



## **Antimicrobial Evaluation of Plant Parts of *Rauwolfia Vomitoria***

**Stephen Chijioke Emencheta<sup>1\*</sup>, Bessie Ifeoma Enweani<sup>2</sup>, Angus Nnamdi Oli<sup>3</sup>,  
Emmanuel Chinedum Ibezim<sup>4</sup> and Ijeoma Eucharía Olaedo Imanyikwa<sup>5</sup>**

<sup>1</sup>*Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmaceutical Sciences,  
University of Nigeria, Nsukka, Nigeria.*

<sup>2</sup>*Department of Medical Laboratory Science, College of Health, Nnamdi Azikiwe University,  
Nnewi Campus, Nigeria.*

<sup>3</sup>*Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmaceutical Sciences,  
Nnamdi Azikiwe University, Awka, Nigeria.*

<sup>4</sup>*Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka,  
Nigeria.*

<sup>5</sup>*Department of Medical Microbiology, Faculty of Clinical Medicine, Enugu State University of Science  
and Technology, Nigeria.*

### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors SCE, BIE and ANO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SCE, BIE and ANO managed the analyses of the study. Authors ECI and IEOL managed the literature searches. All authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/JOCAMR/2020/v12i130197

#### **Editor(s):**

(1) Dr. B. V. Suma, Ramaiah University of Applied Sciences, India.

#### **Reviewers:**

(1) Phatik Tamuli, Gauhati University, India.

(2) B. Premkumar, Tamilnadu Dr. M.G.R Medical University, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/62031>

**Original Research Article**

**Received 18 August 2020**  
**Accepted 22 October 2020**  
**Published 21 November 2020**

### **ABSTRACT**

**Aims:** This study was aimed at evaluating the antimicrobial activities of fractions of *Rauwolfia vomitoria* against some isolates including; *Salmonella typhi*, *Escherichia coli*, *Candida albicans*, *Aspergillus niger*, *Microsporion canis*, and *Trichophyton rubrum*.

**Methodology:** Primary extraction was done on the dried and pulverized plant samples (leaves, barks, and root) using methanol, after which the crude extracts was fractionated using butanol,

\*Corresponding author: E-mail: [stephen.emencheta@unn.edu.ng](mailto:stephen.emencheta@unn.edu.ng);

ethyl acetate, and n-hexane respectively. The samples were duly labelled according to the plant parts and solvents used. Agar diffusion and dilution methods on Mueller-Hinton agar (MHA) and Sabouraud dextrose agar (SDA) were used to determine the minimum inhibitory concentrations (MIC) and minimum bactericidal and fungicidal concentrations (MBC/MFC) respectively. The percentage inhibition diameter growth (PIDG) of the extracts and fractions were also determined.

**Results:** From the results, eleven (11) samples showed inhibitory activity on one or more of the test isolates. Extended spectrum activity was observed with five (5) of the samples, including; RVL ethyl acetate, RVL n-hexane, RVB crude, RVR crude, and RVR ethyl acetate against *S. typhi*, *E. coli*, and *M. canis*. Antifungal activity was observed only against *M. canis*. The ethyl acetate fraction of the leaves (RVLE) and crude methanol extract of the bark (RVBC) gave the least MIC both against *M. canis* at a concentration of 25 mg/ml, while the ethyl acetate fraction of the leaves (RVLE) at 12.5 mg/ml also against *M. canis* gave the least MBC/MFC. Using the inhibition zone diameters (IZD), the obtained PIDG showed apparently that the ethyl acetate fraction of the leaves (RVL ethyl acetate) was the most active against *S. typhi* (28.57%) and *E. coli* (20%).

**Conclusion:** The study lends support to the traditional use of the plant especially in treating gastro intestinal tract and skin infections.

**Keywords:** *Rauwolfia vomitoria*; antimicrobial; methanol; n-hexane; ethyl acetate; butanol.

## 1. INTRODUCTION

Natural medication is employed mostly in the developing world where in many places, they offer a more generally accessible and affordable alternative to conventional drugs [1]. The increased material worth of medical treatment and their strong physiological or chemical effects, contribute to the reasons behind the use of herbal therapy [2]. Many stakeholders including herbal practitioners and drug researchers have consistently looked up to plants to provide treatment to both infectious and non-infectious diseases [3]. The substances that can repress pathogenic microorganisms and have little toxicity to host cells are viewed as candidates for developing new antimicrobials [4]. Additionally, the increasing incidence of drug resistance has evoked the interest in ethnopharmacology. Also, the past few years has demonstrated a rapid rate of plant species extinction, thus the need for studies be done to harness the potentials in these herbs [5].

*Rauwolfia vomitoria* is a shrub that is up to 5 m in height. The stem is glabrous, rigid, and upright. The older sections of the plant do not contain any latex. The branches are whorled and the nodes lumpy and enlarged [6]. It occurs naturally in forests, but where fallow periods are prolonged, it is mostly found in forest regrowth. It is a medicinal plant which is widely distributed in Africa and Asia's humid tropical secondary and low land forests [7]. *Rauwolfia vomitoria* is commonly planted as an ornamental plant and used traditionally in the treatment of various infections. The roots, barks, and leaves are the pieces that are widely used for herbal remedies.

Kutalek and Prinz [8] confirmed that *R. vomitoria* is used against snake bites, nervous disorders and fever. It is used in Ghana and Nigeria as an emetic and purgative substance. Children are treated with the plant for brain cramps, jaundice, and gastrointestinal disorders in the same area. [8] reported that a watery bark solution of *R. vomitoria* can be used to treat parasites infections such as lice and scabies. Reports illustrate that *R. vomitoria* is effective in the treatment of hypertension, impotence, sleeplessness, and nervous disorders [9,10,7], while [11] stated that *R. vomitoria* (Asofeyeje) is good for the prevention of jaundice, fever, diarrhea, dysentery, scabies, mental disorders, worm infestation, and malaria. In Nigeria and other parts of Africa, traditional medicinal practitioners use various parts of the plant in treatment of fever, general weakness, intestinal diseases, and liver problems as stated by [12].

In this study, as reported in their ethnomedicinal use, microorganisms involved in the gastrointestinal tract (GIT) and skin infections were tested against some parts of the plant to investigate the scientific rationale for traditional use.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials

The fresh leaves, barks, and roots of *R. vomitoria* were sourced in September 2016 from Nnewi, Nigeria, latitude 5°58'48.86" and longitude 6°54'30.78". They were identified and authenticated by a Curator in the Department of

Pharmacognosy, Nnamdi Azikiwe University, Nigeria.

## 2.2 Culture Media and Reagents

Culture media used were Nutrient Broth, Mueller-Hinton agar (Oxoid Limited, England) and Sabouraud dextrose agar (Titan Biotech, India). Culture media were prepared according to the instructions of the manufacturers.

Reagents used include McFarland 0.5 turbidity standard (prepared from barium chloride, sulfuric acid and water), sodium hypochlorite solution, and dimethyl sulphoxide (Triveni Chemicals, India).

## 2.3 Microbial Test Isolates

The microorganisms used in the study were isolates of *Escherichia coli* and *Salmonella typhi* stored at 4 – 8°C in Mueller-Hinton Agar slants and four (4) fungal isolates (*Candida albicans*, *Aspergillus niger*, *Trichophyton rubrum*, and *Microsporon canis*). These were clinical isolates previously purified and standardized to McFarland. Their susceptibility to commonly used antibiotics was already established. The isolates were obtained from the Department of Pharmaceutical Microbiology & Biotechnology, Nnamdi Azikiwe University, Awka, Nigeria.

## 2.4 Extraction of Plant Materials

The leaves, barks, and roots of *R. vomitoria* were prepared using the method of [13]. They were dried at room temperature (25°C), pulverized using laboratory mill and stored in a cool dry place. Total extract of each plant material was prepared by mixing with solvent (methanol) in the ratio of 1:10 (plant material/solvents) in a cold maceration system. The plant materials were soaked in 1000 ml solvent in conical flasks for 12 hrs. The extracts were then filtered using Whatman No. 1 filter paper and solvents removed through evaporation under reduced pressure at 45°C using a rotary evaporator (Yamato, USA). The extracts were duly labelled (RVL crude, RVB crude, and RVR crude according to the plant part, were L = leaves, B = barks, and R = roots) and kept in stoppered sample vials at 4°C until needed.

## 2.5 Fractionation of Crude Methanol Extracts

The crude methanol extracts of the different parts of the plant were fractionated using the solvent-

solvent method with n-hexane, ethyl acetate, and butanol in order of increasing polarity. They were duly labelled (RVL butanol, RVL ethyl acetate, RVL n-hexane, RVB butanol, RVB ethyl acetate, RVB n-hexane, RVR butanol, RVR ethyl acetate, RVR n-hexane according to the solvent used and plant part, were L = leaves, B = barks, and R = roots) and kept in stoppered sample vials at 4°C until needed.

## 2.6 Percentage Yield Determination

The percentage yield (% w/w) from all the dried crude methanol extracts was calculated using the following formula:

$$\text{Percentage (\%)} \text{ yield} = \frac{(\text{Weight of extract})100}{\text{Weight of dried plant material}} \quad (1)$$

## 2.7 Preliminary Antimicrobial Assay

The preliminary antimicrobial assay for each of the crude extracts and their respective fractions was carried out using the agar well diffusion assay as described by [14]. The antimicrobial activity of the plant parts (i.e. crude extracts and fractions) was tested against the earlier mentioned standard clinical isolates (two bacterial and four fungal isolates). A 0.5 McFarland standard bacterial/fungal suspension of each of the test isolates was prepared and these formed both the bacterial and fungal stock solutions used in the agar well diffusion assays.

## 2.8 Agar Well Diffusion Assay

The media, Mueller-hinton agar, MHA (Oxoid, USA) and Sabouraud dextrose agar, SDA (Titan Biotech, India), were prepared according to the manufacturers' specifications. After sterilizing, the media were allowed to cool to 50°C and later transferred into 90 mm sterile agar plates and allowed to set. The sterile MHA and SDA plates were inoculated with the test culture from each of the test suspensions, thereafter, 20 ml of the sterile molten agar cooled to 50°C was added to the plate and rocked clockwise and anti-clockwise to ensure even distribution of the test organism. A sterile cork borer was used to make wells (6 mm in diameter) on each of the MHA and SDA plates. Aliquots of 60 µl of the stock concentration (100 mg/ml) of each extract and fraction, reconstituted in DMSO were applied in each of the wells in the culture plates previously seeded with the test organisms. Ciprofloxacin (5 µg) and miconazole (50 µg/ml) served as the

positive controls for the bacteria and fungi respectively, while DMSO served as the negative control. The cultures were incubated at 37°C for 18 - 24 hrs for the bacterial plates and 25 - 27°C for 48 hrs for the fungal plates, respectively. The antimicrobial potential of each extract and fraction was determined by measuring the zone of inhibition around each well (excluding the diameter of the well). For each extract and fraction, three replicates were conducted for each organism. Each extract and fraction was tested against all the bacterial and fungal isolates.

Furthermore, based on the recorded activity, the extracts and fractions that indicated activity were subjected to minimum inhibitory concentration (MIC) and maximum biocidal concentration (MBC) determinations.

## 2.9 Anti-Dermatophytic Activity

The anti-dermatophytic activity was conducted using the method described by [15]. A sterile swab was used to aseptically inoculate each of the fungal suspension (*T. rubrum* and *M. canis*) on the surface of sterilized Sabouraud dextrose agar. The tests were carried out using a stock concentration of 100 mg/ml prepared by dissolving 200 mg of the crude extracts and fractions in 2 ml of DMSO. A well of 6 mm diameter was made in the agar plate then loaded with 60 µl of each of the stock concentration of the crude extracts and fractions of the test samples and incubated at 28 ± 2°C for 15 - 20 days. The inhibition zone diameter was measured in millimeters using a transparent meter rule. The test was conducted in triplicate and results presented as mean. Miconazole 50 µg/ml served as the standard positive control against the *dermatophytic species*.

## 2.10 Determination of Minimum Inhibitory Concentration (MIC)

The MIC was interpreted as the lowest concentration of the test samples (extracts and fractions) that inhibited visible growth. The MICs of the active samples were determined by agar dilution method as described by [15] and [16] for the antimicrobial and anti-dermatophytic activities, respectively. The MIC was determined for the micro-organisms that showed reasonable sensitivity to the test crude extracts and fractions. In this test, a stock solution of the crude extracts and fractions (2,000 mg/ml) was made, then 2-fold serial dilution were done to get graded

dilutions (1000, 500, 250, 125, and 62.5 mg/ml) of each of the crude extracts and fractions. Then 1 ml of each of these concentration was transferred into a sterile Petri dish and properly mixed with 9 ml of molten MHA and SDA, and cooled to 45 - 50°C. After mixing, the final concentrations became 100, 50, 25, 12.5, and 6.25 mg/ml respectively. Finally, the different test isolates were streaked on the solidified agar properly labelled and incubated at 37°C for bacteria and 28 ± 2°C for fungi. The bacteria were incubated for 18 - 24 hours, while *C. albicans* and *A. niger* were incubated for 48 hours and the dermatophytes incubated for 15 - 20 days. Each experiment was performed in triplicate.

## 2.11 Determination of the Minimal Bactericidal, Fungicidal (MBC/MFC) Concentration of the Crude Extracts and Fractions

Plates that showed no visible growth in the MIC test were selected, incubated for additional 48 hours. Thereafter, the plates were examined for signs of microbial growth.

## 2.12 Determination of the Percentage Inhibition Diameter Growth (PIDG)

Following the result of the antimicrobial evaluation, the percentage inhibition diameter growth (PIDG) was determined according to the following equation:

$$\text{PIDG (\%)} = \frac{(\text{Diameter of sample} - \text{Diameter of control})100}{\text{Diameter of control}} \quad (2)$$

## 2.13 Statistical Data Analysis

The results were expressed as mean. Statistical analysis was carried out using one-way analysis of variance (ANOVA) and SPSS (version 20) statistical program. The results were considered significant at  $p < 0.05$ .

# 3. RESULTS AND DISCUSSION

## 3.1 Extraction and Fractionation

The percentage yield of the plant parts of crude methanol extracts is shown in Table 1. The leaves (10.46%) gave the best yield, followed by the bark (6.97%) and then the root (4.48%).

The fractionation of the three (3) crude methanol extracts of the plants components with an

increasing polarity of n-hexane, ethyl acetate, and butanol gave three (3) fractions each of the three (3) secondary solvents; three (3) n-hexane, ethyl acetate, and butanol fractions which gave a total of twelve (12) samples.

**Table 1. The Percentage yield of crude extracts obtained from *Rauwolfia vomitoria***

| Plant part | Percentage yield (%) |
|------------|----------------------|
| Leaves     | 10.46                |
| Barks      | 6.97                 |
| Root       | 4.48                 |

### 3.2 Antimicrobial Activity of *Rauwolfia vomitoria* Extracts and Fractions against the Test Isolates

The samples of *R. vomitoria* showed different IZDs in the range of 2 – 7 mm (Fig. 1). In at least one or more of the pathogenic microorganisms tested, eleven (11) of the twelve (12) crude methanol extracts and fractions of the plant components showed inhibitory activity. No activity against any of the tested organisms was shown only by the butanol fraction of the leaves (RVL butanol). The result showed that five (5) of the samples (RVL ethyl acetate, RVL n-hexane, RVB crude, RVR crude, and RVR ethyl acetate) showed extended spectrum activity against three (3) of the test organisms (*S. typhi*, *E. coli*, and *M. canis*).

Ciprofloxacin (5 µg/mL) was active as a control against the bacterial isolates with a mean diameter of the inhibition zone (IZD) of 7 mm versus *S. typhi* and 5 mm versus *E. coli*. For fungal isolates, the miconazole (50 µg/ml) used as control was active against all the fungal isolates with mean diameter of inhibition zones (IZD) of 18 mm versus *C. albicans* and *M. canis* and 9 mm versus *A. niger* and *T. rubrum*. This demonstrates that susceptible strains were the tests organisms used in the course of this work. None of the test organisms were susceptible to the DMSO serving as the negative control.

The comparison of the various extracts and fractions of *R. vomitoria* by its parts (Fig. 2) showed that for the leaves, there was no antibacterial activity for only the butanol fraction (RVL butanol). The result showed that, although the antimicrobial agents are active in the crude state (methanol), they become more active after fractionation with the bipolar (ethyl acetate) solvent. The antimicrobial activity of the plant bark, as observed with the crude methanol extract (RVB crude) and the n-hexane fraction

(RVB n-hexane), showed that though the principles were active in the crude solvent (methanol), they were potentiated after fractionation in the non-polar solvent (n-hexane). The registered antifungal activity against *M. canis* showed that all but the n-hexane fraction (RVB n-hexane) had no recorded activity. This indicates that the active principle were not contained after fractionation in the non-polar (n-hexane) solvent used. A comparison of the plant's root samples showed that there was no antibacterial activity of the butanol fraction (RVR butanol) against the two (2) bacterial isolates. The n-hexane (RVR n-hexane) fraction also had no activity against *S. typhi*. These indicate that the crude methanol extract (RVR crude) and the ethyl acetate fraction (RVR ethyl acetate) are mostly active antibacterial agents. The four (4) samples all showed inhibition against *M. canis*. However, the ethyl acetate fraction (RVR ethyl acetate) followed by the crude methanol extract (RVR crude) gave the best activities recorded.

### 3.3 Minimum Inhibitory Concentration (MIC) of Extracts and Fractions of *Rauwolfia vomitoria*

The minimum inhibitory concentration (MIC) was considered to be the lowest concentration of the samples (extracts and fractions) of the three plant parts at which no growth of microorganism was observed upon visual observation after incubation. The MIC outcomes confirms that majority of the samples have antimicrobial activity. The range of MICs of the plant samples was 25 – 100 mg/ml.

The lowest MIC value for *R. vomitoria* (Table 2) was recorded with ethyl acetate fraction of the leaves (RVLE) and crude methanol extract of the bark (RVBC); both active against *M. canis* (25 mg/ml). The MIC's were mostly between 50 – 100 mg/ml.

### 3.4 Minimum Bactericidal, Fungicidal Concentration (MBC/MFC) of *R. vomitoria* Extracts and Fractions

The minimal bactericidal concentration (MBC) for bacterial isolates and minimum fungicidal concentration (MFC) for fungal isolates were recorded as the lowest concentrations of the extracts and fractions of the plant parts at which no microbial growth was observed after further incubation of the MIC plates. The lowest value (MFC) (12.5 mg/ml) was given by the ethyl acetate fraction of the leaves (RVLE) against *M. canis* (Table 3).

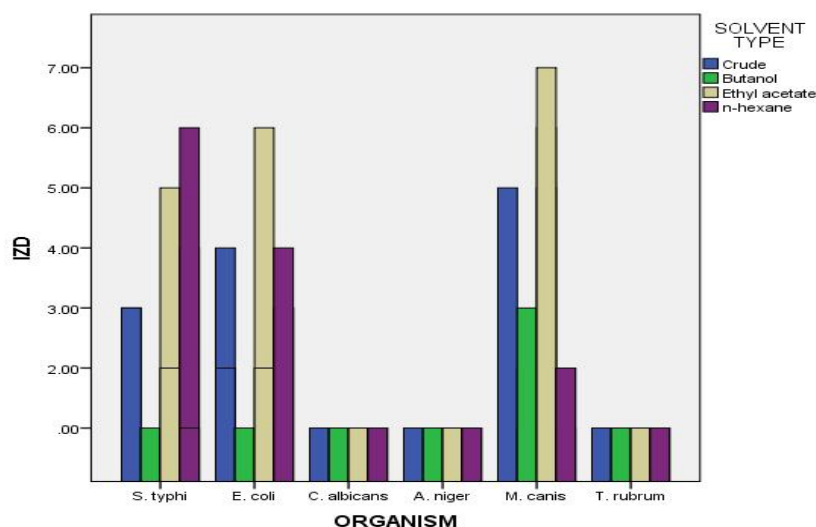


Fig. 1. Susceptibility pattern of the test isolates to the plant extracts/fractions

Table 2. Minimum inhibitory concentration (mg/ml) of the crude extracts and fractions of *R. vomitoria* against tests isolates

| Isolate | MIC (mg/ml) |       |       |       |       |       |       |       |       |       |       |       |
|---------|-------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|         | RV LC       | RV LB | RV LE | RV Ln | RV BC | RV BB | RV BE | RV Bn | RV RC | RV RB | RV RE | RV Rn |
| ST      | 100         | -     | 50    | 50    | 50    | -     | -     | 100   | 50    | -     | 100   | -     |
| EC      | 100         | -     | 50    | 100   | 100   | -     | -     | 100   | 100   | -     | 100   | 100   |
| CA      | -           | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| AN      | -           | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| TR      | -           | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| MC      | -           | -     | 25    | -     | 25    | 100   | 100   | -     | 100   | 100   | 50    | 100   |

Key: ST: *S. typhi*; EC: *E. coli*; CA: *C. albicans*; AN: *A. niger*, MC: *M. canis*, TR: *T. rubrum*, RVLC: *R. vomitoria* leaves (crude extract); RVLB: *R. vomitoria* leaves (butanol fraction); RVLE: *R. vomitoria* leaves (ethyl acetate fraction); RVLn: *R. vomitoria* leaves (n-hexane fraction), RVBC: *R. vomitoria* bark (crude extract); RVBB: *R. vomitoria* bark (butanol fraction); RVBE: *R. vomitoria* bark (ethyl acetate fraction); RVBn: *R. vomitoria* bark (n-hexane fraction), RVRC: *R. vomitoria* root (crude extract); RVRB: *R. vomitoria* root (butanol fraction); RVRE: *R. vomitoria* root (ethyl acetate fraction); RVRn: *R. vomitoria* root (n-hexane fraction)

Table 3. Minimum bactericidal/fungicidal concentration (mg/ml) of the crude extracts and fractions of *R. vomitoria* against tests isolates

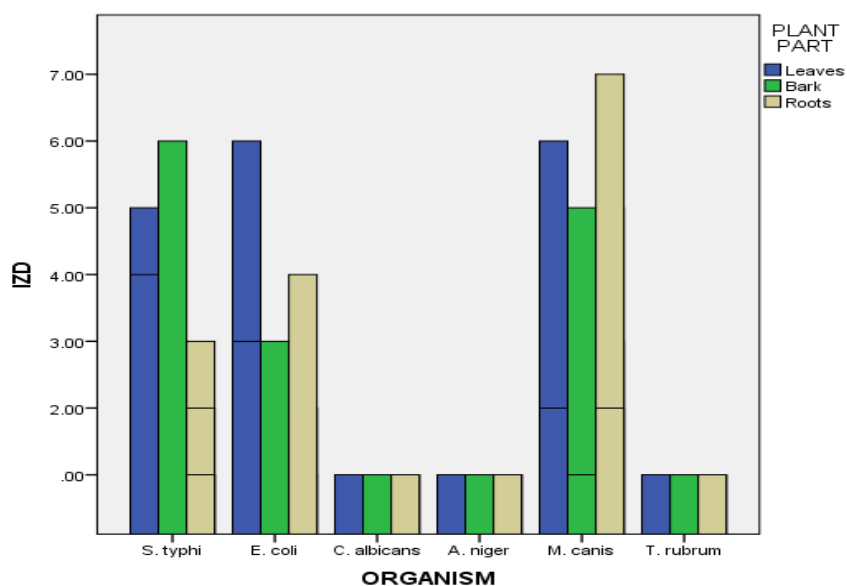
| Isolate | MBC/MFC (mg/ml) |       |       |       |       |       |       |       |       |       |       |       |
|---------|-----------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|         | RV LC           | RV LB | RV LE | RV Ln | RV BC | RV BB | RV BE | RV Bn | RV RC | RV RB | RV RE | RV Rn |
| ST      | 100             | -     | 100   | 50    | 100   | -     | -     | -     | 100   | -     | 100   | -     |
| EC      | 100             | -     | 100   | 100   | 100   | -     | -     | 100   | 100   | -     | 100   | 100   |
| CA      | -               | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| AN      | -               | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| TR      | -               | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| MC      | -               | -     | 12.5  | -     | -     | 100   | 100   | -     | -     | -     | -     | 50    |

Key: ST: *S. typhi*; EC: *E. coli*; CA: *C. albicans*; AN: *A. niger*, MC: *M. canis*, TR: *T. rubrum*, RVLC: *R. vomitoria* leaves (crude extract); RVLB: *R. vomitoria* leaves (butanol fraction); RVLE: *R. vomitoria* leaves (ethyl acetate fraction); RVLn: *R. vomitoria* leaves (n-hexane fraction), RVBC: *R. vomitoria* bark (crude extract); RVBB: *R. vomitoria* bark (butanol fraction); RVBE: *R. vomitoria* Bark (ethyl acetate fraction); RVBn: *R. vomitoria* bark (n-hexane fraction), RVRC: *R. vomitoria* root (crude extract); RVRB: *R. vomitoria* root (butanol fraction); RVRE: *R. vomitoria* root (ethyl acetate fraction); RVRn: *R. vomitoria* root (n-hexane fraction)

**Table 4. PIDGs of test organisms towards different extracts and fractions of *Rauwolfia vomitoria***

| Samples (100 mg/ml) |                   | PIDGs (%) |     |    |    |        |    |
|---------------------|-------------------|-----------|-----|----|----|--------|----|
|                     |                   | ST        | EC  | CA | AN | MC     | TR |
| Leaves              | RVL Crude         | -57.14    | -20 | *  | *  | *      | *  |
|                     | RVL Butanol       | *         | *   | *  | *  | *      | *  |
|                     | RVL Ethyl acetate | 28.57     | 20  | *  | *  | -66.67 | *  |
|                     | RVL n-hexane      | -42.86    | -40 | *  | *  | -88.89 | *  |
| Barks               | RVB Crude         | -57.14    | -60 | *  | *  | -88.89 | *  |
|                     | RVB Butanol       | *         | *   | *  | *  | -88.89 | *  |
|                     | RVB Ethyl acetate | *         | *   | *  | *  | -72.22 | *  |
|                     | RVB n-hexane      | -13.29    | -40 | *  | *  | *      | *  |
| Roots               | RVR Crude         | -57.14    | -60 | *  | *  | -72.22 | *  |
|                     | RVR Butanol       | *         | *   | *  | *  | -83.33 | *  |
|                     | RVR Ethyl acetate | -71.43    | -60 | *  | *  | -61.11 | *  |
|                     | RVR n-hexane      | *         | -20 | *  | *  | -88.89 | *  |

Key: \*: Not applicable; ST: *S. typhi*; EC: *E. coli*; CA: *C. albicans*; AN: *A. niger*; MC: *M. canis*; TR: *T. rubrum*, RVL crude: *R. vomitoria* leaves (crude extract); RVL butanol: *R. vomitoria* leaves (butanol fraction); RVL ethyl acetate: *R. vomitoria* leaves (ethyl acetate fraction); RVL n-hexane: *R. vomitoria* leaves (n-hexane fraction), RVB crude: *R. vomitoria* bark (crude extract); RVB butanol: *R. vomitoria* bark (butanol fraction); RVB ethyl acetate: *R. vomitoria* bark (ethyl acetate fraction); RVB n-hexane: *R. vomitoria* bark (n-hexane fraction), RVR crude: *R. vomitoria* root (crude extract); RVR butanol: *R. vomitoria* root (butanol fraction); RVR ethyl acetate: *R. vomitoria* root (ethyl acetate fraction); RVR n-hexane: *R. vomitoria* root (n-hexane fraction)



**Fig. 2. Susceptibility pattern of the isolates against the plant parts**

**3.5 Determination of the Percentage Inhibition of Diameter Growth (PIDG)**

The results of the Percentage inhibition diameter growth (PIDG) (Table 4) calculated using the inhibition zone diameter (IZD) showed that the most potent sample was ethyl acetate fraction of the leaves (RVL Ethyl acetate) against *S. typhi* (28.57%) and *E. coli* (20%), using the diameters

of the inhibition zones (IZD) in comparison with the positive control (5 µg/ml Ciprofloxacin) as threshold/standard.

**3.6 Discussion**

The results showed that the extracts and fractions inhibited some of the test organisms significantly (p<0.05). The Susceptibility to the

extracts and fractions of *R. vomitoria* of all the test organisms parts showed that *M. canis* is the most sensitive organism with nine (9) of the samples showing inhibitory activity and the best activity observed was with ethyl acetate fraction of the root (RVR Ethyl acetate) having an IZD of 7 mm and MIC of 50 g/ml. This was followed by *E. coli* and *S. typhi* with eight (8) and seven (7) active samples respectively. Susceptibility of *E. coli* and *S. typhi* to methanol extract of the bark has also earlier been reported by Ojo et al. (2012). *T. rubrum*, *C. albicans*, and *A. niger* were found to be resistant as none of the samples had inhibition activity against them. The antimicrobial activity of the aerial parts of the plant extracted from petroleum ether and methanol respectively against *S. typhi*, *E. coli*, and *C. albicans* was reported by [17] and [18] in this present study, several of the fractions were active against *S. typhi* and *E. coli*, however, *C. albicans* was not susceptible to any of the plant parts.

Oseni et al. [19] also assessed the antimicrobial activities of the leaves, stem, and root ethanol and methanol extracts in Akwapem-Mampong, Ghana against microbial isolates, including *S. typhi* and *E. coli*. They reported sensitivity of *S. typhi* to methanol extracts of the plant parts in the order; leaves > stem > root. This is similar to the results of this current study. Against *E. coli*, they reported sensitivity to the methanol extract of the leaves and root, but resistance to that of the stem. Nevertheless, in this current research, *E. coli* was only slightly sensitive to the stem/bark and root samples.

There were also significant differences ( $p < 0.05$ ) among extracts and fractions in terms of range of activity. The ethyl-acetate fraction of the leaves extract across the samples gave the best inhibitory activity, MIC and MBC values against the sensitive organisms; *S. typhi* (IZD: 5 mm, MIC: 50 mg/ml, MBC: 100 mg/ml), *E. coli* (IZD: 6 mm, MIC: 50 mg/ml, MBC: 100 mg/ml), and *M. canis* (IZD: 6 mm, MIC: 25 mg/ml, MBC: 12.5 mg/ml) respectively.

Phytochemical investigation of the aqueous root extracts of *R. vomitoria* by [20]. Revealed the presence of alkaloids, saponins, tannins, carbohydrates, and reducing sugars. These secondary metabolites would, although geographically affected, be relatively present in the other parts of the plant. Alkaloids, among others, are known to possess antimicrobial activities including against gastrointestinal tract infections. [21] reported this ability. Tannin is

known to have antimicrobial abilities, mostly against sexually transmitted diseases [22]. Tannins are complex phenolic polymers that can bind to proteins and carbohydrates, reducing these macromolecules' digestibility and thus inhibiting microbial growth [23,24]. Saponins are recorded as major component acting as antifungal secondary metabolite [25]. They are also surface active agents that interfere with or alter the cell wall's permeability and facilitate the entry of toxic materials or leakage of vital constituents from the cell [26]. The antimicrobial activities observed in this study may be due to these reported secondary metabolites and confirm the ethnomedicinal use of this plant in the treatment of infections.

#### 4. CONCLUSION

This research suggests that the different parts of *R. vomitoria* demonstrated antimicrobial activities as evidenced by their ability to inhibit some of the test organisms. Some of the plant parts have also shown to have extended-spectrum activity against *S. typhi*, *E. coli* and *M. canis*. The antibacterial and antifungal (against *M. canis*) activities of the plant components promote the use of the plants in ethnomedicine for the treatment of diseases including gastroenteritis, GIT, and skin infections. Therefore, in order to identify the exact compounds responsible for these activities further studies are needed.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

#### ACKNOWLEDGEMENTS

We wish to thank the Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi zikiwe University, Agulu campus for their Laboratory assistance.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

1. Ekeanyanwu CR. Traditional medicine in Nigeria: Current status and the future.



- Research Journal of Pharmacology. 2011;5(6):90–94.  
DOI:<http://dx.doi.org/10.3923/rjpharm.2011.90.94>
2. Emencheta SC, Enweani IB, Oli AN, Okezie UM, Attama AA. Evaluation of antimicrobial activities of fractions of plant parts of *Pterocarpus santalinoides*. Biotechnology Journal International. 2019;23(3):1-11.  
DOI:<https://doi.org/10.9734/bji/2019/v23i330081>
  3. Oli AN, Obaji M, Enweani IB. Combinations of *Alchornea cordifolia*, *Cassytha filiformis* and *Pterocarpus santalinoides* in diarrhoeagenic bacterial infections. BMC Research Notes. 2019;12:649.  
DOI:<https://doi.org/10.1186/s13104-019-4687-0>
  4. Okwu GI, Enweani IB, Amengialue OO, Obiazi HAK. Comparative study of the phytochemical and antibacterial properties of two different varieties of *Dioscorea bulbifera* tubers. American Journal of Research Communication. 2016;4(7).
  5. Okwuosa OMTB, Chukwura EI, Chukwuma GO, Okwuosa CN, Enweani IB, Agbakoba NR, Chukwuma CM, Manafa PO, Umedum CU. Phytochemical and antifungal activities of *Uvaria chamae* leaves and roots, *Spondias mombin* leaves and bark and *Combretum racemosum* leaves. African Journal of Medicine and Medical Sciences. 2012;41:99–103.
  6. Orwa C, Mutua A, Kindt R, Jamnadass R, Simmons A. Agroforestry Database: A tree reference and selection guide version 4.0; 2009.  
Available:<http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp>
  7. Olatokunboh A, Kayode YO, Adeola OK. Anticonvulsant activity of *Rauwolfia vomitoria* (Afzel). African Journal of Pharmacy and Pharmacology. 2009;3(6): 319–322.
  8. Kutalek R, Prinz A. Handbook of medicinal plants: African medicinal plants in Yaniv Z and U. Bachrach (Eds) New Delhi, CBS Publishers; 2007.
  9. Lobay D. Rauwolfia in the treatment of hypertension. Integrative Medicine (Encinitas, Calif.). 2015;14(3):40–46.
  10. Javaroni V, Neves MF. Erectile dysfunction and hypertension: Impact on cardiovascular risk and treatment. International Journal of Hypertension, 2012;627278.  
DOI:<http://dx.doi.org/10.1155/2012/627278>
  11. Odugbemi T. A textbook of medicinal plants from Nigeria: Lagos, University of Lagos Press; 2008.
  12. Usunobun U. Review manuscript: A review of some African medicinal plants. International Journal of Pharmaceutical and Biological Sciences. 2012;3(4):1–11.
  13. Ijeh I, Omodamiro O, Nwana I. Antimicrobial effects of aqueous and ethanolic fractions of two species, *Ocimum gratissimum* and *Xylopi aethiopica*. African Journal of Biotechnology. 2005;4(9):53–956.
  14. Waltrich KK, Hoscheid J, Prochnau IS. Antimicrobial activity of crude extracts and fractions of *Vernonia polyanthes* Less (assa-peixe) flowers. Revista Brasileira de Plantas Mediciniais. 2015;17(4, Suppl. 2): 909–914.  
DOI:[https://dx.doi.org/10.1590/1983-084X/14\\_110](https://dx.doi.org/10.1590/1983-084X/14_110)
  15. Jayshree D, Dhruva J. Antidermatophytic activity of some medicinal plants, phytochemical analysis and isolation of active fractions from *Piper longum*. Journal of Pure and Applied Microbiology. 2013;7(4):3207–3312.
  16. Okore V. A standard laboratory technique in pharmaceuticals and pharmaceutical microbiology (2<sup>nd</sup> Edition). Naphtali prints, Nigeria. 2004;6–24.
  17. Ogwuche C, Ayobo P, Odeja K. Antimicrobial properties and chemical compositions of petroleum ether extract of the aerial parts of *Rauwolfia vomitoria* obtained from Agbarho community of Delta State. Scientia Africana. 2016;15:2.
  18. Ogwuche C, Adeyemi O. GC-MS analysis and antimicrobial studies of the methanol extract of aerial parts of *Rauwolfia vomitoria* obtain from Agbarho, Delta State, NISEB Journal. 2016;16(1).
  19. Oseni LA, Ajisope BMU, Sunwiale SS. *In-vitro* bacteria growth inhibition potential of crude extracts of different parts of *Rauwolfia vomitoria* against five standard strains of selected human pathogenic microbes. World Journal of Pharmacy and Pharmaceutical Sciences. 2014;3(6):152–164.
  20. Amole O, Yemitan O, Oshikoya K. Anticonvulsant activity of *Rauwolfia vomitoria* (Afzel). African Journal of

- Pharmacy and Pharmacology. 2009;3(6): 319-322.
21. Othman L, Sleiman A, Abdel-Massih RM. Antimicrobial activity of polyphenols and alkaloids in Middle Eastern plants. *Frontiers in Microbiology*. 2019;10:911. DOI:<http://dx.doi.org/10.3389/fmicb.2019.00911>
22. Borokini T, Omotayo F. Phytochemical and ethnobotanical study of some selected medicinal plants from Nigeria. *Journal of Medicinal Plants Research*. 2012;6(7): 1106–1118,1123. DOI:<http://dx.doi.org/10.5897/jmpr09.430>
23. Nwogu LA, Igwe CU, Emejulu AA. Effects of *Landolphia owerriensis* Leaves extract on the liver function profile and haemoglobin concentration of albino rats. *African Journal of Biotechnology*. 2008;2(12):240-242.
24. Bele AA, Jadhav VM, Kadam VJ. Potential of tannins: A review. *Asian Journal of Plant Sciences*. 2010;9:209–214. DOI:<http://dx.doi.org/10.3923/ajps.2010.209.214>
25. Coleman JJ, Ghosh S, Okoli I, Mylonakis E. Antifungal activity of microbial secondary metabolites. *PloS one*. 2011;6(9):e25321. DOI:<http://dx.doi.org/10.1371/journal.pone.0025321>
26. Onwuliri F, Wonang D. Biochemical and antimicrobial studies of *Zingiber officinale* and *Allium sativum* on the selected micro-organisms. B.Sc. Project in the department of Botany, University of Jos. 2003;59.

© 2020 Emencheta et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*  
The peer review history for this paper can be accessed here:  
<http://www.sdiarticle4.com/review-history/62031>