



## The Antibacteria and Antiglycemic profile of *Chromolaela odorata* and *Tridax procumbens* in wound healing

<sup>1</sup>Nwokorie, Chukwuma Chigozie

[chigo205@yahoo.com](mailto:chigo205@yahoo.com), [chibundo75@gmail.com](mailto:chibundo75@gmail.com), +2348063245155

<sup>2</sup>Ezeagha, Chigozie.Celestina

<sup>3</sup>Okorie Chibundo.Nweze,

<sup>4</sup>Ogbuebuna, Jacinta.Ogechukwu

<sup>5</sup>Nwankwo, Chidiebere

<sup>1</sup>Dept. of Microbiology, Abia State University, Uturu

<sup>2,4</sup>Dept. of Pharmaceutical and Medicinal Chemistry

<sup>3</sup>Dept. of Pharmaceutical Microbiology and Biotechnology, University of Nigeria, Nsukka

Chukwuemeka Odumegwu Ojukwu University, Igbariam Anambra State

<sup>5</sup>Dept. of Microbiology, University of Nigeria, Nsukka

### ABSTRACT

This study evaluated the antibacterial and antiglycemic profile, of *Chromolaela odorata* and *Tridax procumbens* leaf and stem bark extract. A total of One hundred and twenty (120), 8-weeks old male albino wistar rats (220g and 229g bw) were divided into different groups (control and treatment groups), 10g of pulverized plant extracts were homogenized in distilled water. The samples of the plant extract were soaked in ethanol and aqueous solvents. The antibacterial activity was determined using agar well diffusion method. Ethanol showed a clear zone of inhibition *Staphylococcus aureus* (35±0.01mm), for *C odorata* leaf extract and *Pseudomonas aeruginosa* (31±0.01mm) for the stem bark extract, also *T procumbens* had 32±0.01mm and 30±0.01mm for leaf and Stem bark extract with MIC and MBC of 9mg/ml and 10mg/ml. The total viable bacterial cell from the wound excision was determined using 10% and 20% concentrations of the plant extract and gentamycin ointment and normal saline as the control P<0.05. The effect of both Ethanolic and aqueous plant extracts on Streptozotocin –induced diabetic rat were not significant (P<0.05), ranging from (250-500mg/kg) of Total Cholesterol and Triglyceride (mg/dl). The weight of the induced diabetic rat showed a significant values (1-21days)

## INTRODUCTION

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. Medicinal plants represent a rich source of antimicrobial agents and natural antioxidants . Many of the plant materials used in traditional medicines are readily available in rural areas at relatively cheaper than modern medicines . Approximately 80% of the world inhabitants rely on traditional medicine for their primary health care and play an important role in the health care system of the remaining 20% of the population( Akinpelu *et al.*, 2015).

Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of area of the wound. It mainly depends on the repairing ability of the tissue, type and extent of damage and general state of the health of the tissue. The granulation tissue of the wound is primarily composed of fibroblast, collagen, edema, and small new blood vessels.(*Begun et, al.*,2014)

Also Wound healing is the process of re-establishing the integrity of damaged skin . It is an orderly intricate process initiated by a damaged tissue itself, and it involves complex mechanisms which include: Hemostasis, inflammation, proliferation, and remodeling. Each of these mechanisms requires several biochemical substances to occur. Thromboxane A2 and plasminogen activator inhibitor Type 1 ensures hemostasis, heme and heme proteins trigger expression of adhesion molecules, leukocytic infiltration, and release of reactive oxygen species (ROS) also called toxic free radicals or oxidants. The oxidants are detrimental to wound contaminating microorganisms and to the skin tissue itself especially when in excess .



**Fig.3 A scab covering a healing wound**

The aim of this study was to investigate the Antibacterial and Antiglycemic profile of *Chromolaena odorata* and *Tridax procumbens* plant extracts in wound healing.



## **Materials and Methods**

### **Experimental Animals**

A total of 120, 8-week-old male albino Wistar rats weighing between 220 and 229 g were obtained from the laboratory animal unit, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were fed on commercial growers mash (Top feeds®) and water was provided *ad libitum*. These rats were acclimatized for 2 weeks in the animal house at the Department of Veterinary Surgery, University of Nigeria, Nsukka.

### **Plant Collection and Identification**

Fresh *C. odorata* and *Tridax procumbens* leaf and stem bark were collected from Iyiowa Odekpe town in Ogbaru Local Government Area Anambra State, Nigeria, in the month of February, 2016 and was identified at the International Center for Ethnomedicine and Drug Development (InterCEDD), Nsukka, by a plant taxonomist.

## **Preparation of ointments**

The method of Okore *et al* (2006), was adopted in preparation of two herbal ointments *C.odorata* and *T.procumbens* containing 10% w/w and 20% w/w of the extract in sterile soft white paraffin. Immediately after preparation, the ointments were aseptically transferred into sterile cream tubes and sealed until when needed

## **preparation of inocula**

The bacterial isolates ( *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *E-coli*, *Klebsiella pneumonia* and *Bacillus subtilis*) were used in this study were collected from the Department of Pharmaceutics, University of Nigeria, Nsukka. They were clinical wound isolates from patients in the teaching hospital Nsukka, Nigeria, and it were fully identified and maintained on nutrient agar slope at 4°C at the Department of Pharmaceutical Microbiology Laboratory, University of Nigeria, Nsukka. Prior to use, the organisms were sub-cultured on sterile nutrient agar plate, incubated aerobically at 37°C for 24 hrs. Colonies of each organism were homogenized in sterile phosphate buffered saline (PBS) and the turbidity adjusted to correspond to 0.5 McFarland's turbidity standard (equivalent to  $1 \times 10^8$  cfu/ml). The four clinical isolates mentioned were characterized using microbiological and biochemical test for proper identification

## **COLLECTION AND PREPARATION OF BLOOD SAMPLES**

Blood collection were performed by sacrificing the animal under anaesthesia. The blood samples (5ml) were collected and stored in lithium heparin anti coagulant bottle and mixed properly with the reagent. The blood samples were centrifuged at 200ppm for 100mins. to separate the blood cells from plasma. The plasma was stored in the sample container and were labelled for blood assay

## **Extraction of the plant**

One kilogram(kg) each, of the *C. odorata* and *T.procumbens* leaves and stem bark was washed with clean tap water and rinsed with distilled water. After that, they were sliced into smaller pieces; air dried with hot air oven (GL, England) at room temperature of 35 degree for 2 weeks and then pulverized using the laboratory grinding machine at the, Department of Food Science and Technology, Abia State University Uturu. The pulverized leaves and the stem bark were

soaked in 70% ethanol and aqueous solution(hot water) respectively for 48 h with intermittent vigorous shaking . After 48 h, the mixtures was filtered using whatman no.1 filter paper and the extract concentration was produced using a rotary evaporator set at 40°C. The dried samples were weighed and the percentage yield was calculated. The extracts was stored at 4°C in a refrigerator. (Sofowara,1993,Yogeshi *et. al.*,2012)

### **Ethanol Extract**

Ten grammes (10g ) of the leaf and stem bark were washed with clean tap water and rinsed with distilled water .They were blended into smaller pieces; air dried with hot air oven (GL,England) at room temperature for 2 weeks and then pulverized leaf were weighed using Satoric AG Gottingen Electronic Weighing balance.The weighed samples of *C.odorata* and *T.procumbens* leaf and stem bark was soaked in 100mls of ethanol(80%) in a conical flask.The mixture was swirled after 24h elaption with interval stirring.The mixture was filtered using Whatman no.1 filter paper into a clean beaker.The ethanol was recovered using a soxhlet apparatus and it was finally evaporated to dryness using a steam bath at 100<sup>0</sup>C.Ethanol has dark brown colouration after extraction.(Azoro,2002)

### **2.6.2 Aqueous Extract**

Ten grammes(10g) of the pulverized leaves and stem bark were weighed and macerated in 100ml of distilled water.The mixture was vigorously swirled.After the elaption of 24h with interval stirring,the mixture was filtered using Whatman No.1 filter paper into a clean beaker,and the filterate was concentrated to dryness by evaporation using the steam bath at 100<sup>0</sup>C.The filterates has the dark green colouration

The dried samples were weighed and the percentage yield was calculated. (Sofowara, 2008).

### **Characterization of Bacteria Isolates**

The characterization of bacterial isolates was done by Gram staining and biochemical test for proper identifications.

### **GRAM'S STAINING**

This staining was used to differentiate bacterial cells into gram positive and gram negative organism based on the colour reaction exhibited by the bacteria cell wall when they are treated with the dyes.

The method describe by Anderson and Clark,(2013) was used. A colony of bacterial isolate was emulsified (mixed) on a drop of a clean grease free slide and heat fix . The smear was made by flooding the slide with crystal violet for contact time of 1minute ,and immediately it was rinsed with tap water and flooded with grams iodine and allowed to stand for 30 seconds. It was rinsed with water again and decolourized with an alcohol (70%) for 10 seconds and it was counter stained with 0.25% of Safranin for 30 seconds .It was rinsed with tap water until no colour comes off . The smear was allowed to air dry before being viewed under the microscope with x100 oil immersion lens of Bright field light compound microscope (Bright 335 America), Gram-positive cells retained the primary dye (crystal violet while the gram-negative cells retained the secondary dye (safranin),the number of bacterial cells,the pus cells and the cell morphology respectively were revealed.Other biochemical test such as Catalase,Methyl Red,Coagulase,Citrate,Indole,Oxidase,Urease and Sugar fermentation test were carried out

#### **AGAR WELL DIFFUSION METHOD**

The antimicrobial activities of aqueous and ethanol extract were determined by inoculating a standard size of 0.5ml of different isolates into peptone broth for 18hrs. Five holes (6 mm) diameter were bored into the agar plates at strategic points using sterile cork borer(4mm) and labelled to correspond to the extract concentrations. Then each of the holes was filled with 50  $\mu$ l of the plant extract concentration. The plates were allowed to stand for 30mins on the bench to ensure complete diffusion of the extract into the agar and then was incubated at 37°C for 24hrs. After incubation, the zone of inhibition around each well was measured with a meter rule. Each test for each organism was performed in triplicate and the mean inhibitory zone diameter (IZD) was calculated to the nearest whole millimeters.

#### **2.9 Minimum Inhibitory Concentration(MIC)**

The estimation of MIC of the extract was carried out using the method of Akinpelu and Kolawale (2004). Ten fold Serial dilution of the extract was prepared and 2ml aliquots of different concentrations of the suspension was added to 18ml of pre-sterilized molten nutrient agar,the medium was poured into a sterile petri dish and allowed to set.The surface of the medium was allowed to dry under lamina flow,before streaking with 12hrs culture, and incubated at 37°C for 24hrs.Plates were examined for presence or absence of growth.The MIC was taken as the lowest concentration that showed no evidence of growth (turbidity)

### **.Minimum Bacterial Concentration(MBC)**

The MBC of the plant extract was determined by a modification of Method of Spencer and Spencer (2004). Samples were taken from plates with no visible growth in the MIC assay and were sub-cultured on freshly prepared nutrient agar, and were incubated at 37°C for 24hrs. The highest dilution that yields no growth was taken as MBC.

### **Blood glucose.**

#### **Inducement of diabetes**

The animals were fasted for 48hrs, the rats were weighed and anaesthetized by inhalation in a glass dome. A solution of alloxan at 2% diluted in saline at 0.9% was administered to the animals in a single dose corresponding to 40mg of alloxan per kg of animal weight was injected into their penile vein. Food and water were presented to the animal only 30mins after administration.

The animals showed the following signs of the conditions: polydipsia (abnormal thirst), polyuria (increase volume of urine), weight loss (due to lean mass loss).

The animals were later fed with the *C.odorata*, *T.procumbens* leaves and stem bark extract and glibenclamide as positive control.

The level of serum glucose considered to be normal in Rat ranges from 50 to 135mg/l. In this study, rat with glucose levels above 200mg/l was considered as having diabetes and also each group was weighed for weight loss using electronic balance (Bal. China 2W21).

The rats were divided into five main groups i-v. group (i) normal control rats, (ii) Diabetic control, (iii) Diabetic rats treated with glibenclamide (0.25mg/kg), (iv) Diabetic rats treated with ethanolic *C.odorata* (250mg/kg), (v) Diabetic rat treated with ethanolic *T.procumbens* (500mg/kg). All these were determined using colorimeter in principle with (Akinpelu, 2014).

Fasting and Random blood glucose was also measured by the glucose oxidase method, Barham and Trinder (1992), as described in the manual of the Randox glucose kit. Glucose was determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacted with the catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator, was read calorimetrically at 500nm and it was compared with the standard.

### **Excision Wound Model**

Thirty six rats (four groups), i-iv were anaesthetized with 50mg/kg bw of xylazine hydrochloride and ketamine hydrochloride respectively. The dorsal body of the rats were shaved on the dorsum portion using depilatory cream (Reckitt Benckiser, Inc., UK) and was anesthetized. An impression was made on shaved dorsal region and area of the wound created was marked. A full thickness excision wound with a circular area of 480 mm<sup>2</sup> was created along the marking using sterile toothed forceps, a surgical blade, and pointed scissors. The wound was flooded with the broth culture (contaminants) using sterile pipette, the wound was bandaged and kept on the cage of (4ftx2ftx2ft) for 24hrs before treatment. Four treatment groups; i-iv, group i and ii were treated topically with 10% and 20% w/w *Chromolaena odorata* and *Tridax procumbens* ointment respectively, while group iii and iv were treated with sterile white soft paraffin (negative control) and gentamycin (positive control). The simple ointment base, formulated extract ointment, and standard drug (gentamycin) were applied once daily from the day of the operation until the complete healing. In this model, wound contraction and epithelialization period were evaluated. Wound contraction was measured as percentage contraction every 4th day after wound formation. At the end of the study, all the rats were anesthetized and from the healed wounds, specimen samples of tissue were collected from each rat, leaving a 5 mm margin of normal skin around the edges of the healed wound. Specimen tissues were stored in 10% formalin solution and used for histopathological and biochemical studies, Morton and Malone (2013).

### **Incision Wound Model**

Incision wound was created according to the method already described above. The animals were grouped and treated the same as in the excision wound model. All rats were anesthetized using ketamine hydrochloride (50 mg/kg, i.p., body weight). Paravertebral incision of 6 cm length was made through the entire thickness of the shaved skin, on either side of the vertebral column of the rats with the help of a sterile sharp scalpel. After complete hemostasis, the wound was stitched by means of interrupted sutures placed approximately 1 cm apart using black silk surgical thread (number 000) and a curved needle (number 11). After stitching, the wound was left undressed and animals were treated daily for 10 days. On the 10th day, all rats were anesthetized and sutures were removed and tensile strength of cured wound skin is measured using tensiometer.



## Wound Healing Evaluation

### Wound Contraction and Epithelialization

In the excision wound model, wound area was measured by tracing the wound with the help of transparent sheet using millimeter based graph paper on days 0, 3, 7, 14, and 18 for all groups. Wound contraction was measured every 3th day until complete wound healing and represented as percentage of healing wound area . Percentage of wound contraction was calculated taking the initial size of the wound as 100% using the following formula:

**% wound contraction=Initial wound area–Specific day wound area /Initial wound area×100.**

Epithelialization period was calculated as the number of days required for falling off the dead tissue remnants of the wound(scars) without any residual raw wound

### Tensile Strength

The tensile strength of a healing skin wound indicated the degree of wound healing. It represented how much the healed tissue resists to breaking under tension and may identify the quality of healing tissue. On the 10th day, all the animals were anesthetized by injecting ketamine hydrochloride (50 mg/kg, i.p., body weight), the sutures were removed, and the healed tissue was excised from all animals. Tensile strength of excised tissue was measured with the help of tensiometer

### Statistical analysis

Data are presented as the means ( $\pm$ ), standard deviation (SD).Statistical significance of the difference between groups were analysed using student t-test and one way analysis of variance ANOVA,with SPSS,VERSION 1.6 software,Chicago Illinos,USA.Means were considered statistically different at 95% level of confidence( $p<0.05$ ).

## RESULTS/DISCUSSION

Table 1 Minimum of Inhibitory and Bactericidal Concentrations of *c.odorata leaf* extract

Bacterial Isolates	MIC(mg/ml)	MBC (mg/ml)
<i>E-Coli</i>	5	9
<i>Bacillus subtilis</i>	3	7
<i>Pseudomonas aeruginosa</i>	9	10
<i>Staphylococcus aureus</i>	4	8

### KEYS

MIC = Minimum inhibitory concentration

MBC = Minimum bactericidal concentration.

Table 2 .Minimum of Inhibitory and Bactericidal Concentrations of *Tridax procumbens* stembark extract

Bacterial Isolates	MIC(mg/ml)	MBC (mg/ml)
<i>E-Coli</i>	6	8
<i>Bacillus subtilis</i>	7	6
<i>Pseudomonas aeruginosa</i>	9	10
<i>Staphylococcus aureus</i>	8	5

### KEYS

MIC = Minimum inhibitory concentration

MBC = Minimum bactericidal concentration

Zones 3 of inhibitions of *C-odorata* extract against selected organisms(mm)

Conc.mg/ ml	Leaf extract				Stembark extract			
	Bs	Ec	Sa	Pa	Bs	Ec	Sa	Pa
100	25±0.02 <sup>a</sup>	22±0.01 <sup>a</sup>	35±0.01 <sup>a</sup>	29±0.01 <sup>a</sup>	30±0.02 <sup>a</sup>	29±0.01 <sup>a</sup>	29±0.01 <sup>a</sup>	31±0.01 <sup>a</sup>
50	23±0.01 <sup>b</sup>	22±0.01 <sup>b</sup>	32±0.01 <sup>b</sup>	25±0.02 <sup>bb</sup>	25±0.01 <sup>b</sup>	26±0.02 <sup>bb</sup>	28±0.02 <sup>b</sup>	30±0.02 <sup>b</sup>
25	21±0.03 <sup>c</sup>	20±0.02 <sup>c</sup>	30±0.01 <sup>cc</sup>	20±0.01 <sup>c</sup>	21±0.02 <sup>cc</sup>	25±0.01 <sup>c</sup>	28±0.03 <sup>c</sup>	29±0.01 <sup>c</sup>
12.5	20±0.02 <sup>a</sup>	21±0.02 <sup>a</sup>	29±0.02 <sup>a</sup>	15±0.02 <sup>aa</sup>	18±0.02 <sup>aa</sup>	20±0.01 <sup>a</sup>	26±0.01 <sup>a</sup>	30±0.02
6.25	19±0.01 <sup>b</sup>	18±0.01 <sup>b</sup>	25±0.01 <sup>b</sup>	17±0.01 <sup>bb</sup>	15±0.01 <sup>b</sup>	22±0.02 <sup>b</sup>	20±0.02 <sup>bb</sup>	26±0.03 <sup>b</sup>

values with different superscript along a row are significant (P<0.05)

KEYS

Bs = *Bacillus subtilis*

Es = *Esherichi coli*

Pa = *Pseudomonas aeruginosa*

Sa = *Staphylococcus aureus*

Zones.4 of inhibitions of *Tridax procumbens* extract against selected organisms(mm)

Conc.mg/ ml	Leaf extract				Stembark extract			
	Bs	Ec	Sa	Pa	Bs	Ec	Sa	Pa
100	29±0.02 <sup>a</sup>	20±0.01 <sup>a</sup>	32±0.01 <sup>a</sup>	27±0.01 <sup>a</sup>	31±0.01 <sup>a</sup>	27±0.01 <sup>a</sup>	30±0.01	28±0.01 <sup>a</sup>
50	27±0.01 <sup>b</sup>	20±0.02 <sup>b</sup>	30±0.01 <sup>b</sup>	25±0.02	30±0.02 <sup>b</sup>	26±0.02 <sup>b</sup>	28±0.02 <sup>b</sup>	27±0.02 <sup>b</sup>
25	26±0.02 <sup>c</sup>	19±0.02 <sup>c</sup>	31±0.01 <sup>c</sup>	25±0.02 <sup>c</sup>	29±0.001 <sup>c</sup>	26±0.01 <sup>c</sup>	28±0.01 <sup>c</sup>	26±0.03 <sup>c</sup>
12.5	22±0.03 <sup>a</sup>	18±0.02 <sup>a</sup>	28±0.02 <sup>a</sup>	26±0.03 <sup>a</sup>	28±0.01 <sup>a</sup>	25±0.01 <sup>a</sup>	25±0.01 <sup>a</sup>	24±0.01 <sup>a</sup>
6.25	20±0.01 <sup>b</sup>	16±0.01 <sup>b</sup>	26±0.02 <sup>b</sup>	22±0.04 <sup>b</sup>	29±0.01 <sup>b</sup>	26±0.02 <sup>b</sup>	22±0.02 <sup>b</sup>	22±0.02 <sup>b</sup>

values with different superscript along a row are statistically significant (P<0.05)

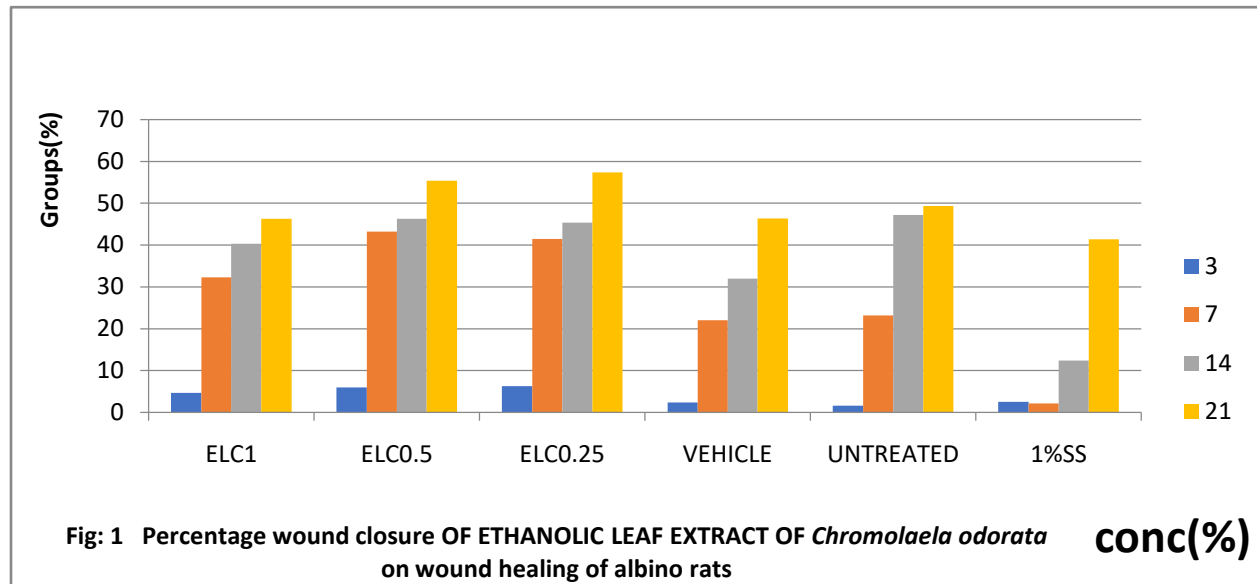
KEYS

Bs = *Bacillus subtilis*

Es = *Esherichi coli*

Pa = *Pseudomonas aeruginosa*

Sa = *Staphylococcus aureus*



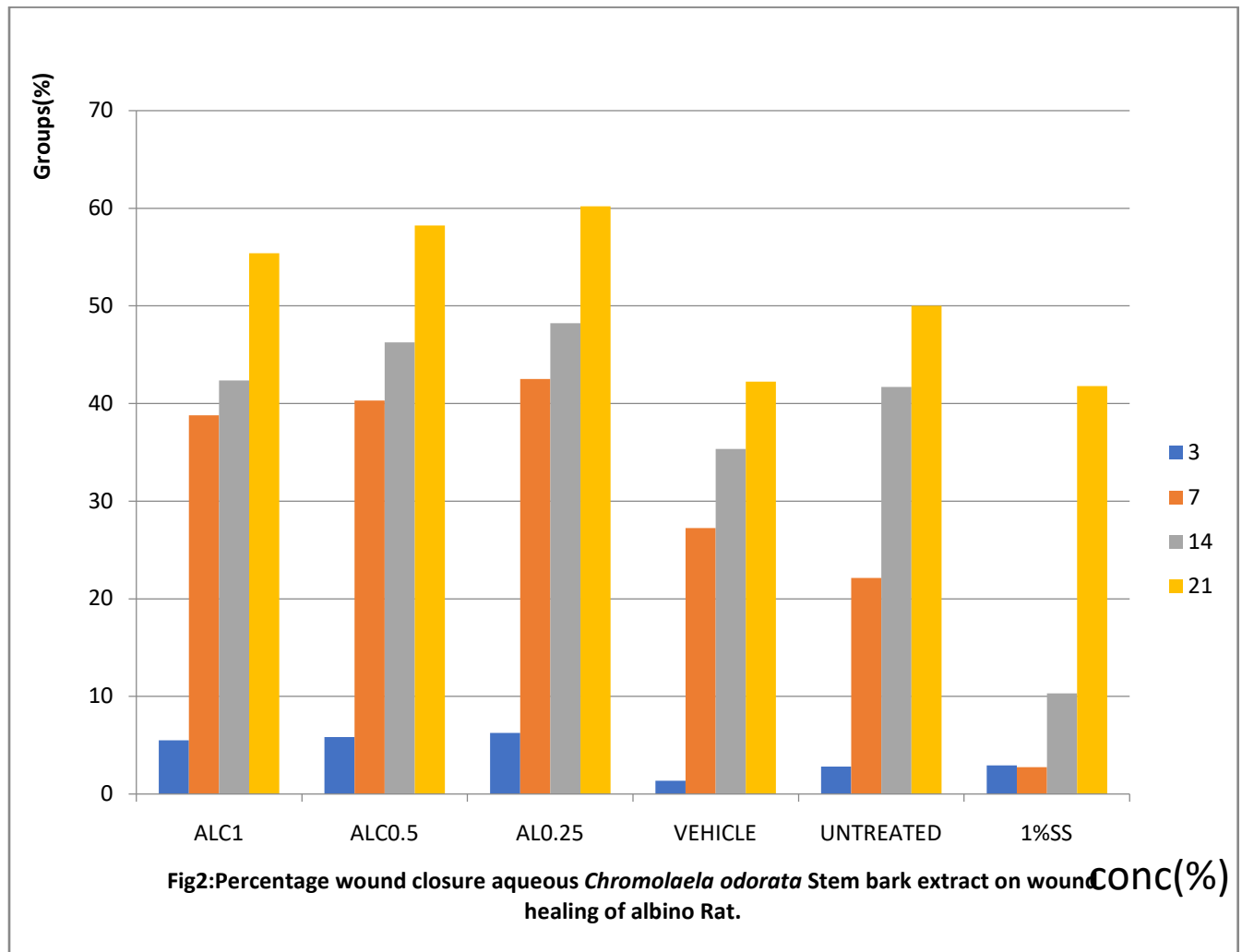


Table.5 .Percentage wound closure of aqueous *T.procumbens* stembark extract on wound healing of albino rat

DAYS	ATP1	ATP0.5%	ATP0.25%	VEHICLE	UNTREAD	1%SS
3	7.49±1.39 <sup>a</sup>	5.35±1.52 <sup>a</sup>	6.45±1.62 <sup>a</sup>	1.37±1.02 <sup>a</sup>	2.82±0.28 <sup>a</sup>	2.93±0.2
7	38.81±2.30	42.3±2.40 <sup>b</sup>	40.5±2.43 <sup>b</sup>	27.25±1.32 <sup>b</sup>	22.12±0.82 <sup>b</sup>	2.75±0.94 <sup>b</sup>
14	40.35±2.35 <sup>c</sup>	42.25±2.45 <sup>c</sup>	42.23±2.48 <sup>c</sup>	35.35±1.36 <sup>c</sup>	41.7±1.6	10.32±0.96 <sup>c</sup>
21	52.4±2.35 <sup>a</sup>	55.25±2.50 <sup>a</sup>	65.2±2.70 <sup>a</sup>	42.25±2.00 <sup>a</sup>	50.0±1.88 <sup>a</sup>	41.78±1.60 <sup>a</sup>

Values with superscript are significant (P<0.05)

**KEYS**

ATP=Aqueous stembark *Tridax procumbens* concentration

**Table.6 .Effect of an Ethanolic leaf extract of *C.odorata* on lipids profile in Streptozotocin-induced**

Treatments	Total Cholesterol(mg/dl)	Triglyceride	HDL	LDL
Normal Control	61.16 ±1.07	70.25±0.81	28.51±0.44	28.391±0.1
Diabetic Control	110.04±0.78	132.12±0.08	16.14 ±0.16	80.64±0.18
<i>C.odorata</i> (250mg/kg)	69.46±0.83	87.25±0.7	25.38±0.03	36.4±1.0
<i>C.odorata</i> (500mg/kg)	65.98±0.55	87.17±0.46	23.37±0.13	39.52±3.6
Glibenclamide (0.25mg)	68.6±0.1	85.12±1.12	26.36±0.33	35.33±0.91

**Table7.Effect of an Aqueou Stembark extract of *C.odorata* on lipids profile in Streptozotocin-induced diabeties**

Treatments	Total Cholesterol(mg/dl)	Triglyceride	HDL	LDL	Days
Diabetic Control	110.04±0.78	132.12±0.08	16.14 ±0.16	80.64±0.18	
<i>C.odorata</i> (250mg/kg)	69.32±0.83	84.05±0.7	26.33±0.03	36.4±1.0	
<i>C.odorata</i> (500mg/kg)	64.93±0.55	87.17±0.46	23.37±0.13	34.52±3.6	
Glibenclamide (0.25mg)	68.4±0.1	84.12±1.12	26.32±0.33	34.33±0.9	
Normal Control	61.16 ±1.07	70.25±0.81	28.51±0.44	28.391±0.1	

**Table 8. Effect of Ethanolic *T.procumbens* leaf extract on body weight of induced diabetic rats**

Groups/Weights(Kg)	1 <sup>st</sup>	7	14	21	28 days
Normal Control	170.2	178.4	180.4	182.6	183.7
Diabetic Control	175.6	168.4	150.2	145.4	139.6
<i>T.procumbens</i> (200mg/kg)	143.4	135.3	120.2	118.2	108.5
<i>T.procumbens</i> (500mg/kg)	142.1	132.4	124.1	119.3	107.6
Glibenclamide(0.25mg)	173.5	174.6	175.6	179.1	182.3

## DISCUSSION

The antibacterial and antiglycemic profile of plant is desirable not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of economic materials. In addition, the knowledge of the constituents of plant would further be invaluable in discovering the actual value for medical remedy. (Iwu 1986, Tiwari and Rao 2002).

The antibacterial activity of the extract is further supported by the MIC result which showed that *T. procumbens* stem bark extract exhibited bacteriostatic activity against the organisms at the concentrations shown in Table 1. The corresponding MBCs of 10 mg/ml, 8 mg/ml, 7 mg/ml and 9 mg/ml in *C. odorata* leaf extract, respectively, further suggest that the extract was bacteriostatic at lower concentrations but bactericidal at higher concentrations Table 2.

In the present study, *in vitro* antimicrobial study revealed that *C. odorata* leaf and stem bark extract inhibited the growth of bacteria - *B. subtilis*, *E. coli*, *S. aureus* and *P. aeruginosa*. This indicates that the plant extract exhibited antibacterial activities. The fact that there was decreased antimicrobial activity with decrease in concentration of the extract, suggests that the antimicrobial effect of the *C. odorata* and *T. procumbens* extract are concentration-dependent. The 100 mg/ml concentration of the extract gave the highest (IZD) for the 4 inhibited organisms (*B. subtilis* [25 mm], *S. aureus* [35 mm], and *P. aeruginosa* [29 mm]), for leaf extract and (30mm), (29mm) and (29mm) which suggests that *C. odorata* leaf and stem bark extract exhibits the best antimicrobial effect at this (100 mg/ml) concentration. The 50 mg/ml concentration gave IZD for *B. subtilis* (23 mm), *S. aureus* (32 mm), and *P. aeruginosa* (25 mm), which is not significant  $p < 0.05$ . The IZD for each organism at 50-6.25 mg/ml concentration

is lower when compared with those of 100 mg/ml concentration in all the plant extracts. This result may suggest that the more the concentration of phytochemicals responsible for the antimicrobial activity, the better the effect. The fact that none of the tested concentrations of the extract inhibited the growth of *P. aeruginosa* which also indicates that *C. odorata* does exhibit anti-pseudomonal effect. This suggests that the *P. aeruginosa* isolate used was not resistant to the extract. **Table 3,4**

The wound contraction of the ethanolic leaf and stem bark extract of *C. odorata* and *T. procumbens* on wound healing shows that the concentration of the extract is directly proportional to the time of healing. Both the plant were able to close the wound at lower concentration ranging from 1%, 0.5% and 0.25% respectively which indicate significant  $P < 0.05$  and is in agreement with the report of Akinpelu *et al.*, (2004) in *Dacryoides edulis*. **Fig 1,2.**

The wound contraction of *C. odorata* and *T. procumbens* extract shows that the concentration of the extract has more impact than the control, which is in agreement with the previous work of (Essien and Okoye, 2009) and it show significant different  $P < 0.05$  **Table 5,6.**

The ethanolic leaf extract of *T. procumbens* on the lipid profile in streptozotocin-induced diabetic rat for total Cholesterol, triglyceride, HDL and LDL. The triglyceride was  $82.05 \pm 0.7$  for group 1 treated with 250mg/kg of *T. procumbens*, followed by the concentration of 500mg/kg when compared with glibenclamide which also had the same trend as  $85.12 \pm 1.12$ , while HDL had  $25.36 \pm 0.13$  and  $23.46 \pm 0.13$  for both the concentration of 250mg/kg and 500mg/kg respectively. **Table 17a.** In the aqueous stem bark of *T. procumbens* on lipid profile, the triglyceride was  $85.17 \pm 0.46$  on *T. procumbens* (500mg/kg) followed by (250mg/kg) of the same triglyceride, while LDL was  $34.4 \pm 1.0$  as least for *T. procumbens* 250mg/kg concentration, the diabetic control was  $132.12 \pm 0.08$  in triglyceride against HDL of glibenclamide (0.25mg)  $35.33 \pm 0.91$  mg/dl respectively.

Also in *C. odorata* leaf extract, triglyceride was (87.17) for 500mg/kg followed by ( $84.05 \pm 0.7$ ) of the same lipid while HDL was least ( $23.37 \pm 0.13$  mg/dl), **Table 17c.** The same trends goes for the stem bark extract.

The effect of ethanolic leaf extract of *C. odorata* on lipid profile, Streptozotocin – induced diabetic rat are shown in **Table 7.** *C. odorata* (250mg/kg) had ( $87.25 \pm 0.07$ ) as highest for triglyceride while HDL was ( $23.37 \pm 0.13$ ) as least. Glibenclamide (0.25mg) was  $85.12 \pm 1.12$  in Triglyceride followed  $68.6 \pm 0.1$  mg/dl for total Cholesterol, while LDL had  $35.33 \pm 0.91$  mg/dl. Also Triglyceride was highest for *C. odorata* extract of the concentration 250mg/kg, while HDL was least  $23.37 \pm 0.13$  mg/dl respectively as presented in **Table 7.**

The aqueous leaf extract of *C. odorata* and the ethanolic stem bark extract had the same trends of values ranging from 144.6-143.6g for (500mg/kg), 140.2-140.4g for 200mg/kg on the 1<sup>st</sup> day of treatment respectively, **Table 8**



The blood glucose level for ethanolic leaf extract of *C.odorata* are presented on table 19a. On the 3<sup>rd</sup> day of treatment *C.odorata* (250mg/kg) had 265.55±2.77mg/dl as highest, while 159.5±1.97mg/dl was observed on the 21<sup>st</sup> day as least, **Table 8**

All other extracts showed the same trend of values, ranging from the 3<sup>rd</sup> day to 7<sup>th</sup> day of the treatment for *C.odorata* 250mg/kg and 500mg/kg respectively.

### CONCLUSION

In this study, the plant extracts of *C. odorata* and *T. procumbens* contains some antibacterial and antiglycemic profile, hence the need to exploit the potentials of this plant extract in this area of traditional medicine and pharmaceutical industries. The extracts also have some healing potentials when administered to the models without any toxic effect. However, *C.odorata* and *T.procumbens* extracts have antidiabetic potentials.

### ACKNOWLEDGEMENT

I thank the peer academic group for providing a platform for this research journal, also my thanks go to the Salvation Foundation for assisting us to publish this article of journal. Finally we appreciate God whose inspiration has been so immense in us.

### REFERENCES

1. **Agarwal PK & Morris H L. (2009).** Medicine and the future- healing-chronic wounds *Britain Medical Journal* 324: 160-163.
2. **Akinpelu D A, Killedar S G, & Adnaik R.S.(2008)** .Anti-diabetic activity of leaf extract of *Tridax procumbens*. *Int J Green Pharm* 2(2):126-128.
3. **Ciswanathan R, Shivashangari K S & Devak T.(2005)** Hepatoprotective activity of *Tridax procumbens* against D- galactosamine/ lipopolysaccharide-induced hepatitis in rats. *J Ethnopharmacol* 101: 55-60.
4. **Oladunmoye M.K.(2006)** Immunomodulatory Effects of Ethanolic Extract of *Tridax procumbens* on Swiss Albino Rats Orogastrically Dosed with *Pseudomonas aeruginosa* (NCIB 950). *Int J Trop Med* (4):152-155.
5. **Nia R, Paper DH, Essien E.E, Oladimeji OH, Iyadi KC, & Franz G.(2003)** Investigation into *in-vitro* radical scavenging and *in-vivo* anti-inflammatory potential of *Tridax procumbens*. *Niger J Physiol Sci* 18: 39-43.

6. **Fareek H, Sharma S, Khajja BS, Jain K,& Jain GC (2009).** Evaluation of hypoglycemic and anti-hyperglycemic potential of *Tridax procumbens* (Linn.). *BMC Comp Alt Med* 9: 48.
7. **Salahdeen H M, Yemitan O K,& Alada A R.(2004)** Effect of aqueous leaf extract of *Tridax procumbens* on blood pressure and heart rate in rats. *Afr J Biomed Res* 7:27-29.
8. **Mahato R.B, &Chaudhary R.P.(2005).** Ethnomedical study and antibacterial activities of Selected plants of the Palpa District, Nepal. *Scientific World* 3(3): 26-31.
9. **Rajkumar S&, Jebanesan A. (2007)** Repellent activity of selected plant essential oils against the malarial fever mosquito *Anopheles stephensi*. *Trop Biomed* 24(2): 71-75.
  
10. **Saxena V K, Albert S.(2005)**  $\beta$ -Sitosterol-3-O- $\beta$ -D- xylopyranoside from the flowers of *Tridax procumbens* Linn. *J Chem Sci* 117(3): 263-266.
11. **Bhalerao SA,& Kelkar TS. (2012)** Phytochemical and pharmacological potential of *Tridax procumbens* Linn. *Int J Adv Biol Res* 2(3): 392-395.
12. **Nalwaya N, Pokharna G, Deb L,& Jain N K.(2009).** Wound healing activity of latex of *Calotropis gigantea*. *Int J Pharm Pharmaceut Sci* 1(1): 176-181.
13. **Kolawole C, Patil MB, Kumar R,& Patil S. (2009)** Preliminary phytochemical investigation and wound healing activity of *Allium cepa* linn (liliaceae). *Int J Pharm Pharmaceut Sci* 2(2):167-175.
14. **Tayade PM, Borde SN, Chandrasekar N, Jagtap SA,& Joshi AS (2011)** Evaluation of wound healing properties of *Psoreliya corolifolia* Linn in diabetic rats. *Pharmaco online* 1; 28- 35
15. **Singh A, Gaurav K, Goel S, Khanna HD,& Goel RK.(2009).** Evaluation of wound healing activity of extracts of plantain banana (*Musa sapientum* var. paradisiaca) in rats. *Indian J Exp Biol* 47:32-40