



Antibacterial activities of aqueous and ethanolic leaf extracts of *Abrus precatorius* against urinary tract pathogens and *Listeria monocytogenes* isolated from vegetables

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Abstract

Medicinal plants have been used traditionally to treat infectious diseases since the origin of mankind. The increase in multidrug resistant organisms associated with conventional agents has ignited a lot of interest in traditional medicine. The aim of this study was to determine the antibacterial activities of ethanolic and aqueous extracts of *Abrus precatorius* leaf against ten clinical microbial isolates mostly (except *Listeria monocytogenes* isolated from leafy vegetable) recovered from urinary tract infected patients. The broth micro-dilution technique was used for the assay. The ethanolic extract was inhibitory to *K. pneumonia*, *P. aeruginosa*, *E. faecalis*, *S. aureus*, *L. monocytogenes* and *P. mirabillis* with minimum inhibitory concentration (MIC) (mg/ml) values of 23.4 ± 7.8 to 46.9 ± 15.6 , 31.3 ± 0.0 to 46.9 ± 15.6 , 23.4 ± 7.8 to 156.3 ± 93.8 , 23.4 ± 7.8 to 250.0 ± 0.0 , 140.6 ± 109.4 and 46.9 ± 15.6 respectively. The aqueous extract was also inhibitory to *K. pneumonia*, *P. aeruginosa*, *E. faecalis*, *S. aureus*, *L. monocytogenes* and *P. mirabillis* at MICs (mg/ml) of 15.6 ± 9.4 , 15.6 ± 9.4 , 7.8 ± 4.7 to 9.4 ± 3.1 , 4.7 ± 1.6 - 4.7 ± 1.6 , 37.5 ± 12.5 and 9.4 ± 3.1 respectively. While the ethanolic extract was bactericidal to *S. aureus* STAPH₂, *L. monocytogenes* and *P. mirabillis*, the aqueous extract was only bactericidal to *P. aeruginosa* PSDO₁ and *S. aureus* STAPH₁. Ofloxacin and gentamycin used as positive controls were effective against all isolates tested at microgram concentrations. The present *in vitro* study scientifically authenticates the traditional use of extracts of *Abrus precatorius* leaf for treatment of some bacterial infections in our region.

Keywords: *Abrus precatorius*, antibacterial, leaf extract, bacteria, urinary tract pathogens

1. Introduction

Plants as source of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. Plants are the largest biochemical and pharmaceutical stores ever known on the planet earth. These living stores are able to generate endless biochemical compounds. Of the 250,000 to 500,000 plants species known to be available on earth today, only a small portion (1-10%) are used by humans and animals [1]. According to the World Health Organization, plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population [2]. Over 50% of all modern clinical drugs are derived from plants [3]. Plant extracts are highly efficient against microbial infections because many of them are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids [4-6] which have been found to have antimicrobial properties [7]. For thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs. Antibiotics provide the main basis for the therapy of microbial infections. Since the discovery of these antibiotics and their use as chemotherapeutic agents, there was a belief in the medical fraternity that this would lead to

the eventual eradication of infectious diseases. However, over use of antibiotics has become the major factor for the emergence and dissemination of multidrug resistant strains of several groups of microorganisms [8, 9]. The recent failure of antibiotics due to the dramatic emergence of multidrug resistance pathogens and the rapid spread of new infections, have prompted World Health Organization and many pharmaceutical industries all over the world to change their strategy and shift more attention to plants as potential alternative sources of natural antimicrobials with different modes of action [10]. Besides, plant based antimicrobial compounds are cheaper and more affordable, and are safer as they have minimal or no side effects.

Abrus precatorius leaf is a member of papilionaceae family and known in various communities with different names. The names include cat's eye, bead tree, rosary pea and jeoquity bean [11]. It is a leguminous slender perennial climber that twines around trees, shrubs and hedges with glabrous internodes and leaves [12]. A pharmacological study has shown that *Abrus precatorius* possesses various biological activities such as antimicrobial, anticancer, antidiabetic, antifertility, anti-inflammatory, antioxidant, antiarthritic etc. [13]. A wide range of active components including a glycoside, abrusic acid, haemagglutinin, a quantity of urease, glycoside abralin and

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albuminous substance 'abrin', the active principle, have been isolated from the plant.

The urinary tract is one of the most common sites of bacterial infection in humans [14]. Lower urinary tract infection (UTIS), such as cystitis, are typically characterized by symptoms including frequency, urgency, and dysuria [15]. If left untreated, these infections can progress to an upper UTI, known as acute pyelonephritis or kidney infections, which can be associated with additional symptoms such as fever, nausea vomiting, and flank pain. These infections also carry the risk of possible progression to bacteraemia. An estimated 40% of women and 12% of men will experience a symptomatic UTI during their lifetime, and approximately a quarter of affected women will suffer recurrent UTI within 6-12 months [16]. Infants and children are also susceptible to UTI. Febrile UTIs in children tend to be associated with vesicoureteral reflux and the potential for renal scarring. Paediatrics UTI might predispose patients to adult disease [17]. In 2006, UTIs were the cause of more than 11 million physician visits, 1.7 million emergency room visits, and half a million hospitalizations; the societal cost of these infections is 3.5 billion dollars annually in the U. S. alone [18]. UTIs can be classified as uncomplicated or complicated. Uncomplicated infections occur in patients who are otherwise considered healthy. Complicated UTIs, on the other hand, occur in patients who are compromised in some way, for example, if they have anatomical or functional abnormalities in the urinary tract are suffering from another illness, are immunocompromised or, undergoing long-term catheterization. The vast majority of UTIs are caused by *Escherichia coli*. By contrast, complicated UTIs especially those associated with catheterization might be poly microbial [19]. These infections are typically caused by *Proteus mirabilis*, *Klebsiella pneumonia*, *E. coli*, *Pseudomonas aeruginosa*.

The main aim of this work was to isolate and identify bacteria from human urine samples, and to determine their susceptibility along with other clinical bacterial isolates to *Abrus precatorius* leaf extract and other conventional antibiotics.

2. Materials and methods

2.1. Source of test organisms

Isolates tested include *Klebsiella pneumonia*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Proteus mirabilis* and *Listeria monocytogenes*. Except *L. monocytogenes* isolate, all other isolates were recovered from UTI patients in Nsukka geopolitical zone. The organisms were confirmed using a battery of biochemical tests and gram staining. Informed consent was obtained from human volunteers who participated in this study before collection of samples leading to the isolation of bacterial isolates. Ethical protocols according to <http://www.wma.net/en/30publications/10policies/b3/17c.pdf> were adhered strictly while performing experiments.

2.2. Identification and characterization of isolates

The experiment was performed in the Department of Microbiology, University of Nigeria, Nsukka, Nigeria. After the organisms have grown, microscopic examination was carried out on which many shapes, size, colour, and member of colonies were noted and recorded. Sub-culturing was also done for each growth on nutrient slants by collecting the single colonies from the master plate. Sub-culturing was done to obtain a pure culture of each organism. From these pure cultures, microscopic examination and biochemical tests were performed.

2.3. Collection and extraction of plant material

Healthy disease-free, matured fresh leaves of *Abrus precatorius* were collected locally from Ezeagu, Udi Local Government Area of Enugu State, Nigeria and identified by an experienced botanist in the department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria. The collected leaves were washed thoroughly two to three times with tap water, rinsed with distilled water and dried under mild sunlight. After drying, the dried leaves were grinded into fine powder using an electric blender. This process breaks the leaves into smaller pieces thus exposing the internal tissues and cells to solvents thus facilitating their easy penetration into the cells to extract the constituents. Ethanol (95%) and warm water were used for the extraction of the plant material using cold maceration method as previously described by Oyagede *et al.* [20] with slight modification. Briefly, one hundred grams (100) of the finely blended dried leaves were extracted in 500 mL of 95% of ethanol and warm water respectively and allowed to stand for 24 hr. The mixtures were separated and concentrated by allowing the solvent to vaporise naturally so that the aqueous rich extract will be obtained.

2.4. Preparation and standardization of the inocula

The McFarland standard was prepared by addition of 0.5 mL of 0.048 M BaCl₂ (1.17% w/v BaCl₂.H₂O) to 99.5 mL of 0.18 H₂SO₄ (1% v/v with constant stirring). This was followed by the adjustment of the turbidity of the solution at 625 nm which equals 0.5 McFarland standard (absorbance between 0.008 to 0.10). This was transferred into screw-cap tubes that have the same size and volume as those used in growing the broth cultures. The tubes were sealed to prevent any loss by evaporation and stored in the dark at room temperature.

Stock inoculum suspensions were prepared by taking five colonies from 24 hr culture and putting into 5 mL sterile saline. Each suspension was shaken for 15 s and density adjusted visually to 0.5 MacFarland turbidity standard by adding more culture when the suspension is light or diluting with sterile saline when it is too turbid.

2.5. Antimicrobial activity of the extracts

2.5.1. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was determined by using broth tube micro-dilution method, a modified method of Andrew [21] that is in accordance with Clinical Laboratory Standard Institute approved guideline for testing bacteria isolates [22]. Serial dilutions of *Abrus precatorius* ethanolic extract (stock solution of 1000 mg/mL) and aqueous (stock solution of 100 mg/mL) extracts were made in test tubes containing 1 mL of Mueller Hinton broth (MHB) to give final concentrations between 500 - 15.6 mg/mL and 50 to 1.3 mg/mL (achieved by adding 1 mL of extract to 1 mL of Mueller Hinton broth and then serially transferring 1 mL from it to the next tube and so on). About 1 mL was removed from the last tube. About 20 μ L of the standardized test organisms were dispensed into the tubes. The negative control tubes were Mueller Hinton broth with different concentrations of each extract, with no organism and the positive control was Mueller Hinton broth with the test organism. Similarly, different concentrations of ofloxacin and gentamycin (128 - 4 μ g/mL) were made with Mueller Hinton broth and used as positive drug control. The tubes were incubated at 37 °C for 24 hr. These were done in duplicate and reported as mean \pm standard error of mean. The minimum inhibitory concentration was determined by visually inspecting the tubes for turbidity by matching each tube with the corresponding negative control tubes of the same concentration. The MIC was reported as the lowest concentration of the test material, which resulted in 100% inhibition of growth of the test organisms.

2.5.2. Determination of minimum bactericidal concentration (MBC)

The minimal bactericidal concentration of the plant extract and antibiotics were determined by further sub-culturing from the tubes which showed no visible growth in the minimum inhibitory concentration assay into fresh sterile nutrient agar plates. The plates were incubated until growth is seen on the positive control plate. The minimum bactericidal concentration was therefore taken as the lowest concentration or the highest dilution that did not show any visible growth on the sub-cultured nutrient agar plate [21].

3. Results

3.1. Characterization and Identification of the isolates from urine samples

Following the culturing of the samples, gram staining, microscopy and biochemical tests, the following organisms were identified: *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Enterococcus faecalis* (Table 1). *Listeria monocytogenes* was identified in a separate study in our laboratory.

3.2. MICs of *Abrus precatorius* plant extract and other conventional antibiotics tested

Minimum inhibitory concentrations of ethanolic and aqueous extracts of *Abrus precatorius* leaf were obtained for clinical urinary pathogens such as *Staphylococcus aureus*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* in addition to *Listeria monocytogenes* which was isolated from vegetable. Our data showed that the extract had more inhibitory effect against *Staphylococcus aureus* STAPH₁ (23.4 \pm 7.8 mg/mL), *Enterococcus faecalis* ENTRO₁ (23.4 \pm 7.8 mg/mL), and *Klebsiella pneumonia* KLB₁ (23.4 \pm 7.8 mg/mL), while the aqueous extract had more inhibitory effect on *Staphylococcus aureus* STAPH₁ (4.7 \pm 1.6 mg/mL) *Proteus mirabilis* (9.4 \pm 3.1 mg/mL) and *Enterococcus faecalis* ENTRO₂ (7.8 \pm 4.7 mg/mL) (Table 2).

The antibiotic, ofloxacin, had more inhibitory effects on *Klebsiella pneumonia* KLB₂ (4.0 \pm 0.0 μ g/mL), *Pseudomonas aeruginosa* PSDO₂ (6.0 \pm 2.0 μ g/mL), and *Enterococcus faecalis* ENTRO₁ (6.0 \pm 2.0 μ g/mL) (Table 3). Other test isolates were inhibited at higher concentration of the antibiotic. The gentamycin had more inhibitory effects on *K. pneumonia* I (6.0 \pm 2.0 μ g/mL), *K. pneumonia* 2 (1.0 \pm 0.0 μ g/mL) and *E. faecalis* I (16.0 \pm 0.0 μ g/mL), compared to other isolates (Table 3).

3.3. MBC of *Abrus precatorius* plant extract and other conventional antibiotics tested

Out of ten isolates tested, the ethanolic extract was biocidal to only *Staphylococcus aureus* STAPH₂ (500.0 \pm 0.0 mg/mL), *Listeria monocytogenes* (250.0 \pm 109.4 mg/mL) and *Proteus mirabilis* PRO₁ (375.0 \pm 15.6 mg/mL). However, the aqueous extract was only biocidal to *P. aeruginosa* PSDO₁ and *S. aureus* STAPH₁ (Table 1).

As shown in Table 3, ofloxacin and gentamycin were both biocidal to all isolates tested. The ofloxacin was biocidal to *Klebsiella pneumonia* KLB₁ and KLB₂ at concentration of 16.0 \pm 0.0 μ g/mL. It was biocidal to other isolates at the concentration of 128.0 \pm 0.0 μ g/mL. The gentamycin was biocidal to *E. faecalis* ENTRO₁, *K. pneumonia* KLB₁ and *K. pneumonia* KLB₂ at concentrations of 64.0 \pm 0.0, 32.0 \pm 0.0 and 16.0 \pm 0.0 μ g/mL respectively. Just like ofloxacin, it was biocidal to other isolates at concentration of 128.0 \pm 0.0 μ g/mL.

Table 1. Characterization and identification of bacterial isolates

| Sample No. | TIS A | | | | | | | | | | | Gram staining | Organisms |
|--------------------|-------|----|---------|--------|----------|--------|-------|------|-----|------------------|----------------|-------------------------------|-----------|
| | MR | VP | Citrate | Indole | Catalase | Urease | Slope | Butt | Gas | H ₂ S | | | |
| PRO ₁ | + | - | + | + | + | + | Y | Y | - | + | Gram (-) rods | <i>Proteus mirabilis</i> | |
| PSDO ₁ | - | - | + | - | + | - | Y | Y | + | + | Gram (-) rods | <i>Pseudomonas aeruginosa</i> | |
| PSDO ₂ | - | - | + | - | + | - | Y | Y | + | + | Gram (-) rods | <i>Pseudomonas aeruginosa</i> | |
| PSDO ₃ | - | + | + | - | + | - | Y | Y | + | + | Gram (-) rods | <i>Pseudomonas aeruginosa</i> | |
| PSDO ₄ | - | + | + | - | + | - | Y | Y | + | + | Gram (-) rods | <i>Pseudomonas aeruginosa</i> | |
| PSDO ₅ | - | + | + | - | + | - | Y | Y | + | + | Gram(-) rods | <i>Pseudomonas aeruginosa</i> | |
| KLB ₁ | - | + | + | - | + | + | Y | Y | + | - | Gram (-) rods | <i>Klebsiella pneumonia</i> | |
| KLB ₂ | - | + | + | - | + | + | Y | Y | + | - | Gram(-) rods | <i>Klebsiella pneumonia</i> | |
| KLB ₃ | - | + | + | - | + | + | Y | Y | + | - | Gram (-)rods | <i>Klebsiella pneumonia</i> | |
| KLB ₄ | - | + | + | - | + | + | Y | Y | + | - | Gram (-)rods | <i>Klebsiella pneumonia</i> | |
| KLB ₅ | - | + | + | - | + | + | Y | Y | + | - | Gram (-)rods | <i>Klebsiella pneumonia</i> | |
| KLB ₆ | - | + | + | - | + | + | Y | Y | + | - | Gram(-)rods | <i>Klebsiella pneumonia</i> | |
| KLB ₇ | - | + | + | - | + | + | Y | Y | + | - | Gram(-) rods | <i>Klebsiella pneumonia</i> | |
| KLB ₈ | - | + | + | - | + | + | Y | Y | + | - | Gram(-) rods | <i>Klebsiella pneumonia</i> | |
| KLB ₉ | - | + | + | - | + | + | Y | Y | + | - | Gram(-) rods | <i>Klebsiella pneumonia</i> | |
| KLB ₁₀ | - | + | + | - | + | + | Y | Y | + | - | Gram(-) rods | <i>Klebsiella pneumonia</i> | |
| KLB ₁₁ | - | + | + | - | + | + | Y | Y | + | - | Gram(-)rods | <i>Klebsiella pneumonia</i> | |
| KLB ₁₂ | - | + | + | - | + | + | Y | Y | + | - | Gram(-) rods | <i>Klebsiella pneumonia</i> | |
| KLB ₁₃ | - | + | + | - | + | + | Y | Y | + | - | Gram(-)rods | <i>Klebsiella pneumonia</i> | |
| KLB ₁₄ | - | + | + | - | + | + | Y | Y | + | - | Gram (-) rods | <i>Klebsiella pneumonia</i> | |
| KLB ₁₅ | - | + | + | - | + | + | Y | Y | + | - | Gram(-) rods | <i>Klebsiella pneumonia</i> | |
| KLB ₁₆ | - | + | + | - | + | + | Y | Y | + | - | Gram(-) rods | <i>Klebsiella pneumonia</i> | |
| KLB ₁₇ | - | + | + | - | + | + | Y | Y | + | - | Gram (-) rods | <i>Klebsiella pneumonia</i> | |
| KLB ₁₈ | - | + | + | - | + | + | Y | Y | + | - | Gram(-) rods | <i>Klebsiella pneumonia</i> | |
| KLB ₁₉ | - | + | + | - | + | + | Y | Y | + | - | Gram(-) rods | <i>Klebsiella pneumonia</i> | |
| KLB ₂₀ | - | + | + | - | + | + | Y | Y | + | - | Gram(-) rods | <i>Klebsiella pneumonia</i> | |
| KLB ₂₁ | - | + | + | - | + | + | Y | Y | + | - | Gram (-) rods | <i>Klebsiella pneumonia</i> | |
| ENTRO ₁ | - | + | - | - | - | - | Y | Y | - | - | Gram(+) cocci | <i>Enterococcus faecalis</i> | |
| ENTRO ₂ | - | + | - | - | - | - | Y | Y | - | - | Gram (+) cocci | <i>Enterococcus faecalis</i> | |
| ENTRO ₃ | - | + | - | - | - | - | Y | Y | - | - | Gram (+) cocci | <i>Enterococcus faecalis</i> | |
| ENTRO ₄ | - | + | - | - | - | - | Y | Y | - | - | Gram (+) cocci | <i>Enterococcus faecalis</i> | |
| STAPH ₁ | + | + | + | - | + | + | Y | Y | - | - | Gram(+) rods | <i>Staphylococcus aureus</i> | |
| STAPH ₂ | + | + | + | - | + | + | Y | Y | - | - | Gram (+) rods | <i>Staphylococcus aureus</i> | |

Key words

| | |
|--------|---------------------------------|
| MR: | Methyl red |
| VP: | Vogel proskauer test |
| TSIA: | Triple sugar iron agar |
| Y: | Presence of lactose |
| Butt: | Presence of glucose |
| Slope: | Presence of lactose and sucrose |
| PRO: | <i>Proteus mirabilis</i> |
| PSDO: | <i>Pseudomonas aeruginosa</i> |
| KLB: | <i>Klebsiella pneumonia</i> |
| STAPH: | <i>Staphylococcus aureus</i> |

Table 2. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of ethanol and aqueous extracts of *Abrus precatorius* leaf extracts (mg/mL) against some selected bacteria isolates

| Test organisms | Ethanol extract | | Aqueous extract | |
|--|-------------------|-------------------|-----------------|----------------|
| | MIC \pm SEM | MBC \pm SEM | MIC \pm SEM | MBC \pm SEM |
| <i>Klebsiella pneumonia</i> (KLB ₁) | 23.4 \pm 7.8 | > 500 | 15.6 \pm 9.4 | > 50 |
| <i>Klebsiella pneumonia</i> (KLB ₂) | 46.9 \pm 15.6 | > 500 | 15.6 \pm 9.4 | > 50 |
| <i>Pseudomonas aeruginosa</i> (PSDO ₁) | 46.9 \pm 15.6 | > 500 | 15.6 \pm 9.4 | 25.0 \pm 0.0 |
| <i>Pseudomonas aeruginosa</i> (PSDO ₂) | 31.3 \pm 0.0 | > 500 | 15.6 \pm 9.4 | > 50 |
| <i>Enterococcus faecalis</i> (ENTRO ₁) | 23.4 \pm 7.8 | > 500 | 9.4 \pm 3.1 | > 50 |
| <i>Enterococcus faecalis</i> (ENTRO ₂) | 156.3 \pm 93.8 | > 500 | 7.8 \pm 4.7 | > 50 |
| <i>Staphylococcus aureus</i> (STAPH ₁) | 23.4 \pm 7.8 | > 500 | 4.7 \pm 1.6 | 9.4 \pm 3.1 |
| <i>Staphylococcus aureus</i> (STAPH ₂) | 250.0 \pm 0.0 | 500.0 \pm 0.0 | 26.6 \pm 23.4 | > 50 |
| <i>Listeria monocytogenes</i> (LM ₁) | 140.6 \pm 109.4 | 250.0 \pm 109.4 | 37.5 \pm 12.5 | > 50 |
| <i>Proteus mirabilis</i> (PRO ₁) | 46.9 \pm 15.6 | 375.0 \pm 15.6 | 9.4 \pm 3.1 | > 50 |

Key: SEM - Standard error of mean

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of antibiotics (μ g/mL) against some selected bacteria isolates

| Test organisms | Ofloxacin | | Gentamycin | |
|--|-----------------|-----------------|-----------------|-----------------|
| | MIC \pm SEM | MBC \pm SEM | MIC \pm SEM | MBC \pm SEM |
| <i>Klebsiella pneumonia</i> (KLB ₁) | 8.0 \pm 0.0 | 16.0 \pm 0.0 | 6.0 \pm 2.0 | 32.0 \pm 0.0 |
| <i>Klebsiella pneumonia</i> (KLB ₂) | 4.0 \pm 0.0 | 16.0 \pm 0.0 | 1.0 \pm 0.0 | 16.0 \pm 0.0 |
| <i>Pseudomonas aeruginosa</i> (PSDO ₁) | 12.0 \pm 4.0 | 128.0 \pm 0.0 | 32.0 \pm 0.0 | 128.0 \pm 0.0 |
| <i>Pseudomonas aeruginosa</i> (PSDO ₂) | 6.0 \pm 2.0 | 128.0 \pm 0.0 | 64.0 \pm 0.0 | 128.0 \pm 0.0 |
| <i>Enterococcus faecalis</i> (ENTRO ₁) | 6.0 \pm 2.0 | 128.0 \pm 0.0 | 16.0 \pm 0.0 | 64.0 \pm 0.0 |
| <i>Enterococcus faecalis</i> (ENTRO ₂) | 16.0 \pm 0.0 | 128.0 \pm 0.0 | 128.0 \pm 0.0 | 128.0 \pm 0.0 |
| <i>Staphylococcus aureus</i> (STAPH ₁) | 32.0 \pm 0.0 | 128.0 \pm 0.0 | 48.0 \pm 16.0 | 128.0 \pm 0.0 |
| <i>Staphylococcus aureus</i> (STAPH ₂) | 48.0 \pm 16.0 | 128.0 \pm 0.0 | 64.0 \pm 0.0 | 128.0 \pm 0.0 |
| <i>Listeria monocytogenes</i> (LM ₁) | 64.0 \pm 0.0 | 128.0 \pm 0.0 | 80.0 \pm 48.0 | 128.0 \pm 0.0 |
| <i>Proteus mirabilis</i> (PRO ₁) | 64.0 \pm 0.0 | 128.0 \pm 0.0 | 64.0 \pm 0.0 | 128.0 \pm 0.0 |

Key: SEM - standard error of mean

4. Discussion

In this study, the antibacterial activity of ethanol and aqueous extracts of *Abrus precatorius* was evaluated *in vitro* against ten bacterial isolates potentially implicated in urinary tract infections and *L. monocytogenes* isolated from leafy vegetable. The ethanolic extract was inhibitory to *K. pneumonia*, *P. aeruginosa*, *E. faecalis*, *S. aureus*, *L. monocytogenes* and *P. mirabilis* at MIC (mg/mL) of 23.4 \pm 7.8 to 46.9 \pm 15.6, 31.3 \pm 0.0 to 46.9 \pm 15.6, 23.4 \pm 7.8 to 156.3 \pm 93.8, 23.4 \pm 7.8 to 250.0 \pm 0.0, 140.6 \pm 109.4 and

46.9 \pm 15.6 respectively. The aqueous extract was inhibitory to *K. pneumonia*, *P. aeruginosa*, *E. faecalis*, *S. aureus*, *L. monocytogenes* and *P. mirabilis* at MIC (mg/mL) of 15.6 \pm 9.4, 15.6 \pm 9.4, 7.8 \pm 4.7 to 9.4 \pm 3.1, 4.7 \pm 1.6 to 4.7 \pm 1.6, 37.5 \pm 12.5 and 9.4 \pm 3.1 respectively. The extracts were bacteriostatic to most of the isolates tested. A similar result was reported by Aibinu et al. [23] with various extracts of *A. precatorius* (MIC range of 4.7 - 250 mg/mL). These results disagree with those of Parekh and Chanda [24] who showed that Gram-positive bacteria are more sensitive to plant extracts than Gram-negative bacteria. Bobbarala and Vadlapudi [25] reported similar results on the antimicrobial activity of *A. precatorius* seed and showed that it was not

effective against *E. faecalis*. This is similar to our findings. In contrast to our findings, Karamoko et al. [26] reported the biocidal effect of *A. precatorius* against *P. aeruginosa*, *E. coli*, and *S. aureus* at lower concentrations. The antibiotics tested were more effective against all isolates tested compared to the extracts. This is similar to the report by Karamoko et al. [26].

There are reports which show that the difference in sensitivity between strains may be due to the differences in the structure and chemical composition of their cell walls and their membrane permeability [27]. Thus, the antibacterial activity observed in our sample could be due to a combined action of molecules such as alkaloids, phenolic acids, tannins, flavonoids, etc previously shown to be present in our plant extract [28]. Thus, *Abrus precatorius* had bacteriostatic as well as bactericidal activity against the bacteria isolates studied. This work confirms the antibacterial activity of *Abrus precatorius* leaf extract as already reported by other authors [25, 26]. The inhibition of growth of the bacteria isolates tested in this study justifies the different traditional uses of *Abrus precatorius* leaf extract in Nigeria folkloric medicine and offers a scientific basis for the traditional use of water and ethanolic extracts of *Abrus precatorius* leaf. This might be a possible source of new and effective herbal medicines in the treatment of bacterial infections caused by both sensitive and multidrug resistant strains that are involved in many different kinds of human and animal infections. However, more studies are still needed. Aside from *in vivo* studies to confirm our *in vitro* observation in the current experiment, it is necessary to determine the toxicity of the active constituents, their side effects and pharmacokinetic properties.

References

1. Boris RP. Natural products as antimicrobial perspectives from a major pharmaceutical company. *J Ethnopharmacol.* 1996;51:29-38.
2. Nayan RB, Shukla VJ. Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* L. An ethnomedicinal plant. *J Adv Pharm Technol Res.* 2011;2(2):104-9.
3. Kirbag S, Zengin F, Kursat M. Antimicrobial Activities of Extracts of some Plants. *Pak J Bot.* 2009;41(4):2067-70.
4. Nweze EI, Onyishi MC. *In vitro* antimicrobial activities of methanolic and ethanolic fruit extracts of *Xylopia aethiopica* and its combination with disc antibiotics against clinical isolates of bacteria and fungi. *J Rural Tropic Public Health.* 2010;9:1-6.
5. Nweze EI, Okafor JI, Njoku O. Antimicrobial activities of methanolic extracts of *Trema guineensis* (Schumm and Thorn) and *Morinda Lucida* Benth used in Nigeria herbal medicinal practice. *J Bio Res Biotechnol.* 2004;2(1):36-9.
6. Twari F, Singh A. Phytochemical and pharmacological properties of *Gymnema Sylvestre*: an important medical plant. *J Biomed Res Int.* 2004;2(10):30-40.
7. Nweze EI, Eze EE. Justification for the use of *Ocimum gratissimum* L. in herbal medicine and its interaction with disc antibiotics. *BMC Complement Altern Med.* 2009;9:37.
8. Nweze EI, Okafor JI, Njoku, OU. Antifungal activities of pair combinations of extracts from *Morinda lucida* Benth by Decimal additive assay. *J Biol Res Biotechnol.* 2005;3(1):99-103.
9. Anikwe LU, Onoja US, Onyeke CC, Nweze EI. Antimicrobial activities of four varieties of *Capsicum annum* fruits cultivated in Southeast Nigeria against multidrug-resistant and susceptible organisms. *J Basic Pharmacol Toxicol.* 2017;1(2):21-26.
10. Nweze EI, Okafor JI. Antifungal activities of a wide range of medicinal plants extracts and essential oils against *Scedosporium apiospermum* isolates. *Am Eurasian J Sci Res.* 2010;5(3):161-69.
11. Ross IA. Medicinal plants of the world. Chemical constituents, traditional and modern medicinal uses. Humana Press: 2003;1:15-31.
12. Budavari S. The Merck Index an encyclopedia of chemicals, drugs and biologicals. 10th ed. Rahway, New Jersey, Merck and Co., Inc. 1989.
13. Ranjuand M, Vidhya VG, Ramya M. Ethnomedical plant used by the traditional healers of Pachamalai Hills. *Ethnomed J.* 2009;3(1):39-41.
14. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Am J Med Sci.* 2002;113(1):5S-13S.
15. Dibua UME, Onyemerela IS, Nweze EI. Frequency, urinalysis and susceptibility profile of pathogens causing urinary tract infections in Enugu State, Southeast Nigeria. *Rev Inst Med Trop Sao Paulo.* 2014;56(1):55-59.
16. Handley MA, Reingold AL, Shiboski S, Padian NS. Incidence of acute urinary tract infection in young women and use of male condoms with and without nonoxynol-9 spermicides. *Epidemiol.* 2002;13(4):431-6.
17. Zore GB, Awad V, Thakre AD, Halde UK, Meshram NS, Surwase BS et al. Activity-directed fractionation and isolation of four antibacterial compounds from *Abrus precatorius* L. roots. *Nat Prod Res.* 2007;21(10):933-40.
18. Litwinet E. Recurrent urinary tract infection in women. *Int J Antimicrob Agents.* 2007;17:259-68.
19. Hooton TM. Uncomplicated urinary tract infection. *N Engl J Med* 2012;366:1028-1037.
20. Oyagede JO, Awotoye OO, Adewunmi JI, Thorpe HT. Antimicrobial activity of some medicinal plants. Screening for antimicrobial activity. *J Biol Sci Comm.* 1993;2(3):193-7.
21. Andrew JM. Determination of minimum inhibitory concentration. *J Antimicrob Chemother.* 2001;8(1):5-16.
22. CLSI (2005). Performance standards for antimicrobial susceptibility testing; fifteenth informational supplement, Clinical and Laboratory Standard Institute, Wayne, Pa. 2005; M100-S15, vol.25, no.1.
23. Aibinu I, Adelowotan O, Ademipekun E, Odugbemi TO. The *in vitro* antimicrobial activity of *Abrus precatorius* (L) Fabaceae extract on some clinical pathogens. *Niger Postgrad Med J.* 2008;15:32-37.
24. Parekh J, Chanda S. *In vitro* antimicrobial activities of extract of *Launea procumbens* (Lubiaceae), *Vitis vinifera* (Vitaceae) and *Cyperus rotundus* (Cyperaceae). *Afr J Biomed Res.* 2006;9:89-93.
25. Bobbarala V, Vadlapudi V. *Abrus precatorius* seed extracts antimicrobial properties against clinically important bacteria. *Int J Pharm Technol Res.* 2009;1(4):1115-1118.
26. Karamoko O, Zoumana T, Idrissa D, Founzegue CA, Adama C. *In vitro* antibacterial activity of ethanol extracts of *Abrus precatorius* linn (fabaceae) on bacteria

- responsible for nosocomial infections. Int Res J Pharm Appl Sci. 2013;3(1):23-27.
27. Bruce J, MacKenzie FM, Cookson B, Mollison J, Vander Meer JW, Krcmery V et al. Antibiotic stewardship and consumption: findings from a Pan-European hospital study. J Antimicrob Chemother. 2009;64(4):853-860.
28. Rashmi A, Naresh S, Sukhwinder K, Deep J. Phytopharmacological evaluation of ethanolic extract of the seeds of *Abrus precatorius* Linn. J Pharmacol Toxicol. 2011;6(6):580-88.