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Studies on Indigenous knowledge in the Management of Typhoid fever and Wound Infections Using *Calotropis Procera* (*Cp*) Leaf Extracts Against selected Microorganisms

Jimba Rai Amos[1], Egbenoma Aigboeghian[2], Aigboeghian Preciousgift[2], Syvernus Chukwudi[2] and J.C. Ogbu[1*]

[1]*Jimba, R.A. Department of Microbiology, Faculty of Science University of Abuja, Nigeria*

[2]*Ugoh, S.C. Department of Microbiology, Faculty of Science University of Abuja, Nigeria*

[*]Corresponding Author
J.C. Ogbu, *Department of Microbiology, Faculty of Science University of Abuja, Nigeria.*

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Abstract

The studies on indigenous knowledge in the management of typhoid fever and wound infections using leaf extract of Calotropis procera plant were carried out. The leaf were plucked and air dried to a constant weighed pulverize, soaked with four different solvent; aqueous, n.hexane, ethanol and methanol. Two hundred and fifty gram of the pulverized dried sample was weighed for each solvent extraction and 28.50±1.00 (g), 12.30±1.00 (g), 16.40±1.0 (g) and 22.60±1.00(g) with correspondence weigh of fractionated pure isolates of the extract obtained from column chromatography of 13.00±1.00, 7.20±1.00, 8.80±1.00 and 10.30±1.00 (ml) was obtained. The result of phytochemical analysis revealed the highest active components of tannins, saponins, alkaloids, flavonoids, cardiac glycoside and terpenoids in methanolic extracts whereas reducing sugar, phenol, and phlobatannins were absent. Using agar well diffusion methods, the different crude extracts showed visible effect on test organisms compared to the fractionated pure isolates of the extracts and the positive control. The methanolic extracts were observed to be more potent than other solvent extracts on the test bacteria with the concentration of 250mg/ml and 500mg/ml on S. typhi. Fractionated pure isolate of the extract showed highest zone diameter of inhibition of 27.50±0.00 and 35.8±0.00 at the concentration of 50 ml and 100 ml respectively on both gram negative and gram positive bacteria compared to the positive control range from 34.00±1.00 to 49.20±1.00. The minimum bactericidal concentration (MBC) of crude and fractionated pure isolates showed effectiveness on both S. typhi and S. aureus at the concentration of 250mg/ml and 500 mg/lm, 50 ml and 100ml respectively. MRSA showed a quick declined in the growth of S. typhi and a little longer in S. aureus after the period of 4h and 12 h at the concentration of 25 mg/ml, 500mg/ml, 50 ml and 100ml of crude and fractionated pure isolates of plant extract. Thus, the results indicates that fractionated pure isolate of the extracts were more significantly higher than the crude extracts at 0<0.5 level of significance compared to the positive control; this could be due to the synergism. The finding therefore concludes that Calotropis procera contains a potential source of bioactive compounds that can be used in management of typhoid fever and wound infections.

Keywords: Calotropis, Procera, Leaf, Extract, Phytochemical, Antibacterial.

1. Introduction

Medicinal plants are part and parcel of human society to combat diseases from the dawn of civilization. The medicinal plants occupy an important place in African pharmacopoeia [1]. Medicinal plants have intensified due to the diverse therapeutic potential that these medicinal plants possess. The evaluation of plants in traditional medicine gives us clues on how these plant parts can be used as antimicrobial agents against many pathogens [2]. The use of plant extracts and physicochemical both known for their antimicrobial properties can be of great importance in therapeutic treatments [3]. Many plants have been used because of their antimicrobial characteristics that are due to their secondary metabolites contain. *Calotropis procera* locally called *Fucheku* plant is used in many African countries by traditional medicine practitioners for the treatment of various ailments including bacterial diseases [4].

In Africa, *C. procera* is widely used in traditional medicine to treat a variety disease including malaria, epilepsy, infectious diseases

[4]. Typhoid fever and wound infections have been a problem and are the field of medicine for a long time [5]. The presence of foreign materials increases the risk of serious infection even with relatively small bacterial inoculums. Advances in control of infections have not completely eradicated this problem because of development of drug resistance. The widespread uses of antibiotics, together with the length of time over which they have been available have led to major problems of multi-drug resistant organisms contributing to morbidity and mortality [6,7].

In Benin, infectious diseases are the primary public health problem [8]. These infectious diseases are often caused by microbial pathogens. To control the pathogens involved in infectious diseases, antibiotic therapy is implemented currently used [9]. Unfortunately, the resistance phenomenon is an increasing cause of treatment failure. One of the options remains to find a local and natural, such as the uses of plants, solution to mitigate these health problems. Among the potential plant, *C. procera* has been identified and used due to its medicinal properties regarding gastric disorders and foodborne diseases [10].

Today, in many parts of the developing world, between 70 and 95% of people continue to rely on plants as a primary form of medicine, and many countries have integrated traditional plant based medicines through regulations into mainstream healthcare systems [11]. Plant-based medicines also continue to make up a key component of intercultural healthcare, encompassing biomedical and traditional medical approaches, in minority and underserved communities [12]. This is proof that traditional medicine still has unexplored potential. However, the main problem with traditional treatments, especially those based on plants, is the lack of scientific knowledge regarding efficacy, mode of action, active ingredients, doses to be administered, indications, lack of properties, safety and quality control. Therefore, the present study aim to evaluate the indigenous knowledge in the management of typhoid fever and wound infection using Calotropis procera leaf extracts against selected gram negative and gram positive microorganisms.

2. Methodology

2.1. Study Area

The study was carried out at the University of Abuja. Abuja is a part of the Federal Capital Territory (FCT). The territory is located just north of the confluence of the River Niger and River Benue. It is bordered by the states of Niger to the West and North, Kaduna to the Northeast and South and Kogi to the Southwest (Wikipedia, 2014). Federal Capital Territory lies between latitude 8.25and 9.20 North of the equator and longitude 6.45 and 7.39 east of Greenwich Meridian. Abuja is geographically located in the centre of the country with a landmass of approximately 7.315km2 of which the actual city occupies 275.3/sq.km. it is situated within the savannah region with moderate climatic condition (Wikipedia, 2014).

2.2. Plant Sample

The fresh samples of plant (leaf) of Calotropis procera was collected from Odulo in Bassa Local Government, Kogi State. The plants were transported with immediate effect to the Botany department, University of Abuja, where it was identified and authenticated by the herbarium of the Department. Part of the plant sample was deposited in the herbarium for reference purposes where the rest of the samples were taken to the department of Microbiology, Faculty of Science all in University of Abuja.

2.3. Preparation of the Extracts

For each of the methanolic, ethanolic, n-hexane and aqueous plant extracts, 250 gm of finely powdered samples were macerated using about 99% methanol, ethanol, n-haxene and aqueous for 24 hours [13]. After fermentation in the room temperature, the mixture was then filtered using filter paper and was concentrated in a water bath at the temperature range 450C – 550C in order to maintain the secondary metabolite present in the plant until the crude extracts were obtained. These crude extracts were both used for phytochemical evaluation, thin layer chromatography, column chromatography and antibacterial susceptibility.

2.4. Antibacterial Assay

The antibacterial assay was conducted using the method modified by Trease and Evans [14]. Crude extracts and fractionated pure isolates obtained from column chromatography and the control antibiotic drug (ciprofloxacin) were used against *Staphylococcus aureus, Bacillus subtile, S. typhi* and *Pseudomonas aeruginosa*. The bacterial isolates used for the study were cultured from Microbiology Laboratory University of Abuja. The isolates were screened morphologically, microscopically and biochemical test was carried out. All the isolates were maintained at the temperature of 40C in the nutrient agar slants.

2.5. Preparation of the Inoculum

The American Type Culture Collection standard reference method was used throughout. A loopful of the test organism was taken from their respective nutrient agar slants and sub-culture into test tubes containing nutrient agar. The test tubes were arranged in rang and incubated overnight at the temperature of 370C. the bacteria obtained were standardized using normal saline solution which doesn't have any alteration effects but it was used to obtained a uniform bacteria population density.

2.6. Standardization of the Plant Extracts

Five test tubes were labeled 1 to 5. A stock concentration solution was made up of 500 mg/ml each of the extracts was prepared in the first test tube. About 5 ml of Dimethylsulfoxide (DMSO4) which does not have any antibacterial effect used to dissolve each of the extracts and was then introduced into the remaining four test tubes. The content of the first test tube was thoroughly mixed. 5 ml of this was drawn and added to the second test tube which was thoroughly mixed to obtain a uniform concentration of 250 mg/ml. another 5 ml was drawn from the second test tube and the introduced into the third test tube and shake vigorously to obtain a concentration of 125 mg/ml. 5 ml was also drawn from the test tube and added to the last test tube and shake thoroughly to have a concentration

of 62.5 mg/l. However, 5 ml was also drawn from the last test tube and discarded.

2.7. Preparation of Media and Zone of Inhibition

The agar diffusion method was used to test the antimicrobial activity of *C. procera* methanolic, ethanolic, n-hexane and aqueous extract against the pathogenic bacteria isolates. According to the method of Jensen et al. (2020), a fresh stock solution of *C. procera* methanolic, ethanolic, n-hexane and aqueous extract was dissolved in DMSO4 (99.9%) with a stuck concentration of 500 mg/mL for each bacterium and then diluted twofold to obtain a serial concentration of 500, 250, 125 and 62.5 mg/mL respectively. In a 96-well format, the MIC for various concentrations of methanolic, ethanolic, n-hexane and aqueous extract was determined [15]. 1 ml of each active bacterial isolate grown on nutrient agar in the test-tubes for 24 h at 37 °C on were spread on the surface Muller Hinton agar plates 0.5 McFarland turbidity (corresponding to 106 CFU mL-1). In the wells containing standard twofold dilutions of methanolic, ethanolic, n.hexane and aqueous extract in a final concentration of µg/mL, the bacterial suspensions were adjusted to 5 × 106 CFU mL−1 in Mueller–Hinton (MH) broth. The plates were incubated at 37 °C for 18–24 h with shaking (300 rpm). All experiments were carried out in triplicate. The MIC was defined as the concentration of methanolic, ethanolic, n-hexane and aqueous extract dissolved in $DMSO_4$ that inhibited the growth of tested bacteria. The MIC reading was taken by measuring the zone diameter with a meter rule [3].

The MBC of methanolic, ethanolic, n-hexane and aqueous extract respectively was performed on both gram negative and gram positive test organisms in comparison to positive control. The highest corresponding minimum inhibitory concentration and the immediately higher concentrations named (MIC_1, MIC_2 and MIC_3) was sub-cultured in two way. First the MIC_1, MIC_2 and MIC_3 was sub-cultured on test tube containing broth agar and incubated for the presence of turbidity. Secondly, it was sub-cultured on Mueller–Hinton agar Petri dishes. The plates were incubated for 24 hours for 37^0C. The MBC was determined and defined after 24 h of incubation as the lowest concentration that inhibited visible growth of the subculture and the MIC_1 and MIC_4 that does not showed significant growth in plate.

2.8. Methicillin Resistant *Staphylococcus Aureus* Test (MRSA)

Methicillin resistant *Staphylococcus aureus* test were grown overnight at 37 °C in nutrient broth No. 2 (Oxoid, CM0067) and diluted in physiological saline (0.9% NaCl) to achieve the 0.5 McFarland turbidity. Bacterial suspensions were adjusted to 106 CFU mL^{-1} in BHI containing 75 and 150 mg/mL of pure isolates of methanolic extract in a final volume of 500 mL and incubated at 37 °C with aeration (150 rpm). Cell counts were determined through tenfold serial dilution on nutrient agar every hour for the first 4 h and 24 h later [16]. Experiments were carried out in duplicate. The MIC of overnight cultures was determined for one of the duplicate experiments to determine susceptibility after prolonged exposure. The MRSA can be determined by allowing the immediately higher concentrations (MIC_1 and MIC_2) to stand undisturbed in an incubator for 72 hours and check for any further growth [3].

3. Analysis

3.1. Results and Discussion

Solvent	CE Yield (g)	FY(ml)	% Yield	Physical Appearance
Methanol	22.60±1.00	10.30±1.00	2.26±1.00	Brown crystalline viscous
Ethanol	16.40±1.00	8.80±1.00	1.64±1.00	Brown crystalline viscous
n.ehaxane	12.30±1.00	7.20±1.00	1.23±1.00	Brown crystalline viscous
Aqueous	28.50±1.00	13.00±1.00	2.85±1.00	Deep-black semi-solid

Table 1: Crude Extract Yield and Physical Appearance of *Calotropis procera*

Key: CE= crude extracts, FY= Fractionated Yield
Values of zone of inhibition are measured in (mm) of three replicates ±standard deviation (SD), $P<0.05$

Sec. Metabolite	N-heE	MeE	EtE	AqE
Tannins	+	+	+	+
Saponins	+	+	+	+
Alkaloids	+	+	+	+
Steroids	-	+	-	-
Flavonoids	+	+	+	+
Cardiac glycoside	+	+	+	-
Anthraquinone	-	-	-	-

Reducing sugar	-	-	-	-
Terpenoids	-	+	+	+
Phenols	-	-	-	-
Phlobatanin	-	-	-	-

Table 2: Phytochemical Screening of *Calotropis procera* extract

Key: Sec = secondary metabolite, N-heE= n-hexane extract, MeE= methanolic extract, EtE= ethanolic extract, AqE= aqueous extract, - = absent, + = present

Extracts	N-heE	MeE	EtE	AqE	PC: Cipro
B. subtilis					
62.5 mg/ml	0.00±0.00	0.00±0.00	16.10±0.00	0.00±0.00	13.30±4.00
125 mg/ml	0.00±0.00	7.60±0.00	18.00±1.00	0.00±0.00	30.10±0.00
250 mg/ml	0.00±0.00	14.00±0.00	15.10±0.00	17.20±0.11	35.00±1.00
500 mg/ml	6.00±2.00	19.00±0.00	15.30±0.00	20.30±4.00	40.30±4.00
S. typhi					
62.5 mg/ml	9.33±0.00	10.50±0.00	0.00±0.00	12.00±0.00	15.00±0.00
125 mg/ml	13.02±0.00	16.22±0.00	18.00±0.00	20.00±0.00	20.00±0.00
250 mg/ml	20.00±0.00	25.00±1.00	20.50±1.00	22.30±1.00	34.00±0.00
500 mg/ml	29.70±0.00	33.80±0.00	25.10±1.00	28.80±0.20	49.20±0.00
S. aureus					
62.5 mg/ml 125 mg/ml	0.60±0.00 5.60±0.00	2.20±1.00 4.80±1.00	2.10±1.20 10.20±1.00	9.00±0.00 13.00±0.00	17.60±0.00 20.60±0.00
250 mg/ml	14.00±1.00	9.20±0.00	13.20±0.00	18.30±1.00	25.60±0.00
500 mg/ml	18.10±0.00	14.70±0.00	22.30±0.00	31.00±0.00	39.90±0.00
P. aeruginosa					
62.5 mg/ml 125 mg/ml	7.60±0.00 10.60±0.00	1.20±1.00 9.80±1.00	2.0±1.20 11.2±1.00	9.00±0.00 12.00±0.00	11.20±0.00 16.10±0.00
250 mg/ml	11.00±1.00	9.20±0.00	17.30±0.00	21.30±1.00	25.60±0.00
500 mg/ml	21.10±0.00	14.70±0.00	22.30±0.00	27.00±0.00	37.90±0.00

Table 3: Antibacterial Activity of Crude Extracts against test organisms

Key: Sec = secondary metabolite, N-heE= n-hexane extract, MeE= methanolic extract, EtE= ethanolic extract, AqE= aqueous extract, - = absent, + = present, PC= Positive Control, Cipro= Ciprofloxacin Values of zone diameter of inhibition are measured in (mm) of three replicates ±standard deviation (SD), $P<0.05$

Extracts	N-heF	MeF	EtF	AqF	PC: Cipro
B. subtilis					
12.5 ml	0.00±0.00	4.00±1.00	10.10±0.00	0.00±0.00	13.30±4.00
25 ml	0.00±0.00	7.90±3.00	14.00±1.00	11.00±0.00	30.10±0.00
50 ml	0.00±0.00	20.00±0.00	15.10±0.00	18.20±0.11	45.00±1.00
100 ml	16.00±2.00	21.00±0.00	25.30±0.00	20.30±4.00	12.30±4.00
S. typhi					
12.5 ml	0.00±0.00	18.00±0.00	9.90±0.00	0.00±0.00	10.00±0.00
25 ml	10.20±0.00	22.20±0.00	16.00±4.00	11.00±2.22	20.00±0.00
50 ml 100 ml	20.00±0.00 29.70±0.00	27.50±0.00 35.80±0.00	22.50±0.00 30.10±1.00	20.30±1.00 26.80±0.20	35.10±0.00 44.20±0.00

S. aureus					
12.5 ml 25 ml	1.20±0.00 6.60±0.00	3.50±1.00 7.40±1.00	22.2±1.20 20.2±1.00	29.00±0.00 23.00±0.00	7.60±0.00 12.60±0.00
50 ml	14.00±1.00	9.20±0.00	13.30±0.00	18.30±1.00	35.60±0.00
100 ml	18.10±0.00	14.70±0.00	22.30±0.00	31.00±0.00	40.90±0.00
P. aeruginosa					
12.5 ml 25 ml	0.00±0.00 0.00±0.00	4.50±1.00 9.0±1.00	2.10±1.20 10.20±1.00	10.00±0.00 13.00±0.00	6.10±0.00 22.20±0.00
50 ml	6.90±1.00	9.20±0.00	13.30±0.00	28.30±1.00	25.60±0.00
100 ml	12.20±1.00	14.70±0.00	22.30±0.00	31.00±0.00	39.90±0.00

Table 4: Antibacterial Activity of Fractionated pure isolates against test organism

Key: Sec = secondary metabolite, N-heF= n-hexane fractionated extract, MeF= methanolic fractionated extract, EtF= ethanolic fractionated extract, AqF= aqueous fractionated extract, PC= Positive Control, Cipro= Ciprofloxacin. Values of zone diameter of inhibition are measured in (mm) of three replicates ±standard deviation (SD), $P<0.05$

Extracts	MIC_1	MIC_2	MIC_3	PC: Cipro
B. subtilis				
62.5 mg/ml	+	+	+	+
125 mg/ml	+	+	+	-
125 mg/ml	+	+	+	-
500 mg/ml	-	+	+	-
S. typhi				
62.5 mg/ml	+	+	+	-
125 mg/ml	-	+	+	-
250 mg/ml 500 mg/ml	- -	- -	+ -	- -
S. aureus				
62.5 mg/ml	+	+	+	+
125 mg/ml	+	+	+	+
250 mg/ml	-	+	+	-
500 mg/ml	-	-	+	-
P. aeruginosa				
62.5 mg/ml 125 mg/ml	+ +	+ +	+	- -
250 mg/ml	+	+	+	-
500 mg/ml	-	+	+	-

Table 5: Minimum Bactericidal Concentration (MBC) of Crude Extracts against *Streptococcus mutans*

Key: MIC_1 = high inhibitory concentration, MIC_2 = moderate Inhibitory concentration, MIC_3 = low inhibitory concentration, - = absence of turbidity, + = presence of turbidity, PC= Positive Control, Cipro= Ciprofloxacin.

4. Discussion

In the present investigation, the active components which are the secondary metabolite of Calotropis procera was studied. Column chromatography and thin layer chromatography and antibacterial screening leaf extract of equeous, methanol, n.hexane and ethanol were used to extract the plant for crude extracts. Fractionated pure isolates obtained from column chromatography using different solvent ratio and the crude extracts were tested against some selected pathogenes, microorganisms such as P. aeruginosa, S. typhi, S. aureus and B. subtile.

Two hundred and fifty (250) gram of the pulverized dried was

weighed for each solvent extraction and 28.50±1.00, 12.30±1.00, 16.40±1.0 (g) and 22.60±1.00 respectively with correspondence weigh of fractionated pure isolates of the extract obtained from column chromatography of 13.00±1.00, 7.20±1.00, 8.80±1.00 and 10.30±1.00 (ml) was obtained (Table 1).

Table (2) showed results of phytochemical analysis which revealed that saponins, tannins, alkaloids, flavonoids and cardiac glycoside were present in n.hexane extracts whereas reducing sugar, steroids, anthroquinone, terpenoids phenol and phlobatannins were absent in n.hexane plant extracts. Methanolic extracts showed the highest active components of tannins, saponins, alkaloids, flavonoids, cardiac glycoside and terpenoids in methanolic extracts whereas reducing sugar, phenol, and phlobatannins were absent. Ethanolic extracts showed the presence of saponins, tannins, alkaloids, flavonoids, terpenoids and cardiac glycoside whereas reducing sugar, steroids, anthroquinone, phenol and phlobatannins were absent. Similarly, in aqueous extracts, saponins, tannins, alkaloids and terpenoid were present whereas reducing sugar, steroids, flavonoids, terpenoids, phenol and cardiac glycoside were absent. This result agreed with Peni et al. [17].

Table 3 showed antibacterial activity of crude extracts against test organisms. The nhexane crude extract showed zone diameter of inhibition of 6.00±2.00 on B. subtile, 9.33±0.00, 13.02±0.00, 20.00±0.00 and 29.70±0.00 on S. typhi, 0.60±0.00, 5.60±0.00, 14.00±1.00 and 18.10±0.00 on S. aureus, 7.60±0.00, 10.60±0.00, 11.00±1.00 and 21.10±0.00 on P. aeruginosa at different concentration of 62.5, 125 mg/ml, 250 mg/ml and 500 mg/ml. Result of methanolic crude extracts showed the mean zone diameter of inhibition of 7.60±0.00, 14.00±0.00 and 19.00±0.00 on B. subtilis, 10.50±0.00, 16.22±0.00, 25.00±1.00 and 33.80±0.00 on S. typhi, 2.20±1.00, 4.80±1.00, 9.20±0.00 and 14.70±0.00 on S. aureus, 1.20±1.00, 9.80±1.00, 9.20±0.00 and 14.70±0.00 on P. aeruginosa. Result of ethanolic crude extracts showed the mean zone diameter of inhibition of 16.10±0.00, 18.00±1.00, 15.10±0.00 and 15.30±0.00 on B. subtilis, 18.0±1.00, 20.50±1.00 and 25.10±1.00 on S. typhi, 2.10±1.20, 10.20±1.00, 13.20±0.00 and 22.30±0.00 on S. aureus; 2.0±1.20, 11.2±1.00, 17.30±0.00 and 22.30±0.00 on P. aeruginosa. Result of aqueous crude extracts showed the mean zone diameter of inhibition of 0.00±0.00, 0.00±0.00, 17.20±0.11and 20.30±4.00 on B. subtilis, 12.00±0.00, 20.00±0.00, 22.30±1.00 and 28.80±0.20 on S. typhi; 9.00±0.00, 13.00±0.00, 18.30±1.00 and 31.00±0.00 on S. aureus; 9.00±0.00, 12.00±0.00, 21.30±1.00 and 31.00±0.00 on P. aeruginosa. The methanolic extracts were observed to be more potent than other extracts on the test bacteria with the mean zone diameter of inhibition of 25.00±0.00 and 33.80±0.00 at the concentration of 500mg/ml on S. typhi.

From table 4, fractionated pure isolate of the extract showed highest zone diameter of inhibition of 27.50±0.00 and 35.8±0.00 at the concentration of 50 ml and 100 ml respectively on S typhi compared to the positive control range 34.00±1.00 and 49.20±1.00. The minimum bactericidal concentration (MBC) of crude and fractionated pure isolates showed effectiveness on both S. typhi and S. aureus at the concentration of 250mg/ml and 500 mg/lm, 50 ml and 100ml respectively. The above indication from agar well diffusion methods, the different crude extracts showed visible effect on crude extracts when compared to the fractionated pure isolates of the extracts and the positive control. This, confirming the assertion that different solvent extracts of same plant have different pharmacological properties. aqueous is the commonly used solvent by traditional healers to extract pharmacologically active compounds because of its easy availability [19].

The results Methicillin Resistant Staphylococcus Aureus Test (MRSA) showed a little longer time of declination in S. aureus within 12 h at the concentration of 250 mg/ml, 500mg/ml, 50 ml and 100ml of crude and fractionated pure extracts after an overnight incubation period. Hashim et al. (2017) reported a time-dependent decline in the case of S. aureus, with a 90% reduction achieved within 8 h of exposure to Cymbopogon schoenanthus essential oil of plant extract [20]. From the results of the findings on MRSA, among the test organisms used, S. typhi revealed a very quick time of declination at the concentration of 250 mg/ml, 500 mg/ml, 50 ml, and 100 ml of crude and fractionated extracts within 4 h of the period of incubation. This was followed by a consistent reduction in the growth of S typi until a constant state of zones of inhibition was established as a benchmark. S. typhi showed less MRSA at concentrations compared to S. aureus that decline at 12 h which is an indication that the plant Calotropis procera leaf extracts has bacteriostatic effects on the test bacteria. Thus, the results deduced that fractionated pure isolate of the extracts were more significantly higher than the crude extracts at $0<0.5$ level of significance compared to the positive control; this could be due to the synergism of the solvent. The finding therefore concludes that the plant Calotropis procera contains a potential source of bioactive compounds that can be used in management of typhoid fever and wound infections.

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