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In Vitro Anti-bacterial Activities of Aqueous, Ethanol and Chloroform Crude Extracts of *Olinia rochetiana* and *Vernonia myriantha*

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Abstract

In the past with the advent of antibiotics, bacterial diseases have been under control. However rapid spread of antibiotic-resistant this success is reversing and searching for newer antibacterial agents is currently a top priority. This study was, thus, aimed at assessing the anti-microbial activities of two traditional medicinal plants: *Vernonia myriantha and Olinia rochetiana*. The crude extracts were tested for their in vitro antibacterial activities and phytochemical content. The extracts were tested against selected 3 clinical and 4 standard test bacterial strains by using agar well-diffusion method and the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC). The ethanol leaves and stem-bark extracts of *O. rochetiana* inhibited the growth of all bacterial strains at a concentration of 250mg/mL. The inhibition zones ranged from 20.33±0.57mm for clinical *Pseudomonas aeruginosa* to 25.66±0.57mm for standard *Salmonella typhi* strains. The values for these same extracts were 20.66±2.51mm and 24.33±1.15mm for standard *P. aeruginosa* and *Staphylococcus aureus* strains respectively. The chloroform extract was similarly effective against all of the strains with inhibition zones between 19.00±1.73mm against *P. aeruginosa* and 22.66±2.51mm for *S. aureus*. Comparatively, the ethanol extract of *O. rochetiana* had the highest MIC (7.81mg/mL) and MBC (62.50mg/mL) were noted against *P. aeruginosa*. On the other hand, chloroform extract of *O. rochetiana* leaf showed the highest MIC (15mg/mL) and MBC (125mg/mL) were recorded against *P. aeruginosa*. The ethanol extract of *V. myriantha* showed growth inhibition only on *S. aureus* (21.00±1.7mm). Both plants tested for terpenoids and glycosides showed positive result, but none for resin. In conclusion, the study suggests that the extracts of *Vernonia myriantha and Olinia rochetiana* can be used in the control of bacterial transmitting diseases because their anti-bacterial activities was high comparable with the reference.

Keywords: Antibacterial activity, phytochemicals, crude extract, pathogenic bacteria

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Introduction

Pathogenic bacteria including pathogenic *Escherichia coli*, Salmonella species, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* are among the common disease-causing bacteria. Bacterial diseases are the major cause of morbidity and mortality in the world, particularly in developing countries. WHO reported that foodborne diseases accounted, almost 420,000 deaths in 2015 [1]. Among this, foodborne diarrheal disease accounts about 230,000 deaths in 2010. *S. typhi* account 52,000 deaths of all food borne death in the same year. Global emergence of drug resistant bacterial strains has been reported and this challenge limits the effectiveness of current drugs and significantly causing failure in the treatment of infectious diseases [2]. The current levels of antibiotic usage often clinically led to increase drug resistance. Resistance of bacteria to antibiotics can be natural or acquired. It is natural upshots of the adaption of the pathogens to the exposures of antimicrobials used in medicine, food, crop production and to disinfections in farms and households. Infection with antibiotic resistant bacteria may cause severe illness, increased mortality rates, and an increased risk of complications and admission to hospital [3]. According to the European Centre for Disease Prevention and Control, 25,000 people in Europe die each year as a result of resistant infection [4]. Antibiotic-resistance leads to an increased amount of healthcare costs. It is estimated that complications associated with antibiotic-resistance cost 9 billion annually in Europe [5].

As consequence, evaluating the medicinal activities of plants is very important as being a source of natural drugs against pathogens. Medicinal plant extracts provide significant potential for development of new antimicrobial agents since they possess different varieties of phytochemicals. Plants have been used for centuries as remedies of human disease and offer new bioactive chemical compounds as antimicrobial agents and that make them richest bio resources for medicinal systems, modern medicine, for folk medicines, pharmaceuticals and chemicals dignified for synthetic drugs [6]. Medicinal use of plants and their products was passed down from generation to generation in various parts of the world throughout the history and has played significant role for the development of different traditional system of medicine. As it is a sum of knowledge, skill practiced, belief and experiences to different cultures, it is wide spread throughout the world. Its acceptance mainly conditioned by cultural factors and much of traditional, for this reason, it may not be readily transferred from one culture to another [7]. According to world health organization, medicinal plants forms base for traditional or indigenous healthcare systems used by majority of inhabitants living in third world countries. Indeed, it is reported that more than 3.5milion people depend on plants for human and livestock aliment [8]. Natural products are an important source of new antimicrobial agents. Anti-microbial agents of plant origin are effective in treatment of infectious disease while simultaneously palliating many of the side effects that are often associated with systematic antimicrobials [9]. Plants *Vernonia myriantha and Olinia rochetiana* are commonly used as traditional medicine in Ethiopia. Therefore the aim of this study was to investigate the antibacterial activity of aqueous, ethanol and chloroform leaf extracts of the two plants against some selected human pathogenic bacteria.

Olinia rochetiana is a shrub, small tree or less often a large tree and evergreen, usually 1.2-16 meters tall, but occasionally reach 27 meters. *O. rochetiana* is a plant included under the family Penaeaceae and genus *Olinia*. It is a tree and shrub endemic to some Eastern and southern African countries. It is characterized by quadrangular branches and branch lets, flowers organized in triads, and an inflorescence placement that is axillary and or terminal.

In Ethiopia, the leave of *O. rochetiana* (locally known as "Tife" in Amharic and Guna in Hadiyyisa) are used traditionally to treat toothache. It was reported by [10] used as powder, infusion or in the form of ointment for the treatment of eczema, acne and scabies. The Ogiek uses the bark as well as freshly forming leaves for chewing as treatment of colds and chest related conditions, toothbrush/mouth freshener and against pneumonia [11]. The plant has wormicidal activities [12].

Vernonia myriantha (family, *Asteraceae*) has more than1000 species growing all over the world with more than 30 species growing in Kenya [13]. It is abundant in Ethiopia and also in

neighbouring countries such as Kenya, Somalia, Tanzania, Nigeria, Cameroon and southward to Angola. It is mostly found in Kambata, Hadiya, Gurage, Sidama and Wolayta zones, Southern Ethiopia, East Welega Zone of Oromia Regional State, and West Ethiopia [14]. Its vernacular name: Reejjichoo in Kambatissa, Barawaa in Hadyissa, Ameraro in Amharic, Reejii in Afan Oromo, Rejicho in Sidamegna and buuzuwaa in Wolaytigna. The use of different parts of this plant for the treatment of various diseases in traditional or folk remedies throughout the world. Traditionally, it has been used as a medicine for a long time and has similarly used as *V. amygdalina*. In Cameroon, the leaf juice is used as eye drops for the treatment of cataract and in Ethiopia to treat toothache [15]. The plant also has application in reproductive health [16].

■ Experimental

Collection and Identification of Plant Materials

Healthy, fresh leaves of *Vernonia myriantha* leaves and stem bark of *Olinia rochetiana* were collected from Hadiyya district, 241km to the southern Ethiopia, from October to January, 2017. Identification and authentication of plant specimens was done at the National Herbarium, Department of plant Biology and Biodiversity Management, College of Natural and Computational Sciences, Addis Ababa University. Then the specimens were deposited for future reference with voucher specimen number AE001 and AE003 for *O. rochetina*, and *V. myriantha*.

Extract Preparation

The leaves and stem-bark of the plants were washed with running tape water, disinfected and rinsed with distilled water and dried in shade place at room temperature (25-30^{o}C) for two weeks as described in [17]. About 1kg of each plant part was powdered by milling and sieved through a fine mesh (Canadian Series sieves with 500µm pores), and the powder were packed in glass bottles and stored at room temperature for further use. From 1Kg powder of the plant extract, 100g powder leaves of *Vernonia myriantha*, leaves and stem bark of *Olinia rochetiana* each was soaked in 1000ml sterile distilled water in Erlenmeyer flasks and placed on shaking water bath for 72 hours according to [18]. After that, macerates were first filtered with four-fold muslin cloth. Then, the filtrate was further filtered through Watt man no 1 filter paper and were kept at refrigerator for 24 hours and concentrated by using (BLOCK, CHRIST, ALPHA1_ 4) lyophilizer at -14^{O}C with vacuum pressure. The dry and concentrated crude extracts of leaves of *Vernonia myriantha*, leaves and stem bark of *Olinia rochetiana* was afforded 18, 10.5 and 12g respectively. Similarly, the same unit (100g) powder of plants were soaked in 1000ml (ethanol 97% and chloroform 99.9%) on separate 500ml Erlenmeyer flascks and placed on shaking water bath at room temperature for 72 hours and filtered through four-fold muslin cloth and then filtered with Watt man no.1 filter paper. The extracts were concentrated in a rotary evaporator at a temperature of 45°C and 40°C. The dry and concentrated ethanol and chloroform crude extracts of leaves of *Vernonia myriantha* was afforded 14 and 8 g respectively. The dry and concentrated ethanol and chloroform crude extracts of leaves of *Olinia rochetiana* was afforded 15 and 9 g respectively. Similarly, the dry and concentrated ethanol and chloroform crude extracts of stem bark of *Olinia rochetiana* was afforded 8 and 5.5 g respectively. The extracts were placed in a small covered bottle at -4°C for further use.

The percentage yield was calculated according to the Formula 1.

$$\% = \frac{\text{Weight of crude extract}}{\text{weight of sample}} \times 100 \qquad \text{(Formula 1)}$$

Test Organisms

Bacterial Strains

Standard (American Type Culture Collection (ATCC) and clinical isolates of different bacterial strains were used for the experiment. These were *E. coli* standard (ATCC 25922) and clinical *E. coli*, *P. aeruginosa* standard (ATCC 2785315) and clinical *P.*

aeruginosa, standard *S. aureus* (ATCC25923), clinical *S. aureus* and standard *S. typhi* (ATCC 13311).

Inoculum Preparation

To obtain pure culture and to avoid contamination the standard and clinical test organisms (*P. aeruginosa*, *S. typhi*, *E. coli*, *S. aureus*) were streaked on selective media; *S. aureus on* Mannitol salt agar (M2A6B00), *P. aeruginosa* on Pseudomonas agar (Sr102), *E. coli* on ethylene methylene blue (EMB) (EMLA-1602). Media was prepared according to manufactures guide line and autoclaved at temperature of 121°C**.** After cooling the media to 45°C, it was poured onto pre-labelled sterile petri dishes aseptically and allowed to solidify for an hour. Then, test organisms were streaked on the respective selective agar media using inoculating wire loop following aseptic condition in a Safety Cabinet and incubated for 18-24 hours at 37°C. The bacterial inoculum of each of bacterium was prepared and standardized by following the guideline of Clinical and Laboratory Standard Institute [19]. Turbidity of the inoculum tube was adjusted visually by either adding bacterial colonies or by adding sterile normal saline solution in comparison with already prepared 0.5 McFarland Standard which is assumed to contain a bacterial concentration of 1-2 ×108 CFU/ml.

Antibacterial Activity Assay of Plant Extract

Agar Well Diffusion

Bacterial susceptibility to crude extracts was assessed by Agar well diffusion. The aliquot of inoculum of the respective bacteria ($1\text{-}2\times10^8$ CFU/ml) were swabed on the sterile MHA plates prepared according to the manufacturer‟s guideline in 90millimetre (mm) diameter sterile petri dish using a sterile swab. On each plate, five equidistant wells were made with a 6mm diameter sterilized cork-borer and finally rim of the agar was swabbed with cotton swab. The corresponding wells were filled with 80 µl/well of 250 mg/ml crude plant extract. In addition, the commercial antibiotic chloramphenicol 80 µl/well and distilled water were used as a positive and negative control respectively. The positive control was selected based on the susceptibility of the bacterium used [19]. Then, the plates were left undisturbed for about 30 minutes at room temperature, in order to give time for pre-diffusion on the inoculated agar. Finally, the plates were incubated at 37°C for 24 hours and then examined to verify inhibitions. Experiments were performed in triplicate and the developed inhibition zones were compared with those of reference.

Determination of Minimum Inhibitory Concentration

The crude extracts that showed antibacterial activity by agar well diffusion method was subjected to serial macro broth dilution technique to determine the MIC of the extracts according to [19]. Values of the plant extracts against the test organisms were estimated to determine the range of MIC values. Consequently, the following concentrations were prepared for each extract, using two-fold serial dilution 125, 62.5, 31.25, 15.62 7.81, 3.9, 1.9, 0.9 and 0.48mg/ml. The bacterial suspension was prepared according to CLSI guideline [19] so that the bacterial concentration was made to be approximately 1×10^6 CFU/ml by diluting the 0.5 MacFarland Standard turbidity equivalents to bacterial suspensions in the ratio of 1:150 in the MHB. Briefly, within 15 minutes of standardization and dilution of bacterial suspension, 1ml of diluted bacterial suspension was added to each test tub except the negative control. After inoculation, the test tubes contain approximately 5×10^5CFU/ml bacteria. The test tube containing only Muller Hinton Broth (MHB) and broth with inoculum (without extract) were included to serve as negative and positive control. Then it was allowed to incubate at 37 for 18 to 24 hours. Finally, the presence of growth was indicated by turbidity and absence of growth was confirmed by clear solution at the end of incubation period. The lowest concentration of the extract showing no growth was regarded as the MIC. All the experiments were performed in triplicates for each bacterium to get the average value.

Determination of Minimum Bactericidal Concentration

The MBC is defined as the lowest concentration of antimicrobial agent that killed

99.9% of the final inoculum after incubation for 18 to 24 hours at 37°C. It was determined by aseptically sub-culturing the contents of test tubes from the MIC results for individual bacterium to antimicrobial free agar as described in different studies [20]. In this technique, the contents of all test tubes containing a concentration of test material above the MIC value from each triplicate was streaked using a sterile wire loop on MHA aseptically and incubated at 37°C for 24 hours. The lowest concentration of the extract which showed no bacterial growth after incubation was MBC. The average value was taken for the MBC of test plant crude extract against each bacterium. It was performed in triplicate to take average.

Preliminary Phytochemical Screening

The crude ethanol extracts of *V. myriantha* leaves, leaf crude ethanol and chloroform extract of O. *rochetiana* and crude ethanol extracts of steam-bark of *O. rochetiana* were screened for the presence of alkaloids, saponins, tannins, steroid, terpenoid, phenol, steroidal glycoside and flavonoids in accordance to standard phytochemical screening methods.

Phenol test

200mg of plant extract mixed with 3ml of distilled water and pipetted 2ml dissolved extract in another test tube and 0.5 ml of 5% $FeCl_2$ (ferric chloride) formation of intense blue colour indicated the presence of phenol [21].

Test for Triterpenoids

Salkolwski‟s test was used for triterpenoids test. For this, 200mg extract was treated with chloroform and a few drops of concentrated H_2SO_4, acetic acid was added, and the test tube was shaken well and allowed to stand for some time. The formation of deep red colour at junction of two layers indicated the presence of triterpenes [22].

Test for saponin

About 200mg of extract was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent broth. Formation of foam indicated the presence of saponins [22].

Test for tannin

About 200mg of plant extract was treated with few drops of 1% lead acetate and observed for formation of yellowish precipitate confirmed the presence of tannins [22]

Steroid test

200mg extract was dissolved with 10ml chloroform and 10ml conc. H_2SO_4 by sides of the test tube. Formation of red colour in the upper part and Sulphuric acid layer showed yellow with green fluorescence confirmed the presence of steroid [23].

Glycoside test

Plant extract was dissolved in glacial acetic acid heated in steam then allowed to cool and treated with 2 drops of 5% $FeCl_3$ (ferric chloride) and 2ml conc. H_2SO_4. Formation of reddish-brown colour ring was observed at junction of two layers confirmed the presence of steroidal glycosides [23].

Test for resins (Turbidity Test)

Ten milliliters of distilled water were added to 1 g of dried extracts, and ultrasonicated for 15 min at 30 °C. The mixture was filtered. Occurrence of turbidity showed the presence of resins [24].

Data Analysis

Data were analysed by using SAS version 9.1.3 (SAS institute 2003) software. The experimental data were expressed as mean plus-or-minus standard error of the mean (mean±SEM) and statistical significance was considered at 95% confidence interval ($P<0.05$). The statistical differences of the mean zone of inhibition of aqueous, ethanol and chloroform crude extract of the three plants leaves and stem-bark of *O. rochetiana* for individual bacteria was carried out by employing one-way analysis of variance (ANOVA) followed by Tukey test.

■ Results and Discussion

Yield of Extracts

The aqueous extract of *V. myriantha* have the highest percentage yield (18.0%) and followed by *O. rochetiana* ethanol leaf extract (15%). The least yield (5.5%) was obtained from chloroform extract of *O. rochetiana* stem-bark (Table1). From the data, it is possible to conclude that the leaf of V. *myriantha* contained more polar compounds compared to *O. rochetiana*. Similarly, the highest (15.0%) yield was obtained from ethanol extracts of *O. rochetiana* leaves whereas the lowest yield (8%) was obtained from stem bark extract. In comparison with other solvents, water yielded more crude extract from leaf and stem-bark of tested plants. In general, the yield obtained from these plants is quite adequate and future work on drug development appears economically feasible (Table1).

Table 1: yields of crude extracts of leaves of V. myriantha and leaves and stem-bark O. rochetiana

Plant	Solvent	Yield (%)
O. rocheatiana leaves	C_2H_5OH	15.0
	H_2O	10.5
	$CHCl_3$	9.0
O. rochetiana stem-bark	C_2H_5OH	8.0
	H_2O	12.0
	$CHCl_3$	5.5
V. myriantha leaves	C_2H_5OH	14.0
	H_2O	18.0
	$CHCl_3$	8.0

Key %: percent; C_2H_5OH: ethanol; $CHCl_3$: chloroform; H_2O: water

Antibacterial Susceptibility Result

The extracts showed varying degree of inhibitory effect against *S. aureus*, *E. coli*, *P. aeruginosa* and *S. typhi* at a concentration of 250mg/mL (Table2). The ethanol and chloroform crude extracts of *O. rochetiana* leaves exhibited significant antibacterial activity against all clinical and standard bacterial strains tested, although the aqueous extract failed. The ethanol leaf extract of this plant exhibited significantly higher inhibitory effect, compared to chloroform crude extract, on both clinical and standard strains. The findings showed that among the tested clinical strains, *S. aureus* was the most susceptible strain and *P. aeruginosa* the least for *O. rochetiana* leaf ethanol extract. Standard *S. aureus* and *E. coli* strains were highly susceptible whereas *P. aeruginosa* again was the least compared to the positive control (chloramphenicol). The inhibitory zone diameter ranged from (20.33±0.57mm) to (22.66±1.15mm) on clinical *P. aeruginosa* and clinical *S. aureus* respectively. The chloroform leaf extract also showed a potent antibacterial growth activity despite its comparatively lower effect than the ethanol extract. Its inhibition zones were (19±1.73 mm) and (22.6±2.51mm) for clinical strains of *P. aeruginosa* and *S. aureus* respectively. As reported [25] 80% methanol crude extract of *O. rochetiana* at concentration of 100mg/ml revealed inhibitory effect on *S. aureus* (25±00mm), *E. coli* (19±0.8mm) and *P. aeruginosa* (22±1.0mm).

Table 2: Antibacterial activity of leaves extracts of O. rochetiana at conc. of 250mg/mL

Bacterial strain	Inhibition zone (mm)±SEM			
	C_2H_5OH	$CHCl_3$	H_2O	Chlora(0.3mg/mL)
Clinical E. coli	21.66b±1.88	20.33b±0.57	-	29.33a±1.15
Standard E. coli	24.33b±0.57	25.33b±0.57	-	31.33a±1.15
Clinical P. aeruginosa	20.33b± 0.57	19.00bc±1.73	-	20.00a±1.73
Standard P. aeruginosa	20.66a±1.15	18.00b±0.00	-	21.66a±3.05
Clinical S. aureus	22.66a±1.15	22.66b±2.51	-	31.33a±1.52
Standard S. aureus	24.33b±1.15	20.00c±1.00	-	31.66a±1.52
Standard S. typhi	25.66b±0.577	23.00c±1.00	-	30.66a±1.15

Key C_2H_5OH: ethanol; $CHCl_3$: chloroform; H_2O; Chlora: chloramphenicol -: no inhibition zone (the aqueous extract didn"t show inhibitory effect on tested bacterial strains.) Treatment mean in the same row having the same subscript have no significant difference (at P-value)

The stem-bark of *O. rochetiana* ethanol extract exhibited growth inhibitory effect on both clinical and standard strains of the test organisms. The inhibitory zone diameter of ethanol extract of *O. rochetiana* ranged from (19.00±1.73mm) to (22.66±1.08mm) on clinical *P. aeruginosa* and clinical *S. aureus* respectively. Among all clinical strains tested, *S. aureus* was the most susceptible one and *P. aeruginosa* the least susceptible though it showed relatively significant inhibition compared to the positive control. The least inhibition zone was observed on standard *P. aeruginosa* (16.66±1.15mm) and the highest on standard *E. coli* (20.66±1.15mm). Both aqueous and chloroform crude extracts of stem-bark of *O. rochetiana* did not show any growth inhibitory effect (Table 3). Inability of growth inhibition of aqueous and chloroform extracts might be because of insufficiency of phytochemicals in the crude.

Table 3: Antibacterial activities of crude stem-bark ethanol, chloroform and aqueous extracts of O. rochetiana at a conc. of 250mg/mL

Bacterial strain	Inhibition zone (mm)±SEM			
	C_2H_5OH	$CHCl_3$	H_2O	Chlora (0.3mg/mL)
Clinical E. coli	18.00c±0.00	-	-	29.33a±1.15
Standard E. coli	20.66c±1.15	-	-	31.33a±1.15
Clinical P. aeruginosa	17.33c±1.15	-	-	21.00a±1.73
StandardP. aeruginosa	16.66b±1.15	-	-	20.66a±3.05
clinical S. aureus	19.00c±1.08	-	-	31.33a±1.52
Standard S. aureus	19.33c±0.57	-	-	31.66a±1.52
standard S. typhi	23.33c±2.08	-	-	30.66a±1.15

Key C_2H_5OH: ethanol; $CHCl_3$: chloroform; H_2O: water; Chlora: chloramphenicol; -: no inhibition zone; Treatment mean in the same row having the same subscript have no significant difference at p-value.

Table 4: Antibacterial activities of crude leaves extracts of V. myriantha at a concentration of 250mg/mL

Bacterial strain	Inhibition zone (mm)±SEM			
	C_2H_5OH	$CHCl_3$	H_2O	Chloral (0.3mg/mL)
Clinical E. coli	-	-	-	29.33a±1.15
Standard E. coli	-	-	-	31.33a±1.15
Clinical P. aeruginosa	-	-	-	21.00a±1.73
Standard P. aeruginosa	-	-	-	20.66a±3.05
Clinical S. aureus	21.00b±1.73	-	-	31.33a±1.52
Standard S. aureus	22.00b±0.00	-	-	31.66a±1.52
Standard S. typhi	-	-	-	30.66a±1.15

Key: C_2H_5OH: ethanol; $CHCl_3$: chloroform; Chlora: chloramphenicol; - : no inhibition zone; Treatment mean in the same row having the same subscript have no significant difference at (p- value <0.0001).

However, ethanol crude extract revealed significant growth inhibition effect, the positive control (chloramphenicol) showed highest inhibition zone in comparison to ethanol crude extract. However, there were significant differences between the activities of either type of the above extracts and that of the positive control ($p<0.0001$). Chloramphenicol, the semisynthetic broad-spectrum antibiotic, showed the highest inhibition zones, 29.33mm±1.15 and 31.33mm±1.15 on clinical *E. coli* and *S. aureus* strains respectively. On the contrary side, both clinical and standard *P. aeruginosa* stains were least susceptible to this antibiotic with inhibition zones of 20.00mm±1.73 and 21.66mm±2.05 respectively, which was almost comparable inhibition zone with the ethanol leaf extract. Antibacterial assay of clinical and standard bacterial strains with crude extract of *V. myriantha* revealed that only ethanol extract showed significant growth inhibitory effect only on standard and clinical isolates of *S. aureus* having inhibition zones of diameter 22±00mm and 21±1.73mm respectively (table 5). Aqueous

and chloroform crude extracts had no inhibitory effect on all tested bacterial strains. The chloramphenicol, however showed the highest growth inhibition zone (21mm to 31mm) on clinical strains of *E. coli* and *S. aureus* respectively. Also, the standard strains showed nearly equal growth inhibition zones with respective clinical strains.

As reported by [26], chloroform crude extract of *V. auriculifera* inhibited growth of *S. aureus* (17.66mm), *E. coli* (18.20mm) *S. typhi* (13mm) at concentration of 100mg/mL and methanol extract at the same concentration inhibited *S. aureus* (19.40mm), *E. coli (*20.60mm) and *S. typhi* (14.30mm). Ethanol extract of *V. auriculifera* exhibited antibacterial activity against bacteria in a previous study [27]. Ethanol extract of another species of the genus, *V. amygdilina,* showed antibacterial activity similar to results reported by different studies on the same genus [28].

Neither any clinical nor standard bacterial strains were inhibited by the leaf extracts of *D. laxata* 250mg/mL concentration. However, positive control showed highest inhibition zone ranging from (21mm) to (31mm) on clinical *P. aeruginosa* and clinical *S. aureus* respectively. Overall, the extracts showed the lowest activity on standard *P. aeruginosa* and highest against standard *S. aureus*. For *O. rochetiana* leaf the ethanol extract showed a lower MIC compared to that of chloroform against all bacterial strains. While the MIC against both clinical and standard *S. aureus* strains was 1.95mg/ml for the ethanol extract, it was 7.8mg/mL for the chloroform. Similarly, the ethanol extract exhibited an MIC of 7.8mg/mL against both clinical and standard strains of *P. aeruginosa* which was much higher than what was required for *S. aureus* inhibition. S. *aureus* was the most inhibited test organism with an MIC of 1.95mg/mL of ethanol *O. rochetiana* leaf extract and the least inhibited organism was *P. aeruginosa* (MIC = 7.8mg/mL). Chloroform extract of *O. rochetiana* leaf showed lowest MIC 3.96mg/mL against *S. aureus* and the highest MIC was recorded on *P. aeruginosa* (15.62mg/mL). On other hand, the ethanol extract exhibited an MIC of 3.9mg/ml and the chloroform extract 7.8mg/mL for clinical and standard *E. coli* (Table 5).

Ethanol crude extract of *O. rochetiana* leaf had the lowest MBC on both standard and clinical strains of *S. aureus* (31mg/mL*)* and highest MBC was observed on *P. aeruginosa* (125mg/mL). Chloroform extract of *O. rochetiana* leaf exhibited an MBC on both clinical and standard strains of *S. aureus, E. coli* and clinical *S. typhi* (62.5mg/mL) whereas the highest MBC (125mg/mL) was shown for *P. aeruginosa*. As noted by [25], 80% methanol crude extract of *O. rochetiana* leaf exhibited MIC values on *S. aureus* 5mg/mL, *P. aeruginosa* 2.5mg/mL and *E. coli* 10mg/mL. [29] reported that the MIC of *S. typi, E. coli* and *P. aeruginosa* were 50mg/ml, 6.25mg/ml and 250mg/ml respectively the same work reported MBC values for *S. typhi, E. coli* and *P. aeruginosa* 75mg/mL 12mg/ml and 75mg/mL respectively. In comparison with the current study, higher MIC value was reported for *S. aureus* and *E. coli* but lower for *P. aeruginosa* by same authors. This apparent disagreement may be due to differences in the procedures followed, solvents used and agro-ecology of the plants tested. Study reported by [30] reported that ethanol crude extract of *O. rochetiana* leaf at concentration ranging from 128µg to 512µg didn‟t show growth inhibition on standard strains of *E. coli, S. aureus* and *P. aeruginosa.*

Ethanol crude extract of *O. rochetiana* steam-bark revealed the lowest MIC value on *S. aureus* (3.9mg/mL), highest on *P. aeruginosa* (15.62mg/mL) and comparatively moderate (7.8mg/mL) was shown on *E. coli*. The most susceptible bacterium was *S. aureus* whereas *P. aeruginosa* was the least. The lowest MBC was observed on both strains of *E. coli, S. aureus* and standard *S. typhi* (62.5mg/mL) whereas the highest MBC was exhibited by both clinical standards of *P. aeruginosa* (125mg/mL). In comparison with leaf ethanol crude extract, leaf ethanol crude extract exhibited lowest MIC value (1.95mg/mL) on both clinical and standard strains of *S. aureus.* The MIC obtained for the ethanol steam-bark extract was 3.9mg/ml on the same bacterial strains. It is possible to conclude that more bioactive compounds are found on leaf than the steam-bark of this particular plant species. *V. myriantha* leaf ethanol extract had an MIC of 31.25mg/mL and MBC 125mg/mL on *S. aureus,* but it did not show any growth inhibitory activities on the other bacterial strains tested.

Table 5: MIC and MBC of crude ethanol and chloroform extracts of O. rochetiana leaves and stem-bark, and V. myriantha leaves on pathogenic bacteria

Bacterial strain	Crude extracts in (mg/mL)						
		O. rochetina leaf		O. rochetiana bark		V. myriantha	
	Solvent	MIC	MBC	MIC	MBC	MIC	MBC
Clinical E. coli	C_2H_5OH	3.90	62.00	7.8	62.5	ND	ND
	$CHCl_3$	7.81	62.50	ND	ND	ND	ND
Standard E. coli	C_2H_5OH	3.90	62.50	7.8	62.5	ND	ND
	$CHCl_3$	7.81	62.50	ND	ND	ND	ND
Clinical P. aeruginosa	C_2H_5OH	7.80	62.50	15.62	125	ND	ND
	$CHCl_3$	15.62	125.00	ND	ND	ND	ND
Standard P. aeruginosa	C_2H_5OH	7.80	62.50	15.62	125	ND	ND
	$CHCl_3$	15.62	125	ND	ND	ND	ND
Clinical S. aureus	C_2H_5OH	1.95	31.25	3.9	62.5	31.25	125
	$CHCl_3$	3.90	62.50	ND	ND	ND	ND
Standard S. aureus	C_2H_5OH	1.95	31.25	3.9	62.5	31.25	125
	$CHCl_3$	3.90	62.50	ND	ND	ND	ND
Standard S. typhi	C_2H_5OH	3.90	62.50	7.8	62.5	ND	ND
	$CHCl_3$	7.81	62.50	ND	ND	ND	ND

Key: C_2H_5OH: ethanol; $CHCl_3$: chloroform: ND ; Not done

Table 6: Preliminary phytochemical screening results for O. rochetiana and V. myriantha

Secondary Metabolites	Plant species, its part and extraction solvent					
	O. rochetiana leaves		O. rochetiana stem-bark		V. myriantha leaves	
	C_2H_5OH	$CHCl_3$	C_2H_5OH	$CHCl_3$	C_2H_5OH	$CHCl_3$
Phenol	+	-	+	-	-	-
Tannin	+	+	+	+	-	-
Steroid	+	+	+	+	-	-
Saponin	+	-	-	-	-	+
Terpenoid	+	+	+	+	+	+
Glycoside	+	+	-	+	+	+
Resin	-	-	-	-	-	-

Key: -: the specific metabolite not detected; +: the specific metabolite detected

Phytochemical Constituents of the Plants

Phytochemical is a broad term meaning plant (phyto) chemical referring to a wide variety of compounds that occur naturally in plants. Positive reactions of phytochemical screening indicate the presence of active compounds in the extracts of medicinal plants. Therefore preliminary phytochemical screening of leaves of *O. rochetiana* , *V. myriantha*, and steam-bark of *O. rochetiana* revealed the presence of different secondary metabolites. There were metabolites that were detected in all extract types of both plants as well as in none. Some were detected only in either of the plant species or extract type. Terpenoids were detected in both ethanol and chloroform extracts of the leaves of both plants and stem-bark of *O. rochetiana*. Similarly, glycosides were extracted by both solvents, in both plants except in the ethanol extract of *O. rochetiana* stem-bark. On the other hand, while tannins and steroids were detected in both ethanol and chloroform extracts of only *O. rochetiana* leaf and stem-bark. A positive test for phenols was obtained on both leaf and stem-bark only for the ethanol extract of *O. rochetiana*. *Saponins* were recorded only from the ethanol extract of *O. rochetiana* leaf and chloroform extract of *V. myriantha.* The preliminary phytochemical screening of *V. auriculifera* from the same genera showed positive test result for the presence of saponins, tannins, alkaloids, flavonoids, terpenoids, and phenolic compounds [26]. Therefore, the

phytochemical screening result reveals that the presence of these phytochemical constituents supports the use of *V. myriantha* and *O. rochetiana* in traditional medications.

Various studies that assessed the antimicrobial activities of the class of phytochemicals listed here in reported the potential of each class of compounds in inhibiting the growth of different microorganisms. Phenolics and polyphenols are among these classes of compounds reported in the literature for having such potential. As noted by [31] eugenol, caffeic acid catechol and pyrogallol were detected to have antibacterial and antifungal effect through reaction of sulfhydryl groups or more non-specific reactions with proteins is thought to be the possible mechanism for phenolic effect to microorganisms. Flavonoids and flavonoid-derived plant natural products have been known to function as antimicrobial agents. As different *in vitro* studies have indicated these molecules are effective antimicrobial substances against a wide spectrum of microorganisms [32]. Intake of tannin comprising beverages, particularly green teas and red wines, was suggested to heal or preclude many varieties of microbial infections [33]. Tannins can cause complexes of proteins through hydrogen bonding, hydrophobic effects and by formation of covalent bonds. A review on the antimicrobial properties of tannins indicated that they inhibit growth and protease activity in many ruminal bacteria. It is reported that tannins bind to cell coat polymers in all strains [34]. They also cause morphological changes in the organisms indicating that the cell wall is the main target of tannin toxicity [35]. Terpenoids and essential oils are other groups of compounds reported to have antimicrobial activities. Results of many studies indicate that terpenes and terpenoids were active against bacteria and fungi. The diterpenoids and sesquiterpenes obtained from *Salvia sclarea* were found to be active against *S. aureus* and the yeast *Candida albicans*. Two terpenoid constituents capsaicin and petalo stemumol were also shown to have an excellent activity against various strains of bacteria and fungi [36].

Conclusion

The ethanol and chloroform extracts of the leaf of *O. rochetiana* and ethanol extract of *O. rochetiana* stem-bark were active against both clinical and standard strains of pathogenic bacteria (*E. coli, P. aeruginosa, S. typhi* and *S. aureus*). But ethanol extract of *V. myriantha* were active only on clinical and standard strains of *S. aureus.* Preliminary phytochemical screening test from ethanol crude extract of *O. rochetiana* leaves revealed the presence of phenol, tannin, steroid, saponin, terpenoid and glycoside on other hand from the same plant part, chloroform crude extract showed presence of tannin, terpenoid and glycoside. Whereas ethanol extract of stem-bark showed presence of phenol, tannin, steroid and terpenoid. The presence of these important phytochemicals in the plants is a justification of the plant use in the traditional treatment against various diseases impacting humans and animals. The presence of these phytochemicals in those plants enhances their pharmaceutical and therapeutic potentials. In conclusion, the study suggests that the extracts of *Vernonia myriantha and Olinia rochetiana* can be used in the control of bacterial transmitting diseases because their anti-bacterial activities was high comparable with the reference.

Conflict of Interest

The author declare there is no conflict of interest.

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