

## Preliminary Antibacterial Screening of the Whole Stem of *Rothmania whitfieldii*

### ABSTRACT

**Aim:** To assess the antibacterial activity of the crude ethanolic and water extracts of the stem of *Rothmannia whitfieldii* on clinically isolated and typed pathogenic bacterial strains.

**Study Design:** This experiment was performed in duplicates under aseptic condition.

**Place and Duration of Study:** Department of Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria, between April 2005 to August, 2005.

**Methodology:** Whole dried stem specimen of *Rothmannia whitfieldii* was extracted by maceration and screened for antibacterial activity using agar well-diffusion technique. Five typed bacterial strains namely, *Staphylococcus aureus* (ATCC 12600), *Pseudomonas aeruginosa* (ATCC 10145), *Escherichia coli* (ATCC 11775), *Salmonella kintambo* (SSRL 113) and *Bacillus subtilis* (ATCC 6051), and six locally isolated clinical strains (*Escherichia coli*, *Salmonella typhi*, *P. aeruginosa*, *S. aureus* and two *Proteus species* designated as *Proteus I* and *Proteus II*), were tested for susceptibility to the plant extract.

**Result:** Phytochemical analysis revealed the presence of flavonoids, saponnins, tannins, carbohydrates and steroidal aglycone. Alkaloid was present in trace amount only in the ethanolic extract. The ethanolic extract exhibited a wider spectrum of activity, being effective against 81.8% of the test organisms compared with the cold-water extract (54.5%) or hot water extracts (27.3%). The minimum inhibitory concentrations (MICs) varied not only with the test organisms but also with the various extracts of the plant.

**Conclusion:** All the extracts had bacteriostatic activity on the susceptible organisms and none was bactericidal. The screening test results authenticate the folkloric claims that *Rothmannia whitfieldii* is medicinal against gastrointestinal diseases and aid in wound healing.

Keywords: *Rothmannia whitfieldii*, Preliminary screening, antibacterial activity, aqueous extract, ethanolic extract and whole stem.

## 1. INTRODUCTION

Medicinal plants used in indigenous and traditional medicine systems have become a focus for providing primary health care needs. Due to the widespread abuse and over-use of antibiotics, most of the pathogenic bacteria have become resistant to many antibiotics in the last few decades and this poses a grave danger to human health [1, 2]. The resistance of pathogenic bacterial strains to antibiotics as well as the evolution of new strains of disease-causing agents is of utmost concern to the global health community [3, 4]. Efficient disease treatment requires the development of new pharmaceuticals or some potential source of novel drugs. Medicinal plants that are frequently used in our community could be an excellent source of drugs to ward off this problem [4]. Medicinal plants contain numerous molecules, a lot of which have antimicrobial and antioxidant properties that can protect humans from pathogenic organisms and cellular oxidation. Thus, assessment of different types of medicinal plants for their antioxidant and antimicrobial potential is of utmost importance [5, 6]. Currently, researches are ongoing towards the development and discovery of newer drugs using many plant products. These researches are being carried out based on the traditional use of these plant products. Plants possess antimicrobial and antiviral activities that can fight against diseases. Consequently, much attention from pharmacognosy has been given to antimicrobial agents gotten from plants [7]. Medicinal plants can provide a wealth of antimicrobial agents, which can be used as an alternate source of antibiotics [8, 9].

A great number of plants have been screened for their antimicrobial activities. Even though not many of them have been used in modern medicine, however, their usefulness in traditional medicine is fairly high. In fact, the continued search for new ones should be a sustained process since some of the most dangerous microbial pathogens have become insusceptible to the available antimicrobial agents as a result of development of resistance to them.

*Rothmannia whitfieldii* (synonym: *Randia malleifera*) is a plant of the Rubiaceae family found within tropical Africa from Senegal to Sudan and in the southern part of Africa: Angola and Zimbabwe to be precise. It is also found in Nigeria and it comprises about 30 species, distributed in tropical Africa,

Madagascar and Asia. About 18 species are present in tropical Africa and are not in danger of genetic erosion. *Rothmania whitfieldii* occurs in forest undergrowth, often in old secondary forest but also in savanna woodland, up to 1700 m altitude. Structurally, the plant is a small tree of about 15 m tall and has fruits of 3 - 7 cm in diameter, with smooth to strongly 10-ribbed, velvety brown pubescent when young but glabrescent when it matures. It has many-seeds crowned by the persistent calyx. The seeds are lens-shaped with a dimension of 7 - 11 mm × 3 - 4 mm [10].

The plant is called uri by the Igbos in the Eastern part Nigeria where their women use juice from the fresh fruit for body decoration. The juice turns blue-black after a while when rubbed on the body, an old art that is no longer trending. It has reported that most plants have both medicinal and dye potentials [11].

Medicinally, the *Rothmannia whitfieldii* plant is considered to possess febrifugal, anti-diarrhoeic and ectobolic properties. The fruit juice is applied to sores and wounds to promote healing and in Tanzania, to leprosy areas of the skin. In East Africa, drinking of cold water in which the root bark has been steeped, provokes abundant expectoration and is a relief for asthma. In southern Africa, the root ash is used as cicatrizant on the wounds and to treat eczema on the toes [10].

*Rothmannia whitfieldii* plant has also been used by the Igbos for the cure of measles [12]. In a part of Ngor okpala in Imo State Nigeria, the plant is used as chewing stick. There, it is believed that using it as a chewing stick clears sore throat.

## **2. MATERIALS AND METHODS**

**2.1 Materials.** – All the reagents and solvents used in this work were of standard grade and purity. Also, the media and nutrient broths were of standard grade and were products of Biotech for Mueller Hinton agar and Lab M for nutrient broth and agar respectively.

### **2.2 Collection, Identification and Preparation of Plant Material.**

The plant, *Rothmania whitfieldii* used for this work was got from the forest of Umuevo Amala Ngor-Okpala Local Government Area in Imo State, Nigeria. This plant known as 'Atu uguru' (as it is used as chewing stick) by the indigenes of this community was identified by Mr A. O. Ozioko of Bioresources Development and Conservation of Project (BDCCP), Nsukka.

### **2.3 Preparation of Extracts**

The fresh stem of this plant was cleaned up by washing with clean tap water to remove dust and sand particles. The extraction was done with cold water, hot water (100 °C) and ethanol (analytical grade). Three 50 g portions each of the pulverized stem were soaked in 200 ml of cold water, hot water and ethanol respectively. The mixtures were shaken thoroughly and left to stand for 24 hours at room temperature. The resulting extracts were first filtered with a clean muslin sieve followed by subsequent filtration with Whatman No.1 filter paper. Each of the filtrates was poured into preweighed clean and sterile petri dishes and then evaporated to dryness in steady air current at warm temperature. The fine residues of the air-dried extracts were sterilized by exposing them to uv rays for 18 hours. Then the dried and sterilized crude extracts were stored 4 °C until when used for further analysis.

The percentage yields of the extracts were calculated with the formula below.

$$\% \text{ yield} = \frac{\text{weight of dried extract}}{\text{weight of pulverized extracts}} \times \frac{100}{1}$$

## 2.4 Phytochemical Screening

The extracts were tested for the presence or absence of flavonoids, tannins, saponins, alkanoid, glycosides, steroidal aglycone, carbohydrates and proteins.

Phytochemical analysis of the extracts was carried out using standard analytical methods described by Harbone [13].

## 2.5 Test Microorganisms

A total of eleven bacterial strains were used in this experiment. They included five standard typed cultures of *Escherichia coli* (ATCC 11775), *Staphylococcus aureus* (ATCC 12600), *Pseudomonas aeruginosa* (ATCC 10145), *Salmonella kintambo* (SSRL 113), and *Bacillus subtilis* (ATCC 6051). The typed cultures were stock cultures obtained from Bioresource Development and Conservation Project (BDGP), Nsukka. Clinical isolates of bacteria used include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Proteus* species I and II. These clinical isolates were got from the medical diagnostic unit of the Department of Microbiology, University of Nigeria, Nsukka Campus.

Each of these test organisms were reactivated and purified by subculturing three times on sterile nutrient agar plates. Afterwards, the cultures were preserved in nutrient agar slants and stored at 4 °C and maintained by subculturing at two weeks interval.

## **2.6 Preliminary Screening of Extracts for Antibacterial Activity**

The standardization of each bacterial strain was performed according to the National Committee for Clinical Laboratory Standards (NCCLS) [14]. Each bacterial strain grown in Mueller Hinton broth for 18 hours at 37 °C was used to achieve the turbidity of 0.5 McFarland units. The turbidity of each bacterium was adjusted to a suspension of  $3 \times 10^6$  cells. Standardized bacterial inoculums containing  $3 \times 10^6$  colony forming units (cfu) per millimeter (ml) were used for the preliminary antibacterial screening.

The preliminary antibacterial screening of the extracts was performed using a modified agar well diffusion method of Okeke *et al.* [15]. This was done to check if the plant has antibacterial activity. The test was carried out using ethanolic, hot and cold water extracts (i.e a total of 3 extracts) of the plant stem. One ml aliquot of the standardized suspension ( $3 \times 10^6$  cfu/ml) of each of the test bacterial strain was spread on petri dishes containing 15 ml of solidified sterile MHA media, to achieve a confluent growth. The plates were allowed to dry before a 7 mm diameter wells were bored in the agar using a sterile cork borer. Four wells were bored into these already seeded media. Each of these wells was numbered. Then 100 µl volume of each of the three extracts (with concentration of 2.5 mg/ml) was introduced into a well. These plates were left to stand for 1 hour to allow the diffusion of the extracts to take place, before they were incubated at 37 °C for 18 hours. Gentamycin at concentration of 8 µg/ml was used as a control. Growth on plates was examined after incubation and the inhibition zone diameters were measured.

## **2.7 Evaluation of the Minimum Inhibitory Concentration (MIC) of the Extracts**

On finding that the extracts had activity against the test organisms, the minimum inhibitory concentrations of these extracts were determined. The MIC was carried out using the agar well diffusion method of Okeke *et al.* [15]. Here, a two-fold serial dilution of the 3 extracts was prepared using 2 ml of sterile distilled water as the diluent to achieve the following concentrations, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml. Following this, each of the standardized test organisms was used to seed 15ml of the solidified sterile Mueller-Hinton agar media plates, making sure that the seeding is uniform over the surface of the media.

A 100 µl sample of each dilution was introduced into duplicate wells in the MHA plates already seeded with the bacterial cells. Each plate received the four different dilutions of a particular extract. The plates were incubated at 37 °C for 18 hours. The lowest concentration showing a zone of inhibition was taken as the MIC.

## 2.7 Evaluation of the Minimum Bactericidal Concentration (MBC) of the Extracts

The minimum bactericidal concentration is taken as the least concentration of the extract that killed off the test organisms within a given time interval. This was done by cutting a 2 mm disc from the area showing very clear zone of inhibition in the MIC test. These cut out discs were aseptically inoculated into fresh sterile nutrient broth medium. The inoculated broth media were incubated at 37 °C for 18 hours, after which a loopful of each incubated broth medium was taken from each of the tubes and was aseptically streaked on sterile MHA medium. The plates were further incubated at 37 °C for 18 hours. After incubation, the plates were examined for growth. The MBC was taken as the lowest concentration of the extracts that killed off (i.e., in which no growth occurred at all on the streaked media plates) the organisms.

## 3. RESULTS AND DISCUSSION

### 3.1 Percentage yield of extracts from ethanol, cold water and hot water

The ethanolic extraction of the whole stem of *Rothmannia whitfieldii* yielded light green extract, while the aqueous extracts of the stem on the other hand were dark coloured with the hot water extract being thicker in consistency.

The yield of the whole stem extracts is shown in Table 1 below. The cold water extract gave the highest yield (6.32 %), followed by the hot water extract (4.08 %) while the least yield was recorded with the ethanolic extract (1.76 %). The yields of the plants were relatively low probably because of the extraction method used. Cold maceration has generally been reported to yield lower extracts of plants, compared to Soxhlet extraction [16]. Among the three extracts, cold water extract of the stem gave the highest percentage yield of 6.32 %<sup>w/w</sup>, followed by the hot water extract which yielded 4.08 %<sup>w/w</sup>, while the ethanolic extract of the stem gave the least percentage of 1.76 %<sup>w/w</sup>.

**Table 1. Percentage yield of extracts from ethanol, cold water and hot water**

Extraction solvent	Plant part code	Yield (g)	Yield (%)
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Ethanol	WS-ETOH	0.88	1.76
Cold water	WS-H <sub>2</sub> O <sub>C</sub>	3.16	6.32
Hot water	WS-H <sub>2</sub> O <sub>H</sub>	2.04	4.08

### 3.2 Phytochemical analysis

Preliminary phytochemical screening of the ethanolic, hot and cold water extracts of the stem of *Rothmania whitfieldii*, revealed the presence of saponins, tannins, flavonoids, alkaloids, steroidal aglycone, carbohydrates and proteins. However, the degree of their concentration in these extracts varied, with some occurring in trace amounts. Steroidal aglycone and saponin occurred in equal proportion in all the 3 extracts of plant's stem. Anthracene, O- and C- as well as cyanogenic glycosides were absent in the 3 extracts. The presence of tannins, saponins, alkaloids and flavonoids suggest antimicrobial activity of a plant as proposed by earlier workers [17]. Table 2 shows the phytochemical analysis of the ethanolic, hot and cold water extracts of the stem of the plant.

**Table 2. Phytochemical Analysis of the Extracts**

Compounds	WS- H <sub>2</sub> O <sub>C</sub>	WS- H <sub>2</sub> O <sub>H</sub>	WS- ETOH
<b>Glycoside</b>	-	-	-
<b>Alkaloid</b>	-	-	+r
<b>Saponin</b>	++	++	++
<b>Flavonoid</b>	++	++	+
<b>Proteins</b>	-	+	+
<b>Carbohydrates</b>	+	+	+
<b>Tannin</b>	++	++	+r
<b>Steroidal aglycone</b>	++	++	++

<b>Anthracene glycoside</b>	-	-	-
<b>O and C glycoside</b>	-	-	-
<b>Cyanogenic glycoside</b>	-	-	-

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#### Key

-: absence of reaction, +r: very low or trace intensity reaction, +: low intensity reaction and ++: medium intensity reaction.

WS- H<sub>2</sub>O<sub>C</sub> = Cold water extract of the whole stem; WS- H<sub>2</sub>O<sub>H</sub> = Hot water extract of the whole stem and WS- ETOH = Ethanolic extract of the whole stem.

### 3.3 Preliminary Sensitivity Test of the Stem Extracts of *Rothmania whitfieldii*

A total of 3 whole stem extracts (hot water, cold water and ethanolic extracts) were tested against 11 bacterial samples.

The cold water extract of the stem of *Rothmania whitfieldii* showed activity against 6 of the test bacterial isolates with *Pseudomonas aeruginosa* (ATCC 10145), *Salmonella typhi* (c), *Bacillus subtilis* (ATCC 6051) and *Staphylococcus aureus* (ATCC 12600) being insusceptible.

The hot water extract produced zones of inhibition against only three of the test bacterial isolates which were *Sal kintambo* (SSRL 113), *Proteus* sp. II and *Proteus* sp. I.

The ethanolic extract of the stem showed activity against 9 out of the eleven test isolates. Only *Bacillus subtilis* (ATCC 6051) and *Staphylococcus aureus* (ATCC 12600) were not susceptible to it. It produced its highest zone of inhibition of 18.70 mm with *Salmonella kintambo* (SSRL 113) and 18.00 mm with *E. coli* (c).

The Gentamycin used at the concentration of 8µg/ml and which served as the control gave IZD ranges from 6-19mm as shown in table 3 below.

Extract obtained with analytical grade ethanol gave a relatively wide spectrum of activity (81.8 %).

The inexplicable observations made were that while two extracts may contain similar secondary metabolites, although not in the same proportion, one shows more antibacterial activity than the other.

For example, no secondary metabolite was more peculiar to the ethanolic extract that gave the widest spectrum of activity against the test isolates. As a result, it is difficult to say which one is the active component responsible for the antibacterial activity of these extracts. This will await the isolation of the active compounds from the crude extract of the plant. Also the hot water extract of the whole stem

showed lower spectrum of antibacterial activity (27.3 %) than the cold water extract of whole the stem (54.5 %) This suggests that the active components of these extracts are heat labile. Judging from the result of the antibacterial screening test of the ethanolic, cold and hot water extracts of the stem of the plant, Gram negative bacteria were the most susceptible. These are especially enteric bacteria such as *Proteus* spp, *Escherichia coli* (ATCC 11775) and *Salmonella kintambo* (SSRL 113). *Sal. kintambo* (SSRL 113), *E. coli* (ATCC 11775) and *Proteus sp* II(c) were the most susceptible, being susceptible to all but one of the various extracts. Whereas *E. coli* (ATCC 11775) and *Proteus sp.* II(c) were resistant to the hot water extract of the stem. Clinical of *Salmonella typhi* showed the highest resistance to these extracts, being susceptible to only the ethanolic extract of the stem.

**Table 3. Preliminary Sensitivity Test of the Stem Extracts of *Rothmania whitfieldii***

Test organism	WS- H <sub>2</sub> O <sub>C</sub>	WS- H <sub>2</sub> O <sub>H</sub>	WS- ETOH	Gentamycin
<i>Pseudomonas aeruginosa</i> (ATCC 10145)	-	-	+++	++++
<i>Pseudomonas aeruginosa</i> (C)	++++	-	+++	+
<i>Salmonella typhi</i> (C)	-	-	++	++++
<i>Salmonella kintambo</i> (SSRL 113)	++++	++++	++++	++++
<i>Escherichia coli</i> (ATCC 11775)	+++	-	+++	++++
<i>Escherichia coli</i> (C)	+++	-	++++	+++
<i>Proteus sp. I</i> (C)	-	+++	+++	++
<i>Proteus sp. II</i> (C)	++	+++	++	++

<b>Staphylococcus</b>				
<b>aureus (ATCC 12600)</b>	-	-	-	++++
<b>Staphylococcus aureus (C)</b>	+	-	+	+
<b>Bacillus subtilis (ATCC 6051)</b>	-	-	-	++++
<b>Proportion susceptible (%)</b>	6/11 (54.5)	3/11 (27.3)	9/11 (81.8)	11/11(100)

#### Key

+ = presence of inhibition; - = absence of inhibition or no activity

Where, 9 – 11 mm = +; 12 – 14 mm = ++; 15 – 17 mm = +++ and > 17 mm = +++++, including the diameter of the hole. (C) means clinical strain of the test isolates.

### 3.4 Minimum Inhibitory Concentration of the various Extracts of the whole Stem of the Plant

The data obtained through MIC revealed variability in the inhibitory concentrations of each extract for a given bacterium. The MIC values of the different plant extracts against the test bacterial isolates were found in the range of 3.125-25.00 mg/ml. The cold water extract of the whole stem suppressed the growth of *Sal. Kintambo* (SSRL 113) and the clinical isolate of *P. aeruginosa* at a minimum concentration of 6.25 mg/ml with inhibition zones of 15.00 and 11.00mm respectively.

The inhibitory effect of the ethanolic extract of the whole stem of *R whitfieldii* started with minimum concentration of 3.125 mg/ml against *Sal. kintambo* (SSRL 113), with a zone of inhibition of 11.00 mm. it had the highest MIC value of 25.0mg/ml against *Proteus* sp. II (c) and *Staphylococcus aureus* (ATCC 12600).The results are as shown in tables 4,5 and 6.

**Table 4. Minimum Inhibitory Concentration of the Cold Water Extract of the Stem of the Plant**

Test organism	Zone of Inhibition in mm of	Concentration in mg/ml	MIC	in
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	mg/ml				
	25.0	12.5	6.25	3.125	
<b><i>Pseudomonas aeruginosa</i></b> (ATCC 10145)	ND	ND	ND	ND	ND
<b><i>Pseudomonas aeruginosa</i></b> (C)	18.00	18.00	11.00	-	6.25
<b><i>Salmonella typhi</i></b> (C)	ND	ND	ND	ND	ND
<b><i>Salmonella kintambo</i></b> (SSRL 113)	19.50	17.50	15.00	-	6.25
<b><i>Escherichia coli</i></b> (ATCC 11775)	16.00	13.00	-	-	12.5
<b><i>Escherichia coli</i></b> (C)	15.50	13.00	-	-	12.5
<b><i>Proteus</i> sp. I</b> (C)	ND	ND	ND	ND	ND
<b><i>Proteus</i> sp. II</b> (C)	12.00	11.00	-	-	12.5
<b><i>Staphylococcus aureus</i></b> (ATCC 12600)	ND	ND	ND	ND	ND

<b><i>Staphylococcus aureus</i> (C)</b>	11.00	10.00	-	-	12.5
<b><i>Bacillus subtilis</i> (ATCC 6051)</b>	ND	ND	ND	ND	ND

### Key

ND = Not determined; - = no activity

**Table 5. Minimum Inhibitory Concentration of the Hot Water Extract of the Stem of the Plant**

Test organism	Zone of Inhibition in mm of Concentration in mg/ml				MIC in mg/ml
	25.0	12.5	6.25	3.125	
<b><i>Pseudomonas aeruginosa</i> (ATCC 10145)</b>	ND	ND	ND	ND	ND
<b><i>Pseudomonas aeruginosa</i> (C)</b>	ND	ND	ND	ND	ND
<b><i>Salmonella typhi</i> (C)</b>	ND	ND	ND	ND	ND
<b><i>Salmonella kintambo</i> (SSRL 113)</b>	19.50	17.50	15.00	-	6.25

<i>Escherichia coli</i> (ATCC 11775)	ND	ND	ND	ND	ND
<i>Escherichia coli</i> (C)	ND	ND	ND	ND	ND
<i>Proteus sp. I</i> (C)	16.00	13.00	-	-	12.5
<i>Proteus sp. II</i> (C)	15.00	11.00	-	-	12.5
<i>Staphylococcus aureus</i> (ATCC 12600)	ND	ND	ND	ND	ND
<i>Staphylococcus aureus</i> (C)	ND	ND	ND	ND	ND
<i>Bacillus subtilis</i> (ATCC 6051)	ND	ND	ND	ND	ND

**Key**

ND = Not determined; - = no activity

**Table 6. Minimum Inhibitory Concentration of the Ethanolic Extract of the Stem of the Plant**

Test organism	Zone of Inhibition in mm	Concentration in mg/ml	MIC in mg/ml
	25.0	12.5	6.25
			3.125

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<i>Pseudomonas aeruginosa</i> (ATCC 10145)	15.00	14.00	-	-	12.5
<i>Pseudomonas aeruginosa</i> (C)	15.00	14.00	-	-	12.5
<i>Salmonella typhi</i> (C)	14.00	13.00	-	-	12.5
<i>Salmonella kintambo</i> (SSRL 113)	18.70	18.00	17.00	11.00	3.125
<i>Escherichia coli</i> (ATCC 11775)	15.00	12.50	9.50	-	6.25
<i>Escherichia coli</i> (C)	18.00	12.00	-	-	12.5
<i>Proteus sp. I</i> (C)	15.50	12.00	-	-	12.5
<i>Proteus sp. II</i> (C)	13.50	-	-	-	25.0
<i>Staphylococcus aureus</i> (ATCC 12600)	ND	ND	ND	ND	ND
<i>Staphylococcus aureus</i> (C)	11.0	-	-	-	25.0

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<i>Bacillus subtilis</i> (ATCC 6051)	ND	ND	ND	ND	ND
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### Key

ND = Not determined; - = no activity

### 3.5 Minimum Bactericidal Concentration of the various Extracts of the Stem of the Plant

None of the extracts produced bactericidal activity against the test bacterial isolates. The stem extracts of the plant *Rothmania whitfieldii* were only bacteriostatic.

**Table 7. Minimum bactericidal concentration of the various Extracts of the Stem of the Plant**

Test organism	Minimum Bactericidal Concentration (MBC) in mg/ml		
	WS- H <sub>2</sub> O <sub>C</sub>	WS- H <sub>2</sub> O <sub>H</sub>	WS- ETOH
<i>Pseudomonas aeruginosa</i> (ATCC 10145)	0.00	0.00	0.00
<i>Pseudomonas aeruginosa</i> (C)	0.00	0.00	0.00
<i>Salmonella typhi</i> (C)	0.00	0.00	0.00
<i>Salmonella kintambo</i> (SSRL 113)	0.00	0.00	0.00
<i>Escherichia coli</i> (ATCC 11775)	0.00	0.00	0.00

<i>Escherichia coli</i> (C)	0.00	0.00	0.00
<i>Proteus I</i> (C)	0.00	0.00	0.00
<i>Proteus II</i> (C)	0.00	0.00	0.00
<i>Staphylococcus aureus</i> (ATCC 12600)	0.00	0.00	0.00
<i>Staphylococcus aureus</i> (C)	0.00	0.00	0.00
<i>Bacillus subtilis</i> (ATCC 6051)	0.00	0.00	0.00

#### 4. CONCLUSION

The result of the MIC and MBC evaluation of the aqueous and ethanolic whole stem extracts of *Rothmannia whitfieldii* showed that they are bacteriostatic. It should be noted that no literature was found to compare and contrast the findings of this study with regards to this plant's extracts, as there has been no previous investigation of the antimicrobial activity of this plant's extract.

This study has mainly provided evidence to show that *R. whitfieldii* exhibits antimicrobial activity against some human pathogenic bacterial strains. It also showed that this plant has bioactive phytochemical compounds with potential medicinal values for treating various bacterial infections. This herb therefore, is a good candidate for further evaluation for integration of ethnomedicine into orthodox medical practice.

#### REFERENCES

- [1] Nasrullah, Suliman, Rahman K, Ikram M, Nisar M, Khan I. Screening of Antibacterial Activity of Medicinal Plants. *International Journal of Pharmaceutical Science Review and Research*, 2012; 14(2): 25-29.

- [2] Pai-Wei, S., Cheng-Hong, Y., Jyh-Ferng, Y., Pei-Yu, S. and Li-Yeh, C. Antibacterial Activities and Antibacterial Mechanism of *Polygonum cuspidatum* Extracts against Nosocomial Drug-Resistant Pathogens. *Molecules* 2015; 20: 11119-11130; doi:10.3390/molecules200611119.
- [3] Rahman, K., Nisar, M. Ullah Jan, A., Suliman, M., Iqbal, A., Ahmad, A. and Ghaffar, R. Antibacterial Activity of Important Medicinal Plants on Human Pathogenic Bacteria. *International Journal of Agronomy and Agricultural Research (IJAAR)*, 2015; 6 (6):106-111.
- [4] Manandhar, S. Luitel, S. and Dahal, R.K. In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. *Journal of Tropical Medicine* 2019, article 1895340. <https://doi.org/10.1155/2019/1895340>.
- [5] Bajpai M., Pande A., Tewari S.K., Prakash D. Phenolic Contents and Antioxidant Activity of Some Food and Medicinal Plants. *International Journal of Food Sciences and Nutrition*, 2005; 56(4): 287-291.
- [6] Wojdylo A, Oszmianski J, and Czemerys R. Antioxidant Activity and Phenolic Compounds in 32 Selected Herbs. *Food Chemistry*, 2007; 105: 940-949.
- [7] Olanrewaju, I.F., Raphael, C. Mordi, R.C., JohnBull. O. and Echeme, J.O. Antibacterial, Antifungal and Anti-tubercular Activities of Chloroform Fraction of the Leaf Extract of *Irvingia Gabonensis* (African Bush Mango). *Anti-Infective Agents*:2019; 17 (1).
- [8] Malik. F., Hussain, S., Mirza, T., Hameed, A., Ahmad, S., Riaz, R., Shah P.A.. and Usmanghani K. Screening for Antimicrobial Activity of thirty-three Medicinal Plants used in the Traditional System of Medicine in Pakistan. *Journal of Medicinal Plant Research*, 2011; 5(14): 3052-3060.
- [9] Walter C, Shinwari Z K, Afzal I and Malik R.N. Antibacterial Activity in Herbal Products Used In Pakistan. *Pakistan Journal of Botany*, 2011; 43: 155-162.
- [10] Jansen, P.C.M. (2005). *Rothmannia whitfieldii* (Lindl.) Dandy. In: Jansen, P.C.M. and Cardon, D., Eds., *Plant Resources of Tropical Africa*, PROTA, Wageningen: 2005; 1-2. <http://www.prota4u.org>
- [11] Osabohien, E. and Otutu, J.O. Extraction and Utilization of Natural Dyestuffs from the Bark of Whistling Pine and the Root of African Peach. *British Journal of Applied Science & Technology*, 2014; 4: 2921-2930. <https://doi.org/10.9734/BJAST/2014/8554>
- [12] Nnorom, O.O. and Onuegbu, G.C. Authentication of *Rothmannia whitfieldii* Dye Extract with FTIR Spectroscopy. *Journal of Textile Science and Technology*, 2019; 5: 38-47. <https://doi.org/10.4236/jtst.2019.52004>.

- [13] Harbone, J.B. *The Flavonoids: Advances in Research Since 1986*. Chapman and Hall Ltd., London.1994; p. 330.
- [14] National Committee for Clinical Laboratory Standards (NCCLS). *Methods for Dilution in Antimicrobial Susceptibility Tests: Approved Standard M2-A5*. National Committee for Clinical Laboratory Standards: Villanova, P.A. 1993.
- [15] Okeke, M.I, Iroegbu, C.U., Eze E.N, Okoli, A.S. and Esimone C.O. Evaluation of Extracts of the Root of *Landolphia owerrience* for Antibacterial Activity. *Journal of Ethnopharmacology.*, 2001;78 (2-3): 119-127.
- [16] Ibrahim, M.B., M.O. Owonubi, and J.A. Onaolapo. Antimicrobial Effects of Extracts of Leaf, Stem and Root Bark of *Anogiessus leicarpus* on *Staphylococcus aureus* NCTC 8198, *Escherichia coli* NCTC 10418, and *Proteus vulgaris* NCTC 4636. *Journal of Pharmaceutical Research and Development* 1007;2:20-26.
- [17] Levan, M., D.A. Vanden Berghe, T. Marten, A. Vilientmick, and E.C. Lomweas. Screening of Higher Plants for Biological Activity. *Planta Medica*, 1979; 36:311-312.