis R. Br. – calotropis
Species	*Calotropis procera* (Aiton) W.T. Aiton – roostertree

Source: (Jain and Sharma, 1996)[37].

Fig 1: Image of *Calotropis procera* where it growing naturally

Geographic distribution

C. procera is drought-resistant, salt-tolerant to a relatively high degree, and it disperses seeds through wind and animals. It quickly becomes established as a weed along degraded roadsides, lagoon edges and in overgrazed native pastures. It has a preference for and is often dominant in areas of abandoned cultivation especially sandy soils in areas of low rainfall; assumed to be an indicator of over-cultivation. *C. procera* is native to India, Pakistan, Nepal, Afghanistan, Algeria, Iran, Iraq, Israel, Kenya, Kuwait, Niger, Nigeria, Oman, Saudi Arabia, United Arab Emirates, Vietnam, Yemen and Zimbabwe [38].

2.0 MATERIALS AND METHODS

Isolation of bacteria from the Clinical Sample

Isolation of Bacteria

The plate streaking technique was used for isolation of bacteria. Swab sticks were used to streak the samples on the already solidified nutrient agar plate, blood agar and chocolate agar, and incubated at 37^{o}C for 24hour. Pure cultures of isolate were obtained by sub-culturing unto freshly prepared plates as appropriate [39].

Identification and Characterization of Bacterial Isolates

The isolated bacteria were identified by using their cultural and morphological characteristics on media. This was followed by microscopic examination of the bacterial isolates under the microscope. The cultural features examined included shape elevation, surface edge and consistency. Physiological and biochemical tests were employed to confirm their identification [40].

Collection and preparation of plant materials:

Fresh leaves of *Calotropis procera* (Bomu-bomu) were collected from a farm from Akure, Ondo State, Nigeria where they were going naturally. The plant material was identified and authenticated at the Crop, Soil and Pest Department, Federal University of Technology, Akure, Nigeria. The leaves were cleaned with tap water and air-dried at room temperature until well dried. The dried part were milled into fine powder using a clean mechanical blender. The powdered sample were collected into sterile cellophane bags and labelled. The sample were kept in cool dry place till further use.

Preparation of Plant Extract

Exactly 300 g each of the dried powdered plant sample was weighted in a beaker and percolated with 3000 ml each of 80% ethanol and distilled cold water. It was allowed to stand for 3 days at room temperature with agitations at intervals. Afterwards, each extract was sieved through a muslin cloth, filtered through a Whatman (No. 1) filter paper and was concentrated *en vacuo* using rotary evaporator. The dried mass was stored in sterile McCartney bottle and kept in the frigerator at 4 ℃ at least 24hrs before subsequently testing [41].

Phytochemical Screening

The preliminary phytochemical analysis of the extracts were carried out to determine the presence of tannins, flavonoids, saponins, alkaloids, phenols and glycosides using standard procedures [42, 43].

Reconstitution and Sterilization of Plant Extracts

Each of the extracts (ethanol and distill cold water) were reconstituted using 0.01% Tween 20 as described by [44]. This was done by dissolving 0.5g of the extract in 10ml 0.01% Tween 20. The resultant solution was filtered using sterile Millipore membrane filter (0.45µm).

Antibacterial activity

Determination of antibacterial activities by leaf extract

Agar well diffusion technique as described by [45] was used to determine the *in-vitro* antibacterial activity of the crude extract. A 1ml aliquot of 18hrs broth culture that had been adjusted to turbidity equivalent of 0.5 McFarland standards was dispensed into sterile Petri dishes previously labeled with the test bacteria. Molten sterile Muller-Hinton was aseptically poured into the plates and gently rotated for the bacteria to be homogenously distributed in the medium. The agar plates were allowed to solidify, after which a sterile cork borer of 6mm in diameter was used to cut uniform wells in the agar plates. The wells were later filled with 0.5ml of the each extracts. In addition, 20% Tween 20 was used as the negative control while Ciprofloxacin served as the positive control. The experiment was conducted in triplicates. All plates were incubated at 37 ^{o}C for 24 hours. Clearance zones around the wells were noted and measured in millimeters.

PURIFICATION OF EXTRACTS

Purification by Column chromatography

The purification of the antibacterial compound was carried out using silica gel column chromatography as described by [46] and [47]. Methanol and petroleum ether in the ratio 3:1 v/v was used as eluting solvent. The column was packed with silica gel (60-120 mesh). The sample to be separated was then added on the top of the packed column and eluted with the solvent at the flow rate of a drop per 3 sec. A collecting conical flask was placed at the bottom of the column to collect the eluted fractions. The collected elute was distilled, leaving the purified fractions. The fractions obtained were spotted unto TLC plates. Fractions with the same retention factor (Rf) were pooled together.

Thin layer chromatography (TLC)

The crude extract was subjected to TLC analysis on 2.6 × 8 cm silica gel plate. These fractions were spotted onto a TLC plate 2 cm above the base. After thorough drying, the plate was placed in a solvent system (methanol:chloroform:petroleum ether (3:1:1)) in a chromatography tank to develop. The plates were removed when the solvent front approached the top of the plates and air-dried. Spots on the plates were visualized in an iodine chamber. The distances travelled by the constituents were measured and used to determine their Rf values [46].

$$R_f = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}}$$

Antibacterial Assay of column fractions of leaf extracts

Antibacterial activity of the column fraction was determined by paper disc diffusion method [48]. Sterile Petri dishes were seeded aseptically with 0.1ml of the standardized test organisms while about 20ml of sterile Mueller Hinton agar was poured aseptically on the seeded plates. Sterile Whatman filter paper discs (6.00mm in diameter) were impregnated with 30mg/ml of the purified fraction reconstituted with 30% dimethyl sulphonamide (DMSO). The impregnated paper discs were allowed to dry and applied with the aid of sterile forceps on the seeded plates. Filter paper disc dipped into DMSO and allowed to dry served as control. The plates were incubated at 37^{o}C for 24 h. Antibacterial activities were determined by the measurement of zone of inhibition around each paper disc.

Spectroscopic analysis of the purified fraction produced by the leaf extract

Infrared (IR) analysis was performed with the aid of infra red spectrophotometer (Perkin-Elmer spectrum bx) at Department of Chemistry, Redeemer`s University, Ede, Osun State. The method used is direct application of sample on cell. A drop of purified extract was placed on fused sodium chloride (NaCl) cell. It was then carefully placed on cell, loosely clamped and fixed on the IR beam and scanned at a range of 350-440nm. After few seconds the spectrum was displayed on the computer screen. The IR data was compared to the table of IR frequencies using the methods of [49].

Data Analysis

All the experiments were carried out in triplicate and data obtained from the study were subjected to analysis of variance. Treatment means were compared using Duncan's New Multiple Range Test (DNMRT) at 5% level of significance using SPPS version 21.

3.0 RESULTS

The following subjected vancomycin and methicillin resistant bacterial isolates were obtained from wound swabs namely; *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis* and *Streptococcus pyogenes.*

Table 2 shows the percentage yield of the extracts by different solvents. 12.37% was the highest yield obtained from Cold water and 9.13% from Ethanol extract. The result of the antibacterial activities of *Calotropis procera* leaf extracts at 500mg/ml as shown in Tables 3

reveals the highest inhibition by the Ethanol extract had the highest zone of inhibition against *Staphylococcus aureus* with 16.03mm and lowest zone of inhibition against *Klebsiella pnuemoniae* with 8.03mm. While for Cold water extract, the highest zone of inhibition was recorded against *Escherichia coli* with 13.30mm and lowest zone of inhibition against *Pseudomonas aeruginosa* with 4.60mm.

Table 2: **Physical Characteristics and Percentage (%) yield of the extracts obtained from *Calotropis procera* leaves**

Solvent (3L)	Input: dried leaf	Output: (extract)	% Recovery	Colour	Odour	Texture
Cold Water	300g	37.1	12.37	Dark-Brown	Pleasant fruity	Oily
Ethanol	300g	27.4	9.13	Black	Slightly repulsive	Oily

Table 3: Antibacterial activity of *Calotropis procera* extracts on the resistant bacterial isolates at 500mg/ml.

	Zone of inhibition (diameter in mm)					
Plant Extract	***Escherichia coli***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***	***Proteus mirabilis***	***Streptococcus pyogenes***
CPX (25mg/ml)	24.12 ± 0.00^{c}	20.45 ± 0.58^{a}	25.61 ± 0.58^{c}	21.67 ± 0.33^{ab}	25.33 ± 0.33^{c}	19.00 ± 0.00^{a}
EET	13.09 ± 0.17^{c}	24.01 ± 0.15^{e}	11.05 ± 0.15^{b}	10.40 ± 0.25^{a}	16.72 ± 0.38^{d}	10.20 ± 0.12^{a}
CWT	15.00 ± 0.11^{c}	12.61 ± 0.58^{b}	7.20 ± 0.10^{a}	7.54 ± 0.15^{a}	12.60 ± 0.15^{b}	17.11 ± 0.10^{d}

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)
KEY: EET= Ethanol Extract**, CWT=** Cold Water Extract**, CPX= Ciprofloxacin.**

Table 4 reveals the qualitative analysis of the phytochemical constituents of *Calotropis procera* Leaf Extracts. Phenolics, saponins, tannins, cardic glycoside, flavonoids and alkaloids were present in both the Ethanol and Cold water extracts.

The quantitative analysis of phytochemicals constituents of *Calotropis procera* Leaf Extract is shown in Figure 2. The Cold water extract had the highest quantity of phenolic (13.38g/ml) The highest quantity of tannins (4.72mg/ml) and saponins (10.34mg/ml) was recorded in Cold water extract. Cold water extract recorded the highest quantity of alkaloid (12.27mg/ml).

Table 4: **Qualitative Phytochemical Constituents of *Calotropis procera* Leaf extract**

Constituents	Crude Extract	Cold Extract	Ethanol Extract
Alkanoids	++	++	++
Flavonoid	++	++	++
Phenolics	++	++	++
Saponin	+	+	+

Tannin	+	+	+
Terpenoids	+	+	+
Cardic Glycoside	+	+	+

KEY

+ = Positive; - = Negative; ++ = Strongly positive

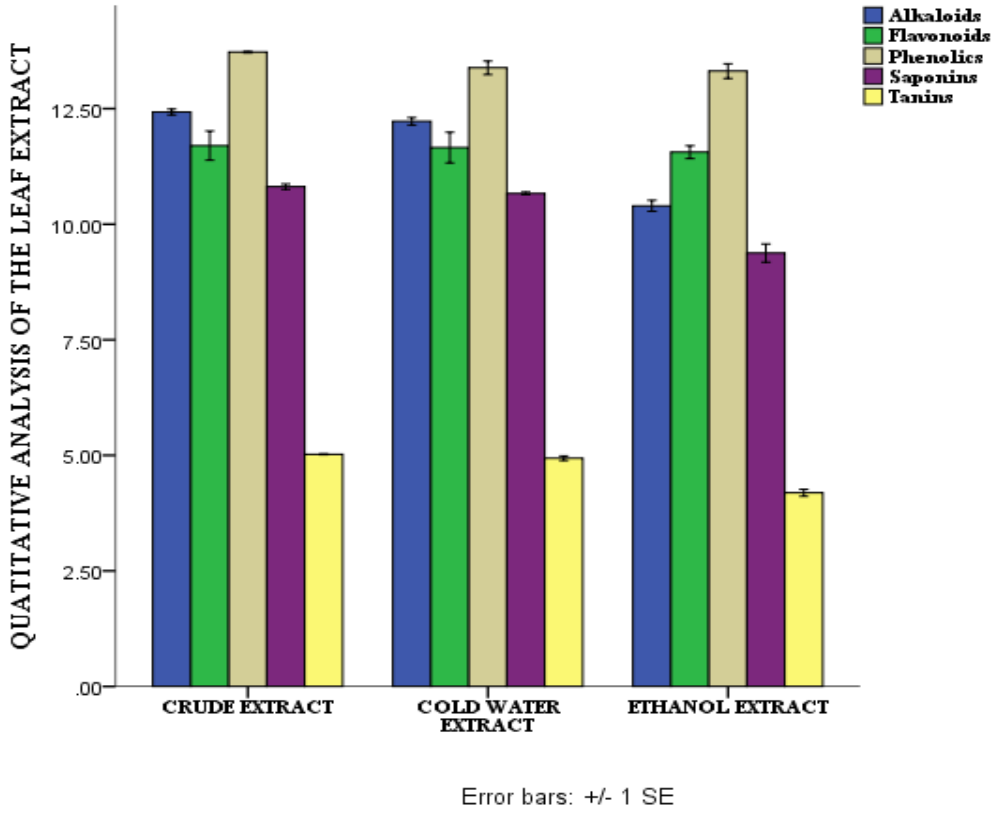

Figure 2: Quantitative phytochemical constituent of *Calotropios procera* leaf extract

Thin Layer Chromatography

Table 5 shows the Column chromatography of the ethanol and cold water extracts. Plate 2 shows Ethanol fractions of *Calotropis procera* leaf extract spotted on the TLC plate. From the thin layer chromatography of fractions, the retention factor values obtained were recorded on Table 6. The highest value was recorded on Emp8a fraction with a green colouration of the value of 0.89 retention factors while the lowest value was recorded in the Cmp1 fraction with a yellowish green colouration of the value of 0.64 refractive factor measured in centimeter (cm).

Table 7 shows the antibacterial activities of the purified extracts on the resistant bacterial isolates. The highest zones of inhibition was recorded by (Emp8a) of ethanol extract against *Pseudomonas aeruginosa* with zones of inhibition of 34.27mm, followed by (Emp10) of ethanol extract against *Staphylococcus aureus* with zones of inhibition of 33.28mm, followed by (Cmp5) of Cold water extract against *Klebsiella pneumoniae* with inhibition of 28.73mm, followed by (Cmp7) of Cold water extract against *Escherichia coli* with zones of inhibition of 28.52mm, followed by (Emp8a) of ethanol extracts on *Streptococcus pyogenes* with zones of inhibition of 28.23mm and followed by (Cmp7) of Cold water against *Klebsiella pnuemoniae* with zones of inhibition of 27.40mm.

Fraction (Emp8a) has the highest zones of inhibition of 34.27mm against *pseudomonas aeruginosa* while the lowest zones of inhibition of 4.26mm against *Proteus mirabilis* for the Ethanol extract. Fraction (Cmp7) has the highest zones of inhibition of 28.52mm against

Escherichia coli while the lowest zones of inhibition of 2.31mm were recorded against *Proteus mirabilis* for the Cold water extract. The infrared spectrum of the antimicrobial agent showed bands corresponding to 17 peaks, (figure 3 and 4; Table 8 and 9).

Table 5: Column chromatography of the ethanol and Cold water extracts

S/N	Fractions	Solvent/eluents	Colour of the fractions
1	Emp1	Methanol: Petroleum ether (75:25)	Light green
2	Emp2	Methanol: Petroleum ether (75:25)	Green
3	Emp3	Methanol: Petroleum ether (75:25)	Light green
4	Emp4	Methanol: Petroleum ether (75:25)	Light green
5	Emp5	Methanol: Petroleum ether (75:25)	Light green
6	Emp6	Methanol: Petroleum ether (75:25)	Lemon
7	Emp7	Methanol: Petroleum ether (75:25)	Light green
8	Emp8	Methanol: Petroleum ether (75:25)	Green
9	Emp9	Methanol: Petroleum ether (75:25)	Dark green
10	Emp10	Methanol: Petroleum ether (75:25)	Light green
11	Emp11	Methanol: Petroleum ether (75:25)	Light green
12	Cmp1	Methanol: Petroleum ether (75:25)	Yellowish green
13	Cmp2	Methanol: Petroleum ether (75:25)	Light green
14	Cmp3	Methanol: Petroleum ether (75:25)	Lemon
15	Cmp4	Methanol: Petroleum ether (75:25)	Light green
16	Cmp5	Methanol: Petroleum ether (75:25)	Light green
17	Cmp6	Methanol: Petroleum ether (75:25)	Light green
18	Cmp7	Methanol: Petroleum ether (75:25)	Light green
19	Cmp8	Methanol: Petroleum ether (75:25)	Lemon
20	Cmp9	Methanol: Petroleum ether (75:25)	Light green

Table 6: Refractive factor of fractions obtained from ethanol and cold leaf extract spotted on the TLC plates.

S/N	Fractions	(R_f) Value(cm)
1	Cmp9+Cmp7+Emp5	0.77
2	Emp1	0.82
3	Emp2+Cmp4	0.75
4	Emp3	0.85
5	Emp4+Emp6+Emp9	0.84
6	Emp7+Emp10	0.87
7	Emp8A	0.89
8	Emp8B+Emp11	0.80
9	Cmp1	0.64
10	Cmp2	0.67
11	Cmp3	0.66
12	Cmp5	0.80
13	Cmp6	0.67
14	Cmp8	0.69

Table 7: Bioassay of the purified extracts on the resistant bacteria isolates

Factions	Zone of Inhibition (diameter in mm)					
	Escherichia coli	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***	***Proteus mirabilis***	***Streptococcus Pyogenes***
Emp7	17.02±0.03^{c}	17.01±0.02^{b}	23.41±0.09^{c}	20.27±0.15^{a}	10.33±0.21^{h}	22.23±0.15^{a}
Emp8A	20.03±0.03^{e}	14.51±0.01^{a}	34.27±0.25^{h}	21.55±0.05^{c}	4.26±0.25^{b}	28.23±0.21^{e}
Emp10	19.06±0.12^{d}	33.28±0.10^{e}	25.13±0.12^{d}	20.33±0.11^{b}	9.07±0.11^{e}	23.57±0.06^{b}
Cmp5	12.06±0.03^{a}	17.02±0.03^{b}	27.58±0.11^{e}	20.07±0.15^{a}	7.53±0.15^{d}	25.23±0.21^{d}
Cmp7	28.52±0.02^{d}	20.29±0.04^{b}	22.10±0.17^{b}	27.40±0.10^{d}	5.30±0.26^{c}	24.53±0.06^{c}
Cmp9	14.11±0.1^{b}	19.36±0.03^{d}	21.24±0.14^{a}	28.73±0.21^{e}	2.31±0.06^{a}	22.27±0.06^{a}

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)

KEY Emp= Ethanol Extract of methanol:petroleum ether ratio 3:1 Cmp= Cold Water Extract methanol:petroleum ether ratio 3:1

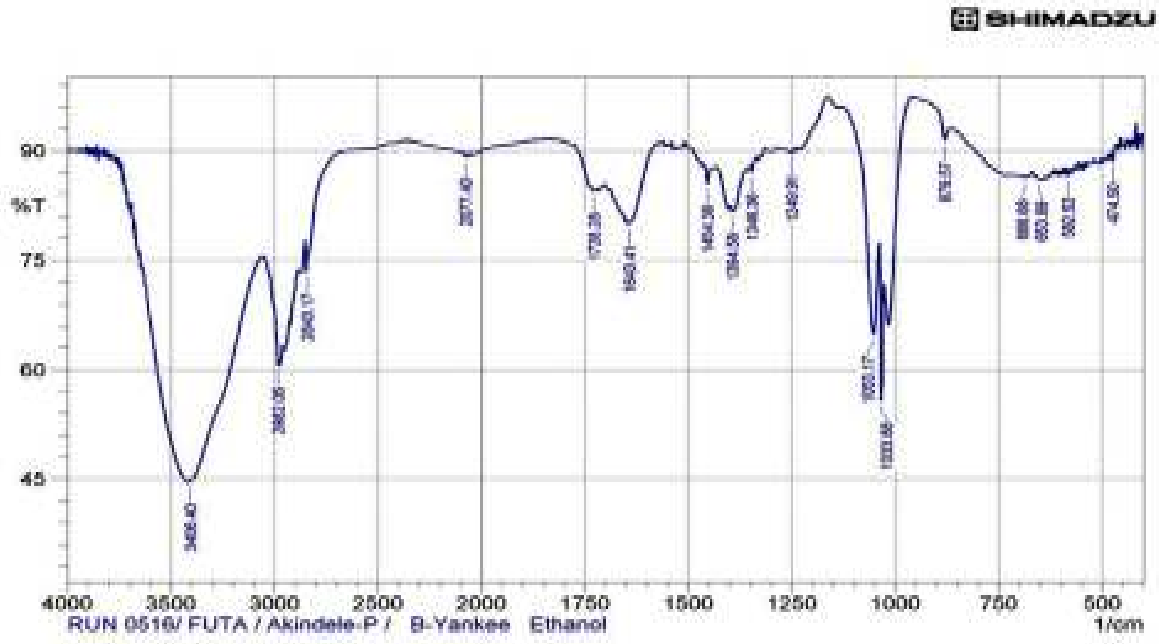

Figure 3: Infrared spectrum of Ethanol fraction showing different peaks

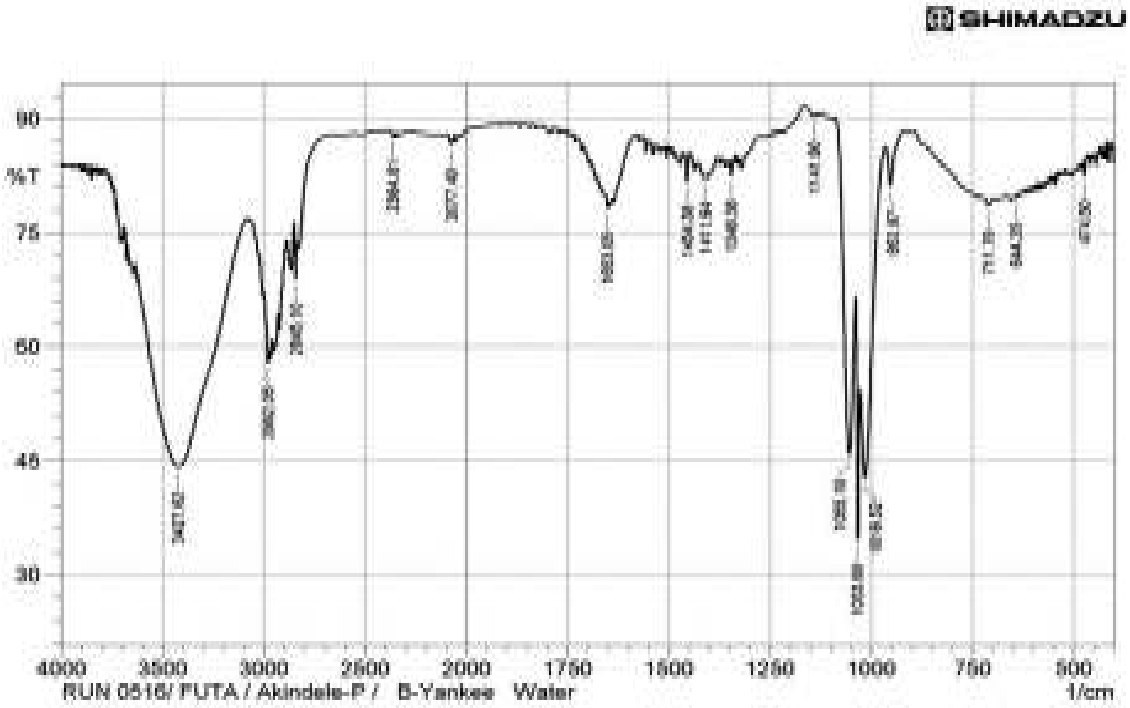

Figure 4: Infrared spectrum of Cold water extract fraction showing different peaks

Table 8: Interpretation of the FT-IR data of the ethanol extracts.

No	Wave Number (cm^{-1})	Stretching or Bending	Intensity	Possible Functional group
1	582.52	Stretching Stretching	Strong	C-I (halo compound) or C-Cl (halo compound) or C-Br (halo compound
2	653.89	Stretching	Strong	C-Br (halo compound)
3	666.66	Stretching	Strong	C-Br (halo compound) or C=C bending alkene disubstituted (cis)
4	1033.86	Stretching	Strong	C-O (vinyl ether) or C-O (alkyl aryl ether) or S=O (sulfoxide)
5	1053.17	Stretching Stretching	Strong	C-O (primary alcohol) or C-O (vinyl ether) C-O (alkyl aryl ether) or S=O (sulfoxide)
6	1249.91	Stretching Bending	Strong Medium	C-F (fluoro compound) or C-O (aromatic ester) C-N (amine)
7	1346.36	Stretching Bending	Strong Medium	C-F (fluoro compound) or N-O (nitro compound) O-H(phenol) or O-H (alcohol)
8	1394.56	Bending Stretching	Medium Strong	O-H (carboxylic acid) or O-H (alcohol) C-F (fluoro compound)
9	1454.36	Bending	Medium	C-H (alkane methylene group)
10	1643.41	Stretching Stretching Stretching Bending	Medium Strong Strong Medium	C=N (imine / oxime) C=O (secondary amide) or C=O (tertiary amide) C=C (alkene disubstituted (cis)) or C=C (conjugated alkene) or C=C (cyclic alkene) or C=C (alkene monosubstituted) or N-H (amine)

11	1728.28	Bending	Weak	C-H (aromatic compound)
		Stretching	Strong	C=O (conjugated anhydride) or C=O (aldehyde) or
		Stretching	Strong	C=O (α,β-unsaturated ester)
12	2077.4	Stretching	Strong	N=C=S (isothiocyanate)
13	2843.17	Stretching	Strong	O-H (carboxylic acid) or N-H (amine salt)
		Stretching	Weak	O-H (alcohol intramolecular bonded)
		Stretching	Medium	C-H (alkane)
14	2982.05	Stretching	Medium	C-H (alkane)
		Stretching	Strong	N-H (amine salt)
15	3406.4	Stretching	Strong	O-H (alcohol intermolecular bonded)
			Medium	N-H (primary amine)

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305 **Table 9: Interpretation of the FT-IR data of the cold water extracts**

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No	**Wave Number (cm^{-1})**	**Stretching or Bending**	**Intensity**	**Possible Functional group**
1	644.75	Stretching	Strong	C-Br (halo compound)
2	711.76	Bending	Strong	C=C (alkene disubstituted (cis)) or C-H (monosubstituted benzene derivative)
3	952.87	Bending	Strong	C=C (alkene disubstituted (trans))
4	1016.62	Stretching	Strong	C-O (vinyl ether) or C-O (alkyl aryl ether)
5	1033.86	Stretching	Strong	C-O (vinyl ether) or C-O (alkyl aryl ether) S=O (sulfoxide)
6	1055.1	Stretching Stretching	Strong	C-O (primary alcohol) C-O (vinyl ether) C-O (alkyl aryl ether) S=O (sulfoxide)
7	1141.9	Stretching Stretching	Strong Strong	C-F (fluoro compound) or C-O (tertiary alcohol) or S=O (sulfone) or C-O (aliphatic ether) or C-N (amine)
	1346.35	Stretching	Strong	C-F (fluoro compound) or N-O (nitro compound)
8		Bending	Medium	O-H (alcohol) or O-H (phenol)
9	1411.94	Bending Bending	Medium Medium	C-H (alkane methyl group) or O-H (carboxylic acid) or O-H (alcohol)
10	1454.38	Stretching	Medium	C-H (alkane methylene group)
11	1653.05	Stretching Stretching Stretching Stretching Stretching Bending	Strong Medium Strong Medium Weak	C=O stretching (primary amide) C=N stretching (imine / oxime) C=O stretching (secondary amide) or C=O stretching (tertiary amide C=C stretching (alkene disubstituted (cis)) or C=C stretching alkene vinylidene) C-H (aromatic compound)
12	2077.4	Stretching	Strong	N=C=S (isothiocyanate)
13	2364.81	Stretching	Strong	O=C=O (carbon dioxide)
14	2845.1	Stretching Stretching Stretching	Weak Medium Strong	O-H (alcohol intramolecular bonded) C-H (alkane) N-H (amine salt) or O-H (carboxylic acid)
15	2982.05	Stretching Stretching Stretching	Weak Medium Strong	O-H (alcohol intramolecular bonded) C-H (alkane) N-H (amine salt) or O-H (carboxylic acid)

16	3427.62	Strong Medium	O-H (alcohol intermolecular bonded) N-H (primary amine)

4.0 DISCUSSION

Finding from this study revealed that the bacterial isolates namely; *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis* and *Streptococcus pyogenes* isolated are the bacteria associated with wound infections. This study is also in agreement with previous study of [50] were the named bacterial isolates are the mostly implicated in wound infections.

In this study, the result of the antibacterial activities of *Calotropis procera* leaf extracts at 500mg/ml as shown in Tables 3 reveals the highest inhibition by the Ethanol extract was recorded against *Staphylococcus aureus* with a zones of inhibition value of 24.01 mm and lowest zones of inhibition recorded against *Klebsiella pnuemoniae* at 10.19 mm. For Cold water extract highest inhibition was recorded against *Escherichia coli* at 15.00 mm and lowest inhibition against *Pseudomonas aeruginosa* at 7.20 mm. The results of this study reveal that Ethanolic extract had highest zone of inhibition against *Staphylococcus aureus* which is in conformity with the work done by [41, 51] but in contrast to the work done by [52] which reveal that the aqueous extract has the highest zones of inhibition against *Staphylococcus aureus*.

Antibacterial activity of medicinal plants and drugs varies in their inhibitory effect depending on the concentration, temperature, nature of organism, size of inoculums [53]. The presence of tannin is also an indication that there are phenolic acids present in the plants which may be responsible for its antibacterial activities [54, 55]. The result from this study has shown that the leaf extracts of *Calotropis procera* has antibacterial properties. Thus the leaf extracts of *Calotropis procera* exhibited a broad spectrum activity, against the test organisms in this work, and hence can be employed in treatment of diseases caused by such. Also the susceptibility of *Staphylococcus aureus* to the plant extracts justifies the traditional use of the leaf in wound healing. This is in agreement with the findings of [54, 55, 24, and 56] which reported that the plant is useful in treatment of wounds, ulcers, spleen complaints, sores, rheumatism, smallpox, epilepsy, hemiplegia, gumboils and skin infections.

It was observed that the purified extracts had higher inhibitory effect on bacterial isolates than crude extracts. [57] reported that crude extracts are liable to deterioration and contamination which reduces their inhibitory activity. The fractions from ethanol and cold water extract which had the highest inhibitory effect were sent for Fourier Transform Infrared (FT-IR) spectroscopic analysis. The infra red signals revealed a compound containing the following principal functional group; an alcohol (O=H), an amine (nitrogen) group, a carbonyl group(C=O), an alkenyl (C=C). The signal at 1346.35 suggest skeletal structure resembling calotropin and calotropegenin which confirmed the presence of amide, alcohols, phenols, alkanes, carboxylic acids, aldehydes, ketones, alkenes, primary amines, aromatics, esters, ethers, alkyl halides and aliphatic amines compounds, which showed major peaks. This is in agreement with the work done by [54, 55]. The presence of an O-H bending phenol which is a very rare functional group in most compound might be responsible for the effectiveness of the leaf extracts and this is an indication that purified active extracts of *Calotropis procera* may be potent material for drug development that could be used in the in the preliminary treatment of wound infections, most especially because of its high inhibitory effect against *Staphylococcus aureus.*

5.0 CONCLUSION

In this study, the microbiological investigation done on *Calotropis procera* leaves obtained in Nigeria have shown activity coherent with the use of this plant in folk medicine.

The extracts could serve as useful sources for new antibacterial agent owing to the increase rate of resistance of bacteria to vancomycin and methicillin antibiotics. Findings from this research indicate that the leaf extracts of *Calotropis procera* posses antibacterial potency which will assist in the preliminary treatment of wound infections, most especially because of its high inhibitory effect against *Staphylococcus aureus.*

ACKNOWLEDGEMENTS

The author are grateful to the Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria, for providing the necessary facilities used in this study.

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