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# Acute toxicity study and antibacterial activity of aqueous and methanol leaf extract of *Crotolaria retusa* Linn. against multidrug resistant bacterial isolates

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ABSTRACT

**Background**: Dealing with drug-resistant pathogens is a serious health care challenge world-over, especially in developing countries. Effective first-line antibacterial agents are usually expensive, have side effects and are not readily available especially in most of Africa's rural areas where plant-based medicinal products such as *Crotolaria retusa* Linn. are commonly used for treatment of infectious diseases among others.

**Methods:** The median lethal dose was investigated using Lorke's method. The antibacterial activity of the aqueous and methanol leaf extracts of *C. retusa* L. (rattle box) against some multidrug resistant (MDR) bacterial isolates were determined using the agar well diffusion method.

**Results:** The acute oral toxicity studies of both the aqueous and methanol extracts of *C. retusa* revealed that the $LD_{50}$ values were 2236.1 mg/kg and 1264.9 mg/kg, respectively. The aqueous and methanol leaf extracts demonstrated a concentration-dependent antibacterial activity against MDR clinical isolates of *S. aureus, K.pneumonia, E. coli* and *P. aeruginosa*. The results of minimum inhibitory concentration (MIC) of the extracts against the four MDR isolates ranged from 15.6 to 250 mg/ml while the minimum bactericidal concentration (MBC) ranged from 62.5 to 250 mg/ml..

**Conclusion:** The plant extracts used in this study significantly inhibited the selected microorganisms with MIC and MBC values comparable to ciprofloxacin and gentamicin. This suggests the plant has potential as a source of effective, relatively safer and affordable antibacterial agent.

## Introduction

Medicinal plants have a pivotal role in the primary healthcare and form the basis of traditional systems of medicines. Plants usually serve as sources of food, spices, flavours, fragrance, and medicines. Since ancient times, plants are being used to treat many diseases or ailments viz: infectious diseases, inflammatory disorders, skin diseases, etc.[1]. Infectious diseases are the major causes of death across the world[2]. Infections due to pathogenic micro-organisms cause a severe concern to human health, hence constitute an important area of drug discovery[2]. Increasing cases of drug resistance, and unwanted side effects of existing antibiotics and the reappearance of earlier known infections compel the need for new, safe, and effective antimicrobial agents[3]. Therefore, current scientific investigations are aimed at identifying new leads from the plant sources possessing significant antibacterial activity[4].

*Crotalaria retusa* Linn., [common names: devil-bean, rattleweed, local names include; fara birana (in Hausa), Akedimwo (in Igbo), Korupo (in Yoruba) and Birijibei (in Fulani)] is a member of the genus Crotalaria and family Leguminosae papilionoideae, cultivated in humid tropical areas but can also grow in semi-arid conditions with average annual rainfall as low as 200 mm. *C. retusa* is common in disturbed areas, roadsides, waste grounds, agricultural lands, pastures, urban areas (i.e., gardens and parks) and grasslands, where it grows as a weed[5-6]. *C. retusa* contains phytochemicals such as glycosides, anthracene glycoside, tannins, sterols, phenolics, flavonoids, alkaloids, saponins and triterpenoids[7]. All parts of the *C. retusa* plant are used in folklore medicine in many countries for treatment of numerous diseases. The whole plant infusion is used to treat skin infections while the roots are used to treat against haemorrhagic cough[8]. The leaves and flowers are used to treat fever and lung diseases[9-10]. The powdered seeds are indicated in leprosy, flatulence and act as an analgesic against the pain of scorpion stings and snake venom[10]. Previous studies reported pharmacological properties of *C. retusa* such as antioxidant, anti-proliferative, antibacterial, thrombolytic, anti-inflammatory and antinociceptive activities[11-13]. *C. retusa* is a pyrrolizidine toxic plant. Its abundant alkaloid monocrotaline is a hepatotoxic compound which is responsible for many intoxications caused by this plant[8].

In recent years, drug resistance to human pathogenic bacteria has been commonly reported all over the world and is challenging in developing countries like Nigeria. The increasing prevalence of multidrug resistant strains of bacteria such *P. aeroginosa, E. coli* and *S. aureus* and the recent appearance of strains with reduced susceptibility to antibiotics raises the spectre of untreatable bacterial infections and add urgency to the search for new infection-fighting strategies. The use of plant extracts for medicinal treatment has become popular, especially among the rural dwellers in Africa. *C. retusa* is frequently used as food, spices, flavours and for its medicinal purpose. Despite this, the antimicrobial activity of the plant is yet to be adequately evaluated, especially against multi-drug resistant (MDR) isolates. Hence, this study was designed to assess the antibacterial activity of aqueous and methanol leaves extracts of *C. retusa* against MDR isolates.

## Materials and Methods

### Reagents

Nutrient agar, antimicrobial sensitivity discs, Sterile distilled water, Peptone water, Methanol, Tetraoxosulphate (vi) acid, Acetic anhydride, Ethanol, Hydrochloride, Fehling's solution, Crystal of resorcinol, Dragendroff's reagent, Meyer's reagent and Wager's reagent.

### The Bacterial Clinical Isolates

Clinical isolates of *Escherichia coli, Staphylococcus aureus*, *Pseudomonas aeroginosa* and *Klebsiella pneumonia* used for this study were obtained from patients' samples analyzed in the Department of Microbiology, UMTH. The isolates were identified via morphological features on culture plates and biochemical analysis. The antibiogram of each isolate was determined as described[14]. Briefly, each of the isolate was suspended in autoclaved peptone water using a sterilized wire loop to match the turbidity of Mc Farland standard. The Nutrient agar was inoculated with the suspension of each of the isolates on separate plate by Lawn culture technique. Using a sterile forceps, the appropriate antimicrobial discs were evenly distributed on the inoculated agar plates and lightly pressed. The plates were then inverted and incubated aerobically at 37°C for 24 hours. After overnight incubation, the size of the zones of inhibition were measured using meter rule and interpreted as sensitive or resistant. Selected multidrug-resistant, *K. pneumoniae*, and *E. coli* were screened for their resistance to more than two different classes of antibiotics following the disk diffusion method protocol (as in the CLSI guidelines) and WHO recommendations[15].

### Collection and Authentication of *C. retusa*

Fresh leaves of *C. retusa* were collected from the premises of the Center for Renal and Urology Research, UMTH in December 2018 and authenticated by a plant taxonomist in

Department of Biological Science, Faculty of Science, University of Maiduguri, Nigeria. A voucher specimen with reference number 2018/001 was deposited in the Pharmacology Laboratory, Department of Clinical Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, College of Medical Sciences, University of Maiduguri, Nigeria.

**Preparation of the Leaf Extracts**

The leaves of *C. retusa* were dried in shade for about a week. The dried leaves were reduced to powdered form. The powdered material (200 g) was placed in two beakers containing 3 L of distilled and 150 mL of methanol. The solutions were allowed to stay for 24 hours with periodic shaking. The solutions were filtered, and the filtrates were allowed to evaporate on standing. The percentage yields were determined for each solvent extract using the formula below[16]:

Percentage (%) yield = *final weight* (*g*) / *initial weight* (*g*) × 100

**Phytochemical screening of the leaf extracts**

**Tests for Cardiac Glycosides**

**Salkowski's Test**

Each of the extracts (0.5 g) was dissolved in 2 mL of chloroform, tetraoxosulphate (vi) acid ($H_2SO_4$) was then carefully added by the side of the test tube to form a lower layer. Appearance of a reddish- brown or yellow colour at the interface was an indication of the presence of steroidal ring[17].

**Liebermann-Burchards's Test**

To 0.5 g of each extract, 2 mL of acetic anhydride was added, dissolved and allowed to cool well in ice. Concentrated $H_2SO_4$ was added carefully and colour development from violet to blue or bluish green was an indication of the presence of a steroidal ring[17].

**Test for Terpenoids**

A little of the extract was dissolved in ethanol and 1 mL of acetic anhydride was added, followed by the addition of concentrated $H_2SO_4$. A colour change from pink to violet indicates the presence of terpenoids[17].

**Test for Flavonoids**

Each of the extracts (0.5 g) was dissolved in ethanol, warmed, and then filtered. Three pieces of magnesium chips were then added to the filtrates with few drops of concentrated HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids[18].

**Tests for Carbohydrates**

**Molisch's Test**

A few drops of Molisch's reagents were added to each of the extracts that was dissolved in distilled water. Followed by addition of 1 mL of concentrated $H_2SO_4$ by the side of the test tube, so that the acid will form a layer beneath the aqueous layer. The mixture was allowed to stay for two minutes and then diluted with 5 mL of distilled water. An interface of red violet colour between the two layers indicates a positive test[19].

**Barfoed's Test (General Test for Monosacccharides)**

One milliliter (1 mL) of extract solution obtained from the Molisch's test above was mixed with 1 mL of Barfoed's reagent in a test tube and then heated on a water bath for 2 minutes. Red precipitate of cuprous oxide indicates the presence of monosaccharides like fructose and glucose[19].

**Fehling's Test (Standard Test for Free Reducing Sugar)**

Two milliliter (2 mL) of the extract solution was heated with 5 mL of equal volume of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide is an indication of a reduced sugar like fructose and glucose[19].

**Test for Combined Reducing Sugar**

Each of the extracts (0.2 g) was hydrolyzed by boiling with 5 mL of dilute hydrochloric acid and the resulting solution neutralized with sodium hydroxide solution. Few drops of Fehling's solution were added to it and then heated on a water bath for two minutes. Formation of a reddish-brown precipitate of cuprous oxide indicates the presence of combined reducing sugars[19].

**Selivanoff's Test (Standard Test for Ketoses)**

Few crystals of resorcinol and 2 mL of concentrated hydrochloric acid (HCl) were added to 2 mL of extract already prepared and boiled for 5 minutes. A reddish coloration indicates the presence of ketose like fructose[19].

**Test for Alkaloids**

Each 0.5 g of the extracts was stirred with 5 mL of 1% aqueous hydrochloric acid (HCl) on water bath and filtered. Of the filtrate, 3 mL was taken and divided into 3 portions in a test tube. To the first portion, a few drops of Dragendroff's reagents were added; the occurrence of an orange-red

precipitate indicates the presence of alkaloids. To the second portion, 1 mL of the Meyer's reagent was added and the appearance of a buff-coloured precipitate indicates the presence of alkaloids. To the third portion, a few drops of Wagner's reagent was added, and a dark-brown precipitate indicates the presence of alkaloids[20].

**Acute Toxicity Test**

**Experimental Animals**

Twenty-four albino rats of both sexes weighing between 120-160g were obtained from the Animal House of the Department of Clinical Pharmacology and Therapeutics, University of Maiduguri Teaching Hospital, Nigeria. The animals were fed on commercial diet (Vital feed, Jos Nigeria) and water *ad libitum* throughout the study period. They were acclimatized for 2 weeks before the study.

**Experimental Design**

The acute toxicity of the *C. retusa* leaf extracts were conducted in accordance with the Lorke's method[21]. The study was conducted in two phases using a total of twenty-four rats. In the first phase, eighteen rats were divided into 6 groups of 3 rats each. Groups 1, 2, 3, 4, 5 and 6 animals received 10, 100 and 1000 mg/kg body weight (b.w.) of aqueous (Groups 1-3) and methanol (Group 4-6) extracts orally, respectively. The rats were closely monitored for the first 24 hours periodically and then daily for 14 days. Untoward reactions such as death, writhing, diarrhoea etc. were looked out for in each group. In the second phase, 1600, 2900 and 5000 mg/kg b.w. each of the aqueous and methanol extracts were administered to three rats (one rat per dose) through the oral route. All animals were observed frequently on the day of treatment and surviving animals were monitored daily for 2 weeks for signs of acute toxicity. $LD_{50}$ was determined for each extract using the formula: $LD_{50} = \sqrt{a \times b}$

Where a = Least dose that killed the animal

b = Highest dose that did not kill the animal

**Determination of the antibacterial activity of the extracts**

The agar well diffusion method was employed for the determination of the antibacterial activity of the extracts as previously described[22-23]. Petri dish containing 20 mL nutrient agar was seeded with 24 hours culture of bacterial strains (*S. aureus*, *P. aeroginosa*, *K. pneumonia* and *E. coli*). Wells about 4 mm in diameter were made using a sterilized cork's borer aseptically. The wells were spaced by at least 30 mm from each other and 4 mm from the edge. A fixed volume of the extracts of varying concentration (50, 100, 200, 400 mg/ml) was introduced into the wells. It was allowed to stand at room temperature for 15 minutes before incubation at 37°C for 24 hours. The clear zones of inhibition were measured using a meter rule.

**Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)**

The MIC value of the extracts were determined as the lowest concentration that completely inhibited bacterial growth after 48 hours of incubation at 37°C. For the determination of MBC, a portion of the liquid (5 µL) from each plates well with no growth was taken and incubated 37°C for 24 hours. The lowest concentration that gave no visible bacterial growth after sub-culturing was taken as MBC. Positive and negative cultures were also prepared.

**Data Analysis**

The data generated from the study were analysed using IBM (USA) Statistical Package for Social Sciences version 21 software[24]. Results are presented in text and Tables and data are expressed as percentages, mean and standard deviation.

**Results**

**Characteristics of *C. retusa* leaf extrac**

The yield of the aqueous and methanol leaf extract of *C. retusa* appeared dark brown and dark green, respectively. The assessment of the percentage yields of the extracts indicated that the extracts yielded different values of 41.7% for the aqueous extract and 10.5% for the methanol extract [Table 1].

**Phytochemical Constituents of *C. retusa* leaf extracts**

In the present study, phytochemical profiling of *C. retusa* was performed for both the aqueous and methanol leaf extracts. The result showed differences in the phytochemical constituents of both extracts as presented in Table 2. Cardiac glycosides, alkaloids, flavanoids, terpenoids and tannins were found to be present in both extracts.

**Acute Toxicity of *C. retusa* leaf extracts**

The results of the oral acute toxicity test of both the aqueous and methanol leaf extracts are shown in Tables 3 and 4, respectively. The oral $LD_{50}$ of the aqueous and methanol extracts were determined to be 2236.1 mg/kg and 1264.9 mg/kg, respectively.

**Antibiogram of the Bacterial Clinical Isolates**

The profile of the four different bacterial isolates obtained from patients seen at the Department of Microbiology, University of Maiduguri Teaching Hospital (UMTH), Nigeria are presented in Table 5. *E. coli*, *P. aeruginosa*, *S. aureus* and *K. pneumonia* were resistant to 8, 6, 6 and 7 antibacterial, respectively.

**Antibacterial Activities of *C. retusa* leaf extracts**

Table 6 and 7 present the zone of inhibition indicating the antibacterial activities of varying concentrations (50, 100, 200 and 400 mg/mL) of the extracts and the standard antibacterial drugs (ciprofloxacin and gentamycin). The results revealed that the aqueous and methanol extracts of *C. retusa* exhibited distinct zones of inhibition at all concentrations i.e 50, 100, 200 and 400 mg/mL towards *E.coli* and *K. pneumonia*. The methanol extract gave the widest zone of inhibition against *K. pneumonia* (23.25±0.43 mg/mL) at concentration of 400 mg/mL. Similarly, the aqueous extract gave the highest inhibitory zone against *K. pneumonia* (22.5±0.50 mg/mL) at 400 mg/mL.

**Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)**

Most of the commercial antimicrobial agents used in the work revealed a verity of sensitivity and resistance against the used microorganisms as described in Table 5. The minimum inhibitory concentration (MIC) of aqueous and methanol extracts against the multidrug resistant (MDR) strains of bacteria ranged from 15.6 to 250 mg/mL while the minimum bactericidal concentration (MBC) of the aqueous and methanol extracts against the multidrug resistant (MDR) strain of bacteria ranged from 62.5 to 250 mg/mL. *E. coli* had MIC value of 62.5 mg/mL for the aqueous extract and 15.6 mg/mL for the methanol extract, while MBC value of the aqueous and methanol extracts was 250 and 125 mg/mL, respectively. *S. aureus* had MIC value of 62.5 mg/mL for the aqueous extract and 31.25 mg/mL for the methanol extract, while the MBC values of the aqueous and methanol extracts were 125 and 250 mg/mL in the same order. The MIC values for *P. aeruginosa* at examination of the aqueous and methanol extracts were 125 and 62.5 mg/mL, while the MBC values for the same organism for the aqueous and methanol extracts were 125 and 250 mg/mL, respectively. The MIC of the aqueous and methanol extracts at examination against *K. pneumoniae* were 62.5 and 31.25 mg/mL, while MBC for the same organism for both extracts was 62.5 mg/mL (Tables 8-11).

**Table 1: Percentage yield of the aqueous and methanol leaf extracts of *C. retusa***

| Extract | Initial Weight (g) | Yield (g) | Percentage yield (%) |
|---|---|---|---|
| Aqueous | 200 | 83.34 | 41.7 |
| Methanol | 200 | 21.00 | 10.5 |

**Table 2: Phytochemical profile of *C. retusa* extracts**

| TESTS | Aqueous extract | Methanolic extract |
|---|---|---|
| Cardiac glycoside | + | - |
| Sakowski's Test | + | - |
| Lieberman Test | + | + |
| Cardenolite | + | - |
| Alkaloid | + | + |
| Flavonoid | + | + |
| Terpenoid | + | + |
| Free Anthraquinone | - | - |
| Combined anthraquinone | - | - |
| Saponin glycoside | - | - |
| Carbohydrate | - | + |
| Phlobatamin | - | - |
| Tanin | + | + |

Key: + Present
- Absent

**Table 3: The oral acute toxicity of *C. retusa* aqueous extract**

| Dose (mg/kg) | Number of animal used | Number of death | % of death |
|---|---|---|---|
| Phase I | | | |
| 10 | 3 | 0 | 0/3 |
| 100 | 3 | 0 | 0/3 |
| 1000 | 3 | 0 | 0/3 |
| Phase II | | | |
| 1600 | 1 | 0 | 1/1 |
| 2900 | 1 | 0 | 0/1 |
| 5000 | 1 | 1 | 0/1 |

$LD_{50}=\sqrt{5000\times1000} = 2236.1$ mg/kg

**Table 4: The oral acute toxicity ($LD_{50}$) of *C. retusa* methanol extract**

| Dose (mg/kg) | Number of animal used | Number of death | % of death |
|---|---|---|---|
| Phase I | | | |
| 10 | 3 | 0 | 0/3 |
| 100 | 3 | 0 | 0/3 |
| 1000 | 3 | 0 | 0/3 |
| Phase II | | | |
| 1600 | 1 | 1 | 1/1 |
| 2900 | 1 | 1 | 1/1 |
| 5000 | 1 | 1 | 1/1 |

$LD_{50}= \sqrt{1600} \times 1000 = 1264.9$ mg/kg

**Table 5: Antibiogram of the Bacterial Clinical Isolates**

| Isolate | Antibiogram | |
|---|---|---|
| | Resistance | Sensitive |
| *E. coli* | Amx, Cn, Cep, CAZ, FEP, Au, ATM, TZP | S |
| *P. aeroginosa* | Amx, DA, CPR, FEP, AU, TZP | S, Cpx |
| *S. aureus* | Amx, Etp, DA, CPR, Au, Sxt | Gem, Pef, E, S, Cn, Cpx |
| *K. pneumoniae* | Amx, Ch, Gem, Rd, CPR, Sxt, Au | Cpx, Pef, Na, E |

Ch Chloramphenicol
Cn Gentamycin
Sxt Septrin®
E Erythromycin
DA Clindamycin
Ceftazidime
TZP Tazobactam
Amx Amoxicillin
Rd Rifampicin
Cpx Ciprofloxacin
Etp Ertapenem
CPR Ceporex
FEP Cefepime
Pef Pefloxacin
Pef Pefloxacin
Cep Cephalexin
S Streptomycin
Gem Gemifloxacin
Au Augumentin CAZ
ATM Aztreonam

**Table 6: Zone of inhibition produced by the *C. retusa* aqueous extract**

| Antibiotics and extract concentration (mg/mL) | Zones of Inhibition (mm) | | | |
|---|---|---|---|---|
| | *E. coli* | *S. aureus* | *P. aeruginosa* | *K. pneumonia* |
| Gentamycin | 16.75±0.82 | 30.00±0.42 | 20.00±0.34 | 12.00±0.25 |
| Ciprofloxacin | 00.00±0.00 | 44.00±0.47 | 35.50±0.57 | 18.00±070 |
| 50 | 10.50±0.50 | 10.37±0.40 | 9.16±0.50 | 8.75±0.43 |
| 100 | 12.50±50 | 12.25±0.43 | 11.83±0.37 | 15.75±0.43 |
| 200 | 15.66±0.47 | 14.83±0.68 | 12.75±0.43 | 14.00±0.70 |
| 400 | 17.83±0.68 | 17.66±0.47 | 25.50±0.50 | 22.00±0.50 |

Values are mean ± standard deviation.

**Table 7: Zone of inhibition produced by the *C. retusa* methanol extract**

| Antibiotics and extract concentration (mg/mL) | Zones of Inhibition (mm) | | | |
|---|---|---|---|---|
| | *E. coli* | *S. aureus* | *P. aeruginosa* | *K. pneumonia* |
| Gentamycin | 16.75±0.82 | 30.00±0.42 | 20.00±0.34 | 12.00±0.25 |
| Ciprofloxacin | 00.00±0.00 | 44.00±0.47 | 35.50±0.57 | 18.00±070 |
| 50 | 9.00±0.50 | 9.75±0.43 | 8.75±0.43 | 6.25±0.43 |
| 100 | 10.00±0.70 | 9.50±0.50 | 9.25±0.43 | 7.00±0.00 |
| 200 | 16.33±1.97 | 13.00±0.70 | 14.75±0.43 | 15.75±0.82 |
| 400 | 19.25±0.43 | 14.00±0.70 | 18.50±0.50 | 23.25±0.43 |

Values are mean ± standard deviation.

**Table 8: MIC of *C. retusa* aqueous extract**

| Bacterial isolates | Aqueous extracts in mg/mL and MIC in wells (1 - 12) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 250 | 125 | 62.5 | 31.25 | 15.6 | 7.8 | 3.9 | 1.95 | 0.98 | 0.49 | - | - |
| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | (11) | (12) |
| *E. coli* | - | - | A | + | + | + | + | + | + | + | + | - |
| *S. aureus* | - | - | A | + | + | + | + | + | + | + | + | - |
| *P. aeruginosa* | - | A | + | + | + | + | + | + | + | + | + | - |
| *K. pneumoniae* | - | - | A | + | + | + | + | + | + | + | + | - |

Key: - =No turbidity seen + =Turbidity seen α = MIC

NB: Well no. 11 contained 50 uL of MHB and 50 uL of 5 % DMSO and 2 uL of isolates to check for inhibitory activity of DMSO while well no. 12 contained 100 uL of the extract as sterility check.

**Table 9: MIC of *C. retusa* methanol extract**

| Bacterial isolates | Methanol extracts in mg/mL and MIC in wells (1 - 12) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 125 | 62.5 | 31.25 | 15.6 | 7.8 | 3.9 | 1.95 | 0.98 | 0.49 | 0.2 | - | - |
| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | (11) | (12) |
| *E. coli* | - | - | - | α | + | + | + | + | + | + | + | - |
| *S. aureus* | - | - | A | + | + | + | + | + | + | + | + | - |
| *P. aeruginosa* | - | A | + | + | + | + | + | + | + | + | + | - |
| *K. pneumoniae* | - | - | A | + | + | + | + | + | + | + | + | - |

Key: - =No turbidity seen + =Turbidity seen α = MIC

NB: Well no. 11 contained 50 uL of MHB and 50 uL of 5 % DMSO and 2 uL of isolates to check for inhibitory activity of DMSO while well no. 12 contained 100 uL of the extract as sterility check.

**Table 10: MBC of *C. retusa* aqueous extract**

| Bacterial isolates | **Concentration of extract without turbidity sub - cultured on MHA(mg/mL)** | | | |
|---|---|---|---|---|
| | **250** | **125** | **62.5** | **31.25** |
| *E. coli* | B | + | + | **NS** |
| *S. aureus* | **-** | B | + | **NS** |
| *P. aeruginosa* | **-** | B | **NS** | **NS** |
| *K. pneumonia* | **-** | **-** | B | **NS** |

Key: - =No growth on MHA + =Growth on MHA NS=Not sub-cultured B =MBC

**Table 11: MBC of *C. retusa* methanol extract**

| Bacterial isolates | **Concentration of extract without turbidity sub-cultured on MHA (mg/mL)** | | | |
|---|---|---|---|---|
| | **250** | **125** | **62.5** | **31.25** |
| *E. coli* | **-** | B | + | + |
| *S. aureus* | B | + | + | **NS** |
| *P. aeruginosa* | B | + | + | **NS** |
| *K. pneumonia* | **-** | **-** | B | **NS** |

Key: - =No growth on MHA + =Growth on MHA NS=Not sub-cultured B =MB

**Discussion**

Biologically active compounds are usually present in different amount in plants. Extraction methods are used to obtain extracts with high yield and with minimal changes to the functional properties of the extract required[25]. The result of the extraction in this study showed that the solvents used were able to extract some components of the plant, but with difference in yields. Studies have shown that the polarity of solvents affects the solubility of the plant constituents and the chemical nature of each constituent of the plant varies, hence their solubility in each solvent[26]. In this study, the aqueous extract had the highest yield recovery of 41.7% while the methanol extract had the least percentage yield of 10.1%. The difference in yield observed between the aqueous and methanol extracts could be explained by the broad extraction spectrum of water. Studies showed that water is a solvent that has capacity to extract a large group of chemical compounds (small, medium, and large molecules)[27]. This could justify the higher yield obtained in the aqueous extract. The high yield of the plant in this study suggests its high pharmacological potential. The extracts also differ in colour with extracting

solvents which indicates difference in the composition of the extracts and each solvent extract varied in component and quantities.

The phytochemical screening of the *C. retusa* extracts confirmed the presence of flavanoid, alkaloid, tannin, terpenoid, cardiac glycoside, cardenolite and carbohydrate. The presence of flavonoids showed that the plant may possess potent antioxidant activity or free radical scavengers[28]. Medicinal plants with alkaloids have been reported to have antimicrobial and analgesic properties, while those with terpenoids have been reported with antibacterial activity in medicine[29]. Terpenoids have been reported as important source of antimicrobial, antifungal, antiparasitic, antiallergenic and anti-inflammatory agents[30]. Flavanoids, tannins and terpenoids have been reported to possess antimicrobial activities and have been suggested for therapeutic purposes[31]. The therapeutic potential of *C. retusa* extract may be attributed to the presence of these bioactive phytoconstituents.

The importance of toxicity testing is that it enables the evaluation of the possible harmful effects attributed to products. Acute toxicity testing involves the estimation of $LD_{50}$ and it is critical in characteristics of all compounds[32]. In the present study, the acute oral toxicity studies of both the aqueous and methanol extracts of *C. retusa* revealed that the $LD_{50}$ values are 2236.1 mg/kg and 1264.9 mg/kg, respectively, which are within the classified range of 500–5000 mg/kg body weight. Hence, the aqueous and methanol extracts of *C. retusa* can be described as slightly toxic according to Hodge and Sterner toxicity scale[33]. This study revealed that the methanol extract of *C. retusa* leaves is more toxic than the aqueous extract. Administration of equal dose of the methanol and aqueous extracts of *C. retusa* produced different behavioral outcomes e.g., tremor and drowsiness which was more pronounced with the methanol extract.

The antibiogram of the clinical isolates used in the study indicated that some of the isolates such as *E. coli, S. aureus, K. pneumonia* and *P. aeruginosa* are multidrug resistant according to previous work[34]. Evaluation of the antibacterial activities of *C. retusa* showed that both the aqueous and methanol leaf extracts have good activities against the multidrug resistant bacteria tested (*S. aureus, E. coli, P. aeruginosa* and *K. pneumonia*).

In the present study, the antibacterial activities of the aqueous and methanol leaf extracts of *C. retusa* were evaluated using Gram-negative and Gram-positive organism. Both extracts produced similar effect against *S. aureus* indicating similarity in some phytoconstituents. Similarly, the effect of both the aqueous and methanol extracts at 200 and 400 mg/mL are comparable to that produced by the control drug gentamicin. The implication of this is that the extracts could serve as a potential source of drug against gentamicin sensitive bacteria such as *S. aureus.* The antibacterial activity demonstrated by the leaf extracts of *C. retusa* against MDR *S. aureus* is like previous studies which demonstrated that *Crotolaria spp.* is active against *S. aureus*[35-36]. It was demonstrated that the ethanol extract of *C. retusa* was active against *S. aureus*[12].

In addition, the aqueous and methanol extracts of *C. retusa* showed similar effect against Gram negative organisms (*K. pneumonia, E. coli* and *P. aeruginosa*), indicating that both solvents were able to extract similar active phytoconstituents. Similarly, the effects of both aqueous and methanol extracts at 400 mg/mL are comparable to that produced by the control drugs (ciprofloxacin and gentamycin). The implication of this is that the extracts could serve as potential source of drugs against ciprofloxacin and gentamicin sensitive bacteria such as *K. pneumonia, E. coli* and *P. aeruginosa.* The antibacterial activity observed against *E. coli* and *P. aeroginosa* in the present study is similar to previous report[12]. Similarly, the present result is in accordance with the previous studies that have shown that *Crotolaria spp.* possess antimicrobial activities against wide range of organisms including *E. coli*[37], *P. aeroginosa*[37-38] and *K. pneumonia*[39].

In general, the results of this research work showed that the methanol extract is more active (had MIC of 31.25 mg/mL) than the aqueous extract (had MIC of 62.5 mg/mL) of *C. retusa* leaf and the antibacterial activity increases with increase in concentration.

## Conclusion

The result of the phytochemical screening of *C. retusa* extracts showed the presence of flavanoid, alkaloid, tannin, terpenoid, cardiac glycoside, cardenolite and carbohydrate. The acute toxicity study showed that *C. retusa* is slightly toxic when administered orally. Also, the aqueous and methanol extracts of *C. retusa* demonstrated a concentration-dependent antibacterial activity against *S. aureus, E. coli, P. aeruginosa* and *K. pneumonia.* This could contribute to effective medicinal use of the plant and provide novel effective therapeutic agent for the treatment of MDR bacterial infection, especially when purified.

## Author Contributions

AOA and STB: conceptualization, methodology, data

curation, formal analysis and writing—original draft, review, and editing. FHA, LMP, SM, JJ and EIB: resources, investigation and supervision. OAS, AOA, STB and LMP: resources, investigation, supervision, and review. All authors contributed to the article and approved the submitted version.

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**Conflicts of Interest**

The authors declare no conflict of interest.

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