

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

Abstract: The Phytochemical and antibacterial activities of *Calotropis procera* Leaf Organic Fractions were tested against vancomycin and methicillin 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 pneumoniae*. 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, cardiac 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* possess 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 *Staphylococcus aureus* (MRSA) and Vancomycin- Resistant *Staphylococcus aureus* (VRSA) are very virulent in humans and are referred as professional pathogens. Which has also

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

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

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

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

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

81 **Description of *Calotropis procera***

82 *Calotropis procera* is member of plant family Asclepiadaceae, a shrub of about 6m
83 high and is widely distributed in the tropics. The plant is erect, tall, large, much branched and
84 perennial with milky latex throughout. It is found in most parts of the world in dry, sandy and
85 alkaline soils and warm climate and is more common in south western and central India and
86 western Himalayas. It is found in waste lands and grows as a weed in agricultural lands. In
87 ancient Ayurvedic medicines, the plant *Calotropis procera* was known as “Rakta arka” [19].
88 It is a common plant in Nigeria but it is more abundant in the northern part of the country
89 [20].

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

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

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

100 *Calotropis procera* is a well known plant and has been traditionally used for the
 101 treatment of a wide range of infections globally such as wound, otitis media, rheumatism,
 102 epilepsy, hemiplegia, sores, ulcers, spleen complaints, gumboils, smallpox, protracted labor
 103 and skin infections [22, 23, 24]. The antimicrobial activity of *C. procera* plant extracts
 104 against bacteria and fungi is well documented [25, 26, 27, 28, 29]. Pharmacological studies of
 105 *Calotropis* species showed anti-inflammatory, anti-tumoral [30], antioxidant [31, 32],
 106 antibacterial [33], antidiarrheal [34], antifungal [35] and Nanoparticles Synthesize
 107 [36]activities.

108 **Table 1: Scientific 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	<i>Calotropis</i> R. Br. – calotropis
Species	<i>Calotropis procera</i> (Aiton) W.T. Aiton – roostertree

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

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

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Geographic distribution

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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

123 **Isolation of bacteria from the Clinical Sample**

124 **Isolation of Bacteria**

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

129 **Identification and Characterization of Bacterial Isolates**

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

135 **Collection and preparation of plant materials:**

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

143 **Preparation of Plant Extract**

144 Exactly 300 g each of the dried powdered plant sample was weighted in a beaker and
145 percolated with 3000 ml each of 80% ethanol and distilled cold water. It was allowed to
146 stand for 3 days at room temperature with agitations at intervals. Afterwards, each extract
147 was sieved through a muslin cloth, filtered through a Whatman (No. 1) filter paper and was
148 concentrated *en vacuo* using rotary evaporator. The dried mass was stored in sterile
149 McCartney bottle and kept in the frigerator at 4 °C at least 24hrs before subsequently testing
150 [41].

151 **Phytochemical Screening**

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

155 **Reconstitution and Sterilization of Plant Extracts**

156 Each of the extracts (ethanol and distill cold water) were reconstituted using 0.01%
157 Tween 20 as described by [44]. This was done by dissolving 0.5g of the extract in 10ml
158 0.01% Tween 20. The resultant solution was filtered using sterile Millipore membrane filter
159 (0.45µm).

160 **Antibacterial activity**

161 **Determination of antibacterial activities by leaf extract**

162 Agar well diffusion technique as described by [45] was used to determine the *in-vitro*
163 antibacterial activity of the crude extract. A 1ml aliquot of 18hrs broth culture that had been
164 adjusted to turbidity equivalent of 0.5 McFarland standards was dispensed into sterile Petri
165 dishes previously labeled with the test bacteria. Molten sterile Muller-Hinton was aseptically
166 poured into the plates and gently rotated for the bacteria to be homogenously distributed in
167 the medium. The agar plates were allowed to solidify, after which a sterile cork borer of 6mm
168 in diameter was used to cut uniform wells in the agar plates. The wells were later filled with
169 0.5ml of the each extracts. In addition, 20% Tween 20 was used as the negative control while
170 Ciprofloxacin served as the positive control. The experiment was conducted in triplicates. All
171 plates were incubated at 37°C for 24 hours. Clearance zones around the wells were noted and
172 measured in millimeters.

173 PURIFICATION OF EXTRACTS

174 Purification by Column chromatography

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

183 Thin layer chromatography (TLC)

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

$$191 R_f = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}}$$

193 Antibacterial Assay of column fractions of leaf extracts

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

203 Spectroscopic analysis of the purified fraction produced by the leaf extract

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

211 Data Analysis

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

216 3.0 RESULTS

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

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

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

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

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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

232

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

Plant Extract	Zone of inhibition (diameter in mm)					
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumoniae</i>	<i>Proteus mirabilis</i>	<i>Streptococcus pyogenes</i>
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

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

238 **KEY: EET= Ethanol Extract, CWT= Cold Water Extract, CPX= Ciprofloxacin.**

239

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

243 The quantitative analysis of phytochemicals constituents of *Calotropis procera* Leaf
 244 Extract is shown in Figure 2. The Cold water extract had the highest quantity of phenolic
 245 (13.38g/ml) The highest quantity of tannins (4.72mg/ml) and saponins (10.34mg/ml) was
 246 recorded in Cold water extract. Cold water extract recorded the highest quantity of alkaloid
 247 (12.27mg/ml).

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

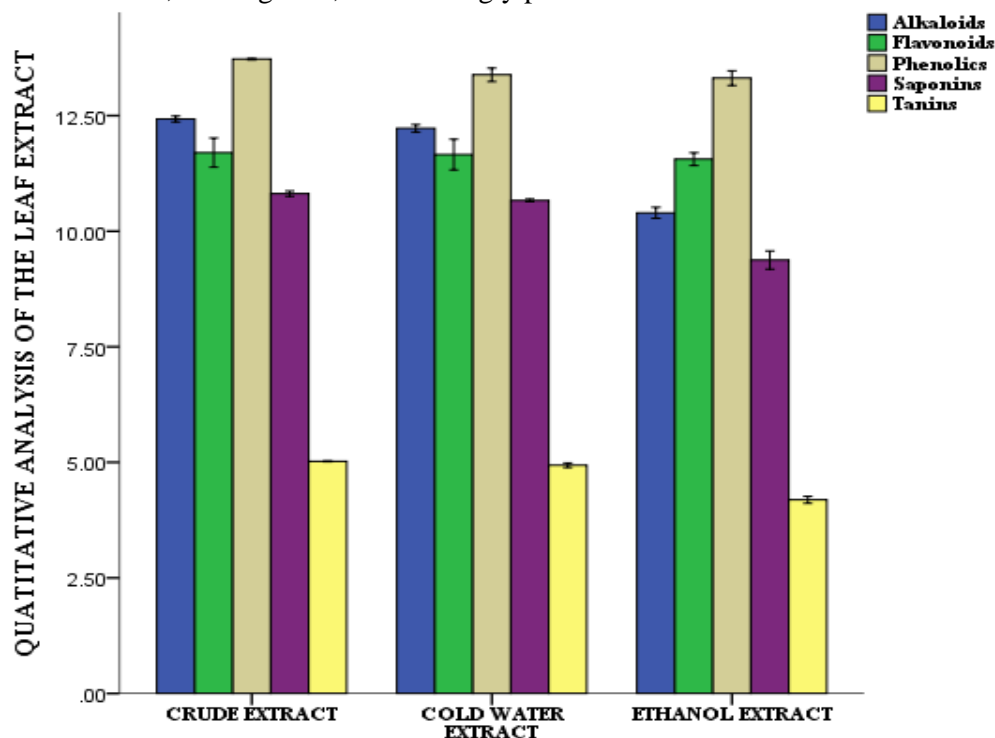
Constituents	Crude Extract	Cold Extract	Ethanol Extract
Alkanoids	++	++	++
Flavonoid	++	++	++
Phenolics	++	++	++
Saponin	+	+	+

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

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KEY

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



Error bars: +/- 1 SE

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Figure 2: Quantitative phytochemical constituent of *Calotropis procera* leaf extract
Thin Layer Chromatography

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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).

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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 pneumoniae* with zones of inhibition of 27.40mm.

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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

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

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

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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

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280 **Table 6: Refractive factor of fractions obtained from ethanol and cold leaf extract**
 281 **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

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

Factions	Zone of Inhibition (diameter in mm)					
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumoniae</i>	<i>Proteus mirabilis</i>	<i>Streptococcus Pyogenes</i>
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

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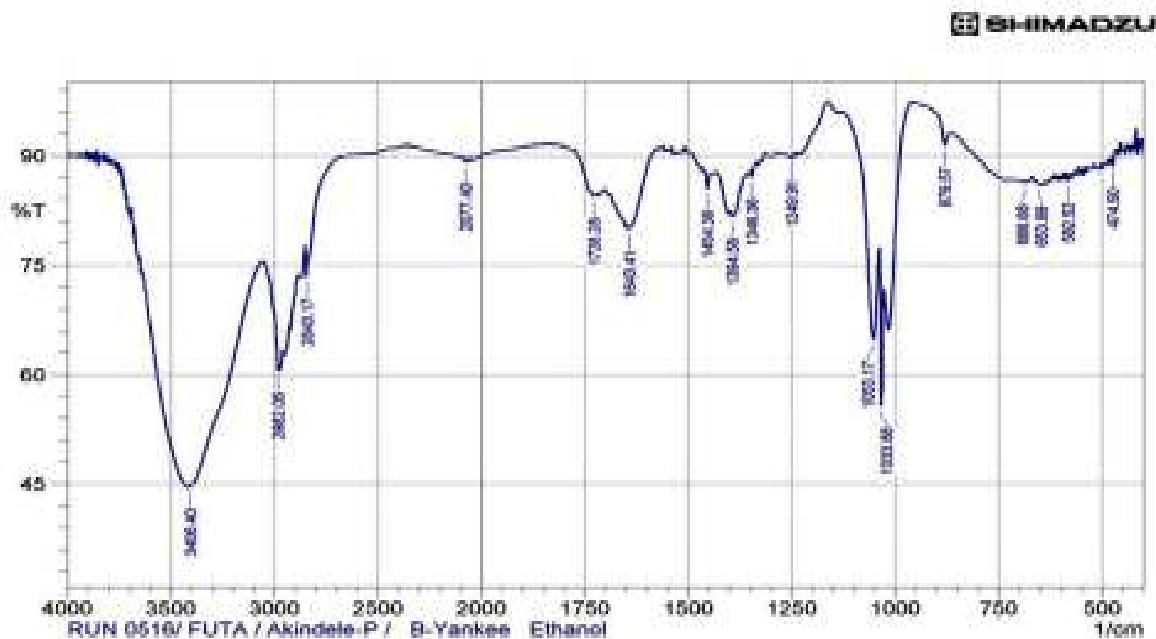
291 **Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along**
292 **the same column are not significantly different (P<0.05)**

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

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methanol:petroleum ether ratio 3:1

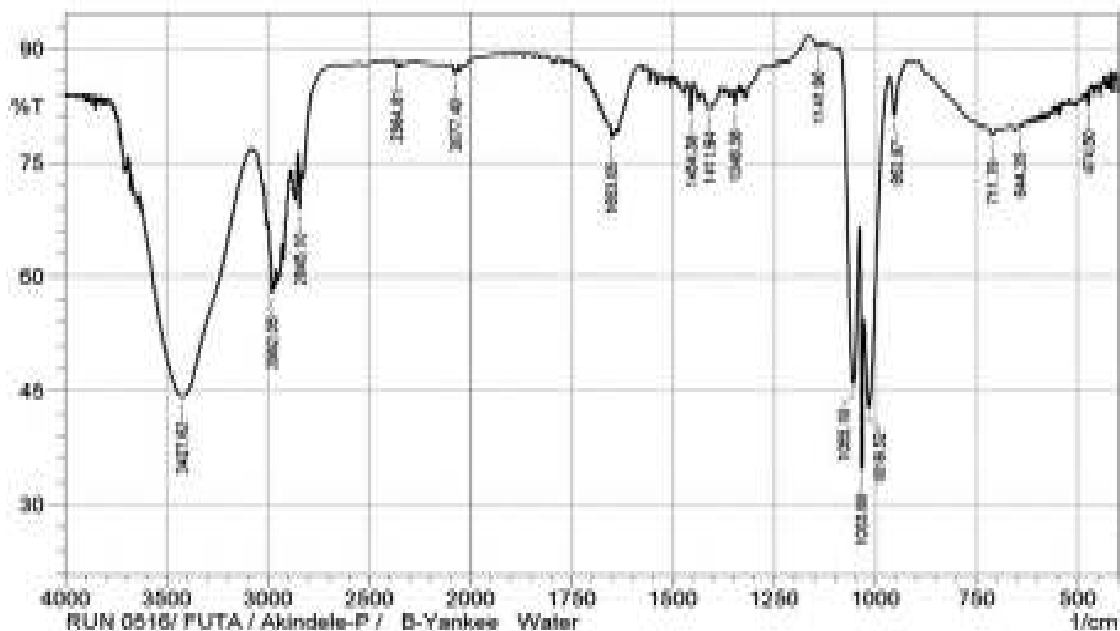


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Figure 3: Infra-red spectrum of Ethanol fraction showing different peaks



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300 **Figure 4: Infra-red spectrum of Cold water extract fraction showing different peaks**

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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	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	Strong	C-O (primary alcohol) or C-O (vinyl ether)
6	1249.91	Stretching	Strong	C-O (alkyl aryl ether) or S=O (sulfoxide)
		Bending	Medium	C-F (fluoro compound) or C-O (aromatic ester)
7	1346.36	Stretching	Strong	C-N (amine)
		Bending	Medium	C-F (fluoro compound) or N-O (nitro compound)
8	1394.56	Bending	Medium	O-H(phenol) or O-H (alcohol)
		Stretching	Strong	O-H (carboxylic acid) or O-H (alcohol)
9	1454.36	Bending	Medium	C-F (fluoro compound)
10	1643.41	Stretching	Medium	C-H (alkane methylene group)
		Stretching	Strong	C=N (imine / oxime)
		Stretching	Strong	C=O (secondary amide) or C=O (tertiary amide)
		Stretching	Strong	C=C (alkene disubstituted (cis)) or C=C (conjugated alkene)
		Bending	Medium	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
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	Strong	C-O (primary alcohol) C-O (vinyl ether)
		Stretching		C-O (alkyl aryl ether) S=O (sulfoxide)
7	1141.9	Stretching	Strong	C-F (fluoro compound) or C-O (tertiary alcohol) or
		Stretching	Strong	S=O (sulfone) or C-O (aliphatic ether) or C-N (amine)
8	1346.35	Stretching	Strong	C-F (fluoro compound) or N-O (nitro compound)
		Bending	Medium	O-H (alcohol) or O-H (phenol)
9	1411.94	Bending	Medium	C-H (alkane methyl group) or O-H (carboxylic acid) or
		Bending	Medium	O-H (alcohol)
10	1454.38	Stretching	Medium	C-H (alkane methylene group)
11	1653.05	Stretching	Strong	C=O stretching (primary amide)
		Stretching	Medium	C=N stretching (imine / oxime)
		Stretching	Strong	C=O stretching (secondary amide) or C=O stretching (tertiary
		Stretching	Medium	amide
		Stretching	Weak	C=C stretching (alkene disubstituted (cis)) or C=C stretching
12	2077.4	Bending	Strong	alkene vinylidene)
		Bending		C-H (aromatic compound)
13	2364.81	Stretching	Strong	N=C=S (isothiocyanate)
		Stretching		O=C=O (carbon dioxide)
14	2845.1	Stretching	Weak	O-H (alcohol intramolecular bonded)
		Stretching	Medium	C-H (alkane)
		Stretching	Strong	N-H (amine salt) or O-H (carboxylic acid)
15	2982.05	Stretching	Weak	O-H (alcohol intramolecular bonded)
		Stretching	Medium	C-H (alkane)
		Stretching	Strong	N-H (amine salt) or O-H (carboxylic acid)

307

308 **4.0 DISCUSSION**

309

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

315 In this study, the result of the antibacterial activities of *Calotropis procera* leaf
316 extracts at 500mg/ml as shown in Tables 3 reveals the highest inhibition by the Ethanol
317 extract was recorded against *Staphylococcus aureus* with a zones of inhibition value of 24.01
318 mm and lowest zones of inhibition recorded against *Klebsiella pneumoniae* at 10.19 mm. For
319 Cold water extract highest inhibition was recorded against *Escherichia coli* at 15.00 mm and
320 lowest inhibition against *Pseudomonas aeruginosa* at 7.20 mm. The results of this study
321 reveal that Ethanolic extract had highest zone of inhibition against *Staphylococcus aureus*
322 which is in conformity with the work done by [41, 51] but in contrast to the work done by
323 [52] which reveal that the aqueous extract has the highest zones of inhibition against
324 *Staphylococcus aureus*.

325 Antibacterial activity of medicinal plants and drugs varies in their inhibitory effect
326 depending on the concentration, temperature, nature of organism, size of inoculums [53]. The
327 presence of tannin is also an indication that there are phenolic acids present in the plants
328 which may be responsible for its antibacterial activities [54, 55]. The result from this study
329 has shown that the leaf extracts of *Calotropis procera* has antibacterial properties. Thus the
330 leaf extracts of *Calotropis procera* exhibited a broad spectrum activity, against the test
331 organisms in this work, and hence can be employed in treatment of diseases caused by such.
332 Also the susceptibility of *Staphylococcus aureus* to the plant extracts justifies the traditional
333 use of the leaf in wound healing. This is in agreement with the findings of [54, 55, 24, and
334 56] which reported that the plant is useful in treatment of wounds, ulcers, spleen complaints,
335 sores, rheumatism, smallpox, epilepsy, hemiplegia, gumboils and skin infections.

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

352 **5.0 CONCLUSION**

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

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

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