



***Aframomum melegueta* [Roscoe] K. Schum) Ethanollic Seed Extract against Asymptomatic Bacteriuria Isolates from Pregnant Women Attending Antenatal Clinic in Ondo State, Nigeria**

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The purpose of this research work is to compare the activity of medicinal plant(*Aframomum melegueta*) and conventional antibiotic against Asymptomatic Bacteriuria isolates from pregnant women attending ante-natal clinic in a major primary health center in Akoko, south ,Ondo state Nigeria. The target Asymptomatic Bacteriuria isolates which is inherent in pregnant women with no observable features. The rate of growth/ death of Asymptomatic Bacteriuria Isolates were x-rayed. Bacteria were isolated from Urine of pregnant women attending antenatal clinic in Public health care Akungba Akoko, Ondo state, Nigeria and were identified using conventional method. The antibiotic susceptibility test and antimicrobial screening of ethanol seed extract of *Aframomum melegueta* were determined using disc diffusion and agar well diffusion methods respectively. The eleven(11) bacteria identified were *Klebsiella pneumoniae* (three,3), *Proteus mirabilis*, *Proteus vulgaris*, *Bacillus subtilis*(Two,2),*Pseudomonas aeruginosa*, *Enterobacter cloacae* (Two,2), *Escherichia coli* (three,3), *Bacillus cereus*, *Serratia mercesiensis*, *Enterobacter aerogens*. *E. coli* and *klebsiella pneumoniae* were the most common isolates. The second most common isotales was

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Enterobacter cloacae.. *Klebsiella pneumonia* and *Proteus mirabilis* were resistant to Ampicillin, Ceporex, Nalixadic acid and Septrin. *E. coli*, the most common isolate was sensitive to Gentamycin and most of the antibiotics used. The antimicrobial screening of ethanol seed extract of *Aframomum melegueta* shows zones of inhibition with diameter ranging from 1-25mm. Secondary metabolite screening indicates the presence of flavonoid, tannins, saponins, alkaloids, cardiac glycosides. Ultraviolet spectrophotometer was also used to determine the Growth dynamic /Death rate of the isolates, the addition of antibiotics to the organism at the 48th hour speed up the death rate of the isolates, the addition of ethanol seed extract at the 48th hour also speed up the death rate of the isolates from the urine samples. The results of this study validate the use of *Aframomum melegueta* seed in the traditional treatment of Asymptomatic bacteriuria in pregnant women.

Keywords: *Aframomum melegueta*; asymptomatic bacteriuria.

1. INTRODUCTION

Asymptomatic Bacteriuria refers to the presence of bacteria in the urine of a patient without showing any signs and symptoms. It is a condition in which urine culture reveals a significant growth of pathogens that is greater than 10⁵ bacterial/ml, but without the patient showing symptoms [1]. Asymptomatic bacteriuria is common during pregnancy, the apparent reduction in immunity of pregnant women appears to encourage the growth of both commensal and non-commensal organisms. Asymptomatic bacteriuria is more common among women than among men but few of these women will develop symptomatic infections because the body's normal defense mechanisms prevent symptomatic infection in most cases [2]. In the elderly, it is thought that incomplete bladder emptying contributes to the increased incidence of asymptomatic bacteriuria. Asymptomatic bacteriuria is a common clinical finding. While less than 0.5% of infants and toddlers have asymptomatic bacteriuria, the incidence increases with age. *Escherichia coli* is the most common bacteria identified in asymptomatic bacteriuria [3].

The physiological increase in plasma volume during pregnancy decreases urine concentration and up to 70% pregnant women develop glucose-urea, which encourages bacterial growth in the urine. [4,5]. Other risk factors include multiparity, low educational level, social economic status, diabetes mellitus, age, delayed medical care bad hygiene and nephrolithiasis [6]. Pregnancy in females predisposes such individual to UTIs and they are two times more commonly affected than age-matched non-pregnant females. This is because of urinary stasis due to the effect of progesterone during pregnancy; in addition to various anatomic

changes occurring during gestation [7]. Pregnancy enhances the progression of asymptomatic bacteriuria to symptomatic bacteriuria which can lead to pyelonephritis, urethritis, cystitis, and adverse pregnancy outcomes that includes prematurity, low birth weight and higher foetal mortality rates. These complications seem to result from the effect of bacterial/microbial exotoxins and endotoxins that damage the body's tissues including endothelium, although not all causes associated with them are fully proven [8]. Several factors are thought to increase the likelihood of asymptomatic bacteriuria [9].

Diagnosis of asymptomatic bacteriuria is by urine culture. Either a properly collected clean-catch specimen or a catheterized specimen is acceptable. Urine dipstick for leukocyte esterase will reliably identify pyuria, but it is not specific for asymptomatic bacteriuria. (Pyuria may result from other inflammatory disorders of the genitourinary tract.) Urinary dipstick for nitrites is also of limited usefulness because of infection with non-nitrite-producing organisms, the delay between collection and testing of the specimen, and insufficient time since the last voiding for nitrites to be produced at detectable levels [10]. Urinalysis with microscopic exam for bacteria is a useful, but non-quantitative, way to identify bacteriuria. Pregnant women should be screened for asymptomatic bacteriuria with a urine culture. there are few indications to screen for asymptomatic bacteriuria. Researchers have identified a number of risk factors, including pregnancy:, diabetes, increasing age, immunosuppressive disorders, such as HIV, AIDS, and some cancers, taking medication that suppresses the immune system, undergoing a procedure that affects the urinary tract, receiving a Kidney transplant [11].



Plate 1. *Aframomum melegueta* seeds [24]

The taxonomical classification of the plant is as follows: Plantae (kingdom), Tracheophyta (phylum), Liliopsida (class), Zingiberales (order), Zingiberaceae (family), *Aframomum* (genus) and *Aframomum melegueta* (species). This plant is also known as alligator pepper, grains of paradise, guinea pepper or *melegueta* pepper. It is native to tropical African countries such as Ghana, Nigeria and Cameroon [12,13]. This plant can grow up to 1.5 m in height with orange-coloured lips and pinkish-orange upper flowers that can develop into fleshly and indehiscent pods. The size of the pods are 5-7 cm in length, are edible and contain numerous small, reddish brown seeds with a pungent scent of ginger and cayenne pepper. The stem is short and covered with scars of fallen leaves. The leaves are about 30 cm long, 12 cm wide, and have close nerves underneath [14]. A common condiment in West and North African cuisine, *melegueta* pepper has been used as a spice for meats, sauces and soups. Traditionally, *Aframomum melegueta* is mixed with other herbs for the treatment of common ailments such as body pains, diarrhoea, sore throat, catarrh, congestion and rheumatism in West Africa [15; 16; 12].

Traditionally, *Aframomum Melegueta* is mixed with other herbs for the treatment of common ailments such as body pains, diarrhoea, sore throat, catarrh, congestion and rheumatism in West. It is a perennial herbal plant that is cultivated because of its valuable medicinal and pharmacological effects such as antimicrobial, hepato-protective, anti-cancer and anti-diabetic effects [15;16;12]. With reference to the current

literature, *Aframomum melegueta* contain 6-Gingerol, 8-Gingerol and Methyl-6-Gingerol, 6-Shogaol, 6- and Rac-6-Dihydroparadol, 6-Gingeredione, 2-(5-butylfuran-2-yl) ethyl-2-methoxyphenol, While 6-Paradol is said the active ingredient among these composition of *Aframomum melegueta* extract. Cancer-battling antioxidants, flavonoids, have been found in relative abundance in *Aframomum melegueta* [17].

2. MATERIALS AND METHODS

2.1 Locations / Place of Sample Collection

The samples were collected from pregnant women attending antenatal clinic in Public health care centre, Akungba Akoko, Akoko south-west local government in Ondo state. It has a boundary with neighboring on east- Edo and Delta, on west – Ogun and Osun, on the north – Ekiti and Kogi and south – bright Atlantic Ocean. Ondo state is located on the latitude 5° 45° and 7° 52° and longitude 4° 20° and 6° 05° E. The health care centre is one of the referred maternity hospital in Akoko south west. The health care centre was chosen to enable obtain the required sample size from volunteers.

2.2 Collection of Urine Samples

Mid-stream, clean-catch urine samples were obtained from randomly selected 30 (Thirty) pregnant women attending antenatal clinic. The

Urine samples were collected around 8am in the morning. Sterile wide mouth containers were given to the subjects on arrival at the health care, They were instructed on how to collect their urine specimen, 15 minutes of collection to avoid contamination. The urine specimens were taken to the Microbiology laboratory for immediate analysis and where delay was envisaged, they were preserved in bag refrigerator at -4 °C.

2.3 Collection of *Aframomum melegueta* Seed Extract

Aframomum melegueta seeds were collected across farms in Akungba community Ondo state. The plant part was authenticated at the herbarium of Department of Plant science and biotechnology Adekunle Ajasin University Akungba Akoko, Ondo state, Nigeria.

2.4 Extraction and Preparation of Extract

Aframomum melegueta seed extraction were done using soaking method. The ground sample was extracted using ethanol as solvent. 100g of the powdered sample was extracted with 1000ml of distilled water while 100g of the sample was extracted with 1000ml of 70% ethanol. The sample was soaked overnight for 24 hours. After 24 hours, the sample was filtered ten times with muslin cloth and the extract was collected in a round bottom flask, filtered and concentrated using a rotary evaporator and then oven dried at 70%. The crude extract was thus kept in the refrigerator at -4°C for further screening [18].

2.5 Standardization of Plants *Aframomum melegueta* Seed Extracts

At aseptic condition, the extracts were reconstituted by adding 1g of each extract to 2.5ml of Dimethylsulphoxide (DMSO) and 7.5ml of sterile distilled water, making it 100mg/ml. For each extract, 5ml of distilled water is measured into four sterile bijou bottles. In bijou bottle A, 5ml from the 100mg/ml bijou bottle was drawn and added, making it 50mg/ml. The serial concentration was prepared to get concentrations of 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml respectively [19].

2.6 Test Organism

2.6.1 Source of Clinical test organism

The test organism was isolated from urine samples of pregnant women attending antenatal

clinic at Public Health Care laboratory, Akungba Akoko, Ondo state, Nigeria.

2.6.2 Preparation of Urine sample for culture

9ml of distilled water was dispensed into 5 test tubes and the mouth was corked with cottonwool wrapped with aluminum foil and then sterilized at 121°C for 15 minutes using an autoclave. After sterilization, the water was allowed to cool for few minutes, each test tube was then labeled as 10⁻¹ - 10⁻⁵ respectively. One ml of urine sample was added to the first tube to provide an initial dilution of 10⁻¹ and then, 1 ml of mixture was taken from it and added to the next 9 ml diluents serially, the final dilution for 10⁻⁵.

2.6.3 Bacteriological analysis of Urine sample

Using the pour plate method of inoculation, 0.5ml of the five -fold dilution of 10⁻³ and 10⁻⁵ urine samples (inoculum) was aliquoted into sterile Petri dishes.

MacConkey medium was prepared by dissolving 55g grams of the Agar, into 1 liter of distilled water in a sterile conical flask, corked with cotton and aluminum foil and then homogenized to dissolve. It was sterilized in an Autoclave at a temperature of 121°C for 15 minutes. After the sterilization, the medium was allowed to cool but not solidify. Twenty (20) ml amount was then poured into different sterile Petri dishes containing the 0.5ml of the inoculum and allowed to set at ambient temperature. Then the plates were incubated at 37°C for 48hrs. After 48hrs, the cultural characteristics of the plates were studied and recorded. Colonies isolated from media were checked for significant growth and subcultured on fresh Nutrient agar. The inoculated media were incubated for 24 h at 37°C [19].

2.7 Identification of Isolates

2.7.1 Microscopic examination and biochemical test for identification of urine isolates

Identification of the isolates was based on the colony morphological characteristics, gram staining & biochemical tests [20].

2.7.1.1 Gram staining technique

Working solution of reagents used for the Gramstaining technique was prepared according

to manufacturer's instruction. Staining was carried out by emulsifying approximately one isolated 18- 24hours old colony in a drop of water placed at the centre of a clean grease free slide until a thin smear was made. The smear was air heat fixed by passing the slide through a Bunsen burner flame and then air dried. The heat fixed smear was flooded with a basic aniline dye (crystal violet) for 60 seconds. This was flooded with Lugol's iodine and allowed to remain for 60 seconds. This was then rinsed off with running tap water. The smear was decolorized with 70% ethanol which was immediately washed out to avoid total decolorization. The smear was counter stained with Safranin for 60 seconds, washed off with running tap water and blot-dried. The slide was then examined under oil immersion objective microscope. Organisms that retained the purple colour of crystal violet- iodine complex (CV-1 complex) were recorded as Gram- positive, while those that appeared pink were Gram- negative [21].

2.7.2 Biochemical characteristics of the isolate

2.7.2.1 Catalase test

This test detects the presence of catalase enzyme when present in a bacterium, it catalyse the breaking down of hydrogen peroxide with the release of oxygen as bubble. With a wire loop, a colony was picked from the pure culture and was transferred to the centre of a glass slide. 1- 2 drops of 3% hydrogen peroxide was added to the bacterial isolates. Immediate production of bubbles indicated positive result and if no bubble indicated negative [21].

2.7.2.2 Indole test

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole which then accumulates in the medium for indole production. Bacterial isolates were inoculated in peptone water medium contained in a sterile test .tubes then incubated at 37°C for 48 hours. After the incubation period about 3 drops of kovac's indole reagent was added to the peptone water culture. The bottles were shaken thoroughly and allowed to stand and observed for colour development. A red colour ring at the interface of the medium denotes a positive result. and if the isolate is negative, the reagent layer will remain yellow or slightly cloud [22].

2.7.2.3 Oxidase test

The isolated organisms were inoculated and grown in Nutrient broth for 24 hrs at 37°C. After 24 hrs Oxidase strip was dipped into the broth and colour change was observed. Bacteria Isolates were oxidase positive when the colour changes to purple within 15 seconds to 30 seconds and oxidase negative when the colour did not change [20].

2.7.2.4 Urease test

The Urease test is used to identify those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. In this test each isolate was inoculated into test tubes containing sterilized urea agar medium and incubated at 37°C. The medium was observed for a colour change at 24 hrs and everyday up to 6 days. Urease production was indicated by a bright pink colour throughout the medium [23,22].

2.8 Fermentation of Other Sugars (Dextrose, Lactose and Sucrose) and Hydrogen Sulfide, Gas Production

This test shows the ability of microorganisms to ferment certain sugars. Five sugars were used; mannitol, sucrose, maltose, galactose and fructose using. Sugar is utilized in the presence of oxygen such reactions are known as oxidation. Sterilization of the basal medium was done using an autoclave at 1210C for 15min. Ten percent (10%) sterile solution of the test sugar (glucose, fructose, mannitol, dextrose and galactose) were added, inverted Durham tubes were added into each tube. Different isolates were inoculated into each test tube according to the labeling using a sterile inoculating loop. Uninoculated tubes serve as controls. The results were examined daily for up to 7days in which methyl red indicator changed to yellow. A yellow coloration indicated growth and acid production. Also, the upper part of the Durham tubes were examined to detect any accumulated gas which indicated gas production [24].

2.9 Antibiogram (Antibiotic Susceptibility Test) of Isolates

The test was performed to determine the phenotypic resistant of the bacterial isolates to commonly used antibiotics. These tests were carried out following the Kirby-Bauer disc diffusion method.. Inoculum from culture of

bacteria isolates on nutrient agar slants were inoculated into test tubes containing sterilized nutrient broth and incubated at 37°C for 18h which serve as the stock for the test. Mueller-Hinton agar was prepared and sterilized, then dispensed into sterilized Petri dishes. The plates were allowed to cool for about 15min so as to allow it to gel and excess surface moisture to be absorbed. The inoculum was introduced into plates by streaking before applying the antibiotics impregnated discs. Two types of discs were used; Cephalosporin antibiotic discs (Oxoid); Cefuroxime (30 µg), Cefotaxime (30 µg), Cefoxitin (30 µg), Cefpodoxime (10 µg), Cefepime (30 µg) and Multi-test Predetermined commercial Gram negative and Gram positive discs which were applied to the surface of the well labeled inoculated agar plated aseptically using sterile forceps. The discs were then placed firmly by slightly pressing on the inoculated plates with the sterilized forceps to ensure complete contact with the agar. After 24h of incubation, each plates was examined, susceptibility to each antibiotics were indicated by a clear zone. The zone of inhibition were measured using a calibrated ruler was held on the back of the inverted petri plate and was recorded [25] guidelines [24].

2.10 Antibiogram (Antibiotic Susceptibility Test) of Ethanol Seed Extract *Afr. melegueta*

The antimicrobial screening of *Aframomum melegueta* extract against the bacterial isolates was carried out using the agar well diffusion method. A stock of 100 mg/ml was constituted by dissolving 1g each of the extracts in 50ml of ethanol diluted with sterile distilled water in ratio 1:3. Inoculum from culture of bacteria isolates on the slants were inoculated into test tubes containing sterilized Nutrient Agar broth and incubated at 37°C for 15 hours to achieve the 0.5 McFarland standards. A sterile swab stick was dipped inside the broth suspension of 1ml of 10⁻⁴ sterile water dilution of 24 hours broth culture in the test tube and pressed firmly against the wall to remove excess inoculum and used to streak the entire dried surface of sterile Mueller Hinton Agar plates. Wells were bored on the agar using 6 mm cork borer. 50µl of each concentration of the extracts was poured into each well and incubated at 37°C for 24 hours. The diameter zones of inhibition were measured and recorded in millimeter [25].

2.11 Secondary Metabolite (Phytochemical Screening) of Ethanol Seed Extract *Afr. melegueta*

Phytochemical analysis demonstrated the presence of the common phytoconstituents alkaloids, flavonoids, saponins, tannins, cardiac glycosides and phenols [26].

2.12 Qualitative Method of Ethanol Seed Extract *Afr. Melegueta*

2.12.1 Test for alkaloids

One ml of the *Afr. melegueta* extract was mixed with 5 mL of 2% HCl in a test tube, heated on water bath, and filtered. Of the filtrate, 2 ml was divided into two aliquots of 1 ml each. To the first portion, few drops of Wagner's reagent were added; occurrence of reddish-brown precipitate is taken as a positive test. To the second aliquot, 1 ml of Mayer's reagent was added and appearance of buff-coloured precipitate will be an indication for the presence of alkaloids [27].

2.12.2 Test for phenols

The Million's test was employed. Two mL of *Afr. melegueta* extract was added to 3ml of Million's reagent in a test tube and heated in a water bath for 2 min at 70°C. Pink-red colour formation indicates the presence of phenolic groups [28].

2.12.3 Test for tannins

About 0.5g of the dried powdered *Afr. melegueta* extract was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue black colouration [29].

2.12.4 Test for saponins

(Frothing test). Distilled water (3 ml) was added to 1 ml of the *Afr. melegueta* extract . 0.5 ml filtrate was diluted to 5 mL with distilled water and shaken vigorously for 2 minutes. Formation of stable froth head indicates the presence of saponin [27].

2.12.5 Test for flavonoids

About 1 ml of the *Afr. melegueta* extract, 1 ml of 10% lead acetate solution was added. Appearance of a buff-coloured precipitate indicates the presence of flavonoids [29].

2.12.6 Test for cardiac glycosides

5ml of the *Afr. melegueta* extract were treated with 2ml of glacial acetic acid containing 1 drop of ferric chloride solution (0.1%). This was underlaid with 1ml of concentrated H₂SO₄. A brown ring of the interface indicated a deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring, while in the acetic layer, a greenish ring may form just gradually throughout thin layer [30].

Measurement of growth Dynamic and Death Rate (killing kinetics) of Isolates Using Ultra Violet (UV) Spectrophotometer.

Growth dynamic refers to the rate at which cells of microorganism grow at a given time. This test was done to determine the rate of growth of the isolates as well as their killing kinetics. Colony was picked from the stocked culture slant and inoculated into nutrient broth which was incubated for 24 hours at 37°C. A loopful of organism was picked from the broth culture into nutrient broth in three sets which are set A, B, and C respectively. Ultraviolet spectrophotometer was set at 480λ wavelength, warmed up for 15 minutes and then the control was first read, the first reading was taken at zero hour and it continues after every 12 hours for 8 times. At the 5th reading, which is the 48th hour of set B and set C, ciprofloxacin was added into the set B and ethanol seed extract of *Aframomum melegueta* was added into set C to determine the killing time respectively [31].

3. RESULTS

The organisms were isolated using microscopic and macroscopic examination, biochemical tests and sugar fermentation were used to identify the isolates. Ethanol seed extract of *Aframomum melegueta* was extracted and used for Antimicrobial Assay. Growth dynamic/death rate were determined using Ultraviolet spectrophotometer.

Table 1: Shows the type of sample collected, the numbers of sample, place and time of collection. In this table, Urine sample were collected from pregnant women attending antenatal clinic, 30(thirty) number of sample were collected.

Table 2: Shows the dilution factors of samples and colony count cultured of isolates on MacConkey agar. Each plate contains inoculum in serial dilutions, the highest dilution factors are the 10⁻³ and the lowest dilution factor are the 10⁻⁵

. PHC 1, PHC 2, PHC 3, PHC 4, PHC 8, PHC 11, PHC 16 and PHC 17 has dilution factor 10⁻³, while PHC 5, PHC 7, PHC 9, PHC 14 has dilution factor 10⁻⁵. The table explains the number of colony found in each plate, PHC 11 and PHC 3 has the highest colony count of 88 and 40 respectively, while PHC 8 and PHC 5 has the lowest colony count 10 and 5 respectively.

Table 3: Shows the cultural characteristics and date of analysis of isolates cultured on MacConkey agar. In this table, it was observed that PHC 1, PHC 2, and PHC 8 has same cultural characteristics of rise dark pink and smooth edges. PHC 3, PHC 5, PHC 5, PHC 9, PHC 11, PHC 14, and PHC 16 has cultural characteristics of rise light pink and rough edges. PHC 17 has cultural characteristics of rise light pink and smooth edges. While PHC 4 and PHC 7 has same cultural characteristics of rise creamy and smooth edges.

Table 4: Shows sample code, morphological characteristics and date of analysis of isolates subcultures from the MacConkey plates. In this table, it was observed PHC 1 × 10⁻³, PHC 11 × 10⁻³, PHC 14 × 10⁻³ has morphological characteristics of rise, whitish, swamy, opaque and smooth edges, PHC 2 × 10⁻³, PHC 3 × 10⁻³, PHC 4 × 10⁻³, PHC 5 × 10⁻⁵, PHC 7 × 10⁻⁵, PHC 8 × 10⁻³ has rise creamy, swamy, opaque and smooth edges morphological characteristics. PHC 9 × 10⁻⁵, PHC 16 × 10⁻³ has rise, creamy, swamy, opaque and rough edges.

Table 5: Shows Gram staining and microscopic examination of isolates. It was observed in this table, PHC 1 × 10⁻³, PHC 2 × 10⁻³, PHC 3 × 10⁻³ B, PHC 4 × 10⁻³, PHC 5 × 10⁻⁵, PHC 7 × 10⁻⁵, PHC 8 × 10⁻³, PHC 9 × 10⁻⁵, PHC 11 × 10⁻³, PHC 14 × 10⁻³ B, PHC 16 × 10⁻³ A, PHC 16 × 10⁻³ B, PHC 17 × 10⁻³ A and PHC 17 × 10⁻³ B were all negative to Gram staining, it was observed, PHC 1 × 10⁻³, PHC 5 × 10⁻⁵, PHC 8 × 10⁻³, 11 × 10⁻³, PHC 17 × 10⁻³ A and PHC 17 × 10⁻³ B were all tiny rods, PHC 2 × 10⁻³, PHC 3 × 10⁻³ B, PHC 4 × 10⁻³, PHC 14 × 10⁻³ A, PHC 14 × 10⁻³ B were short rods, PHC 7 × 10⁻⁵, PHC 9 × 10⁻⁵, PHC 16 × 10⁻³ A, PHC 16 × 10⁻³ B were long rods.

Table 6: Shows biochemical tests of recovered isolates. It was observed, PHC 16 × 10⁻³ A was negative to motility test while other isolates were positive to motility test. PHC 1 × 10⁻³, PHC 2 × 10⁻³, PHC 5 × 10⁻⁵, PHC 7 × 10⁻⁵, PHC 8 × 10⁻³, 9 × 10⁻⁵, 11 × 10⁻³, PHC 14 × 10⁻³ B, PHC 17 × 10⁻³ A and PHC 17 × 10⁻³ B were negative to indole

test, PHC 3 × 10⁻³ A, PHC 3 × 10⁻³ B, PHC 4 × 10⁻³, PHC 9 × 10⁻⁵, PHC 14 × 10⁻³ A, PHC 16 × 10⁻³ B were positive to indole test. PHC 1 × 10⁻³, PHC 2 × 10⁻³, PHC 3 × 10⁻³ B, PHC 4 × 10⁻³, PHC 7 × 10⁻⁵, PHC 8 × 10⁻³, PHC 14 × 10⁻³ A, PHC 14 × 10⁻³ B, PHC 16 × 10⁻³ A were positive to urease test, PHC 5 × 10⁻⁵, PHC 9 × 10⁻⁵, PHC 11 × 10⁻³, PHC 16 × 10⁻³ B, PHC 17 × 10⁻³ A and PHC 17 × 10⁻³ B were negative to urease test. PHC 1 × 10⁻³, PHC 3 × 10⁻³ B, PHC 4 × 10⁻³, PHC 7 × 10⁻⁵, PHC 8 × 10⁻³, PHC 9 × 10⁻⁵, PHC 14 × 10⁻³ B, PHC 16 × 10⁻³ B were positive to hydrogen Sulphide test, PHC 2 × 10⁻³, PHC 3 × 10⁻³ A, PHC 5 × 10⁻⁵, PHC 11 × 10⁻³, PHC 14 × 10⁻³ A, PHC 16 × 10⁻³ A, PHC 17 × 10⁻³ A, PHC 17 × 10⁻³ B were negative to hydrogen sulphide

test. PHC 1 × 10⁻³, PHC 2 × 10⁻³, PHC 3 × 10⁻³ A, PHC 9 × 10⁻⁵, PHC 16 × 10⁻³ B were negative to gas production test, PHC 3 × 10⁻³ B, PHC 4 × 10⁻³, PHC 5 × 10⁻⁵, PHC 8 × 10⁻³, PHC 11 × 10⁻³, PHC 14 × 10⁻³ A, PHC 14 × 10⁻³ B, PHC 16 × 10⁻³ A, PHC 16 × 10⁻³ B, PHC 17 × 10⁻³ A, PHC 17 × 10⁻³ B were positive to gas production test.

Table 7: shows the continuation of the biochemical tests of isolated bacteria from Urine samples. In this table, it was observed, PHC 1 × 10⁻³, PHC 3 × 10⁻³ B, PHC 4 × 10⁻³, PHC 5 × 10⁻⁵, PHC 7 × 10⁻⁵, PHC 8 × 10⁻³, PHC 11 × 10⁻³, PHC 14 × 10⁻³ A, PHC 17 × 10⁻³ B were negative to Oxidase test, PHC 2 × 10⁻³, PHC 9 × 10⁻⁵, PHC 14 × 10⁻³ A, PHC 14 × 10⁻³ B,

Table 1. Sample collected, number of sample collected, place and time of collection

Sample collected	Number of samples collected	Place of collection	Collection time
Urine	15	PHC 1	10:00 A.M
Urine	15	PHC 2	10:00 A.M

Table 2. Dilution factors and colony count of asymptomatic bacteriuria isolated from pregnant women attending antenatal clinic urine samples (MacConkey Agar)

Sample code	Dilution factor	Number of colony
PHC 1	10 ⁻³	18
PHC 2	10 ⁻³	16
PHC 3	10 ⁻³	40
PHC4	10 ⁻³	17
PHC 5	10 ⁻⁵	5
PHC 7	10 ⁻⁵	14
PHC 8	10 ⁻³	10
PHC 9	10 ⁻⁵	20
PHC 11	10 ⁻³	88
PHC 14	10 ⁻⁵	16
PHC 16	10 ⁻³	30
PHC 17	10 ⁻³	25

Table 3. Cultural characteristics and date of analysis of asymptomatic bacteriuria isolated from pregnant women attending antenatal clinic urine samples (MacConkey Agar)

Sample code	Cultural characteristics	Date of analysis
PHC 1	Rise dark pink, smooth edges	17/05/2021
PHC 2	Rise light pink, smooth edges	17/05/2021
PHC 3	Rise light pink, star shape edges	17/05/2021
PHC4	Rise creamy, smooth edges	17/05/2021
PHC 5	Rise light pink, rough edges	18/05/2021
PHC 7	Rise creamy, smooth edges	24/05/2021
PHC 8	Flat dark pink with smooth edges	24/05/2021
PHC 9	Rise light pink with rough edges	24/05/2021
PHC 11	Rise pink, smooth edges	25/05/2021
PHC 14	Rise light pink, slightly rough edges	25/05/2021
PHC 16	Rise light pink, rough edges	27/05/2021
PHC 17	Rise light pink, smooth edges	27/05/2021

Table 4. Sample code, morphological characteristics and date analysis of subcultures asymptomatic bacteriuria isolated from pregnant women attending antenatal clinic urine samples (MacConkey Agar to Nutrient Agar)

Sample code	Morphology characteristics	Date analysis
PHC 1 × 10 ⁻³	Rise whitish, swarmy, opaque, smooth edges	21/05/2021
PHC 2 × 10 ⁻³	Rise creamy, transparent, smooth edges	21/05/2021
PHC 3 × 10 ⁻³ A	Rise creamy, swarmy, opaque, smooth edges	21/05/2021
PHC 3 × 10 ⁻³ B	Umbolate, creamy, swarmy, opaque, smooth edges	21/05/2021
PHC 4 × 10 ⁻³	Rise creamy, swarmy, opaque, rough edges	21/05/2021
PHC 5 × 10 ⁻⁵	Elevated, creamy, swarmy, opaque, smooth edges	21/05/2021
PHC 7 × 10 ⁻⁵	Rise creamy, swarmy, opaque, smooth edges	26/05/2021
PHC 8 × 10 ⁻³	Rise mucoid, creamy, opaque smooth edges	26/05/2021
PHC 9 × 10 ⁻⁵	Rise creamy, swarmy, opaque, irregular edges	26/05/2021
PHC 11 × 10 ⁻³	Rise whitish, swarmy, opaque, smooth edges	26/05/2021
PHC 14 × 10 ⁻⁵ A	Elevated whitish, swarmy, opaque, rough edges	27/05/2021
PHC 14 × 10 ⁻⁵ B	Elevated whitish, swarmy, transparent, irregular edges	27/05/2021
PHC 16 × 10 ⁻³ A	Rise creamy, mucoid, opaque, rough edges	28/05/2021
PHC 16 × 10 ⁻³ B	Elevated creamy, swarmy, opaque, irregular edges	28/05/2021
PHC 17 × 10 ⁻³ A	Flat whitish, opaque, rough edges	28/05/2021
PHC 17 × 10 ⁻³ B	Rise whitish, opaque, smooth edges	28/05/2021

Table 5. Gram Staining and Microscopic Examination of Asymptomatic Bacteriuria Isolated from Pregnant Women Attending Antenatal Clinic Urine Samples

Isolates	Gram stain	Shape
PHC 1 × 10 ⁻³	-ve	Tiny rods
PHC 2 × 10 ⁻³	-ve	Short rods
PHC 3 × 10 ⁻³ A	-ve	Short rods
PHC 3 × 10 ⁻³ B	+ ve	Short rods
PHC 4 × 10 ⁻³	- ve	Short rods
PHC 5 × 10 ⁻⁵	- ve	Tiny rods
PHC 7 × 10 ⁻⁵	- ve	Long rods
PHC 8 × 10 ⁻³	- ve	Tiny rods
PHC 9 × 10 ⁻⁵	- ve	Long rods
PHC 11 × 10 ⁻³	+ ve	Tiny rods
PHC 14 × 10 ⁻⁵ A	+ ve	Short rods
PHC 14 × 10 ⁻⁵ B	- ve	Short rods
PHC 16 × 10 ⁻³ A	- ve	Long rods
PHC 16 × 10 ⁻³ B	- ve	Long rods
PHC 17 × 10 ⁻³ A	- ve	Long rods
PHC 17 × 10 ⁻³ B	-ve	Tiny rods

PHC 16 × 10⁻³ A, PHC 17 × 10⁻³ A were positive to Oxidase test. PHC 1 × 10⁻³, PHC 2 × 10⁻³, PHC 5 × 10⁻⁵, PHC 8 × 10⁻³, 9 × 10⁻⁵, PHC 14 × 10⁻³ A, PHC 14 × 10⁻³ B, PHC 17 × 10⁻³ A and PHC 17 × 10⁻³ B were negative to Catalase test, PHC 3 × 10⁻³ A, PHC 3 × 10⁻³ B, PHC 4 × 10⁻³, PHC 7 × 10⁻⁵, PHC 11 × 10⁻³, were positive to Catalase test. PHC 1 × 10⁻³, PHC 7 × 10⁻⁵, PHC 8 × 10⁻³, were all Gamma hemolysis, PHC 2 × 10⁻³, PHC 3 × 10⁻³ B, PHC 4 × 10⁻³, PHC 5 × 10⁻⁵, PHC 7 × 10⁻⁵, PHC 8 × 10⁻³, PHC 9 × 10⁻⁵, PHC 11 × 10⁻³, PHC 14 × 10⁻³ B, PHC 16 × 10⁻³ A, PHC 16 × 10⁻³ B, PHC 17 × 10⁻³ A and PHC 17 × 10⁻³ B were all Beta hemolysis. The suspected

isolated organisms include *Klebsiella pneumonia* (3), *Proteus mirabilis*, *Proteus vulgaris*, *Bacillus subtilis* (2), *Pseudomonas aeruginosa*, *Enterobacter cloacae* (2), *Escherichia coli* (3), *Bacillus cereus*, *Serratia mercesiensis*, *Enterobacter aerogens*.

Table 8. Shows the Secondary metabolite (phytochemical) constituents of seed of *Aframomum melegueta*. It was observed that seed extract contains some secondary metabolites. Like Tannin, Saponin, Flavonoid, Steroid, Terpenoids, Cardiac Glycoside, Alkaloid were all present.

Table 6. Biochemical tests of asymptomatic bacteriuria isolated from pregnant women attending antenatal clinic urine samples

Isolates	Motility	Indole	Urease	Lactose	Sucrose	Dextrose	H₂S	Gas production
PHC 1 × 10 ⁻³	+	—	+	+	+	+	+	—
PHC 2 × 10 ⁻³	+	—	+	+	+	+	—	—
PHC 3 × 10 ⁻³ A	+	+	+	+	+	+	—	—
PHC 3 × 10 ⁻³ B	+	+	+	+	+	+	+	+
PHC 4 × 10 ⁻³	+	+	+	+	+	+	+	+
PHC 5 × 10 ⁻⁵	+	—	—	+	+	+	—	+
PHC 7 × 10 ⁻⁵	+	—	+	+	+	+	+	+
PHC 8 × 10 ⁻³	+	—	+	+	+	+	+	+
PHC 9 × 10 ⁻⁵	+	+	—	+	+	+	+	—
PHC 11 × 10 ⁻³	+	—	—	+	+	+	—	+
PHC 14 × 10 ⁻⁵ A	+	+	+	+	+	+	—	+
PHC 14 × 10 ⁻⁵ B	+	—	+	+	+	+	+	+
PHC 16 × 10 ⁻³ A	—	+	+	+	+	+	—	+
PHC 16 × 10 ⁻³ B	+	+	—	+	+	+	+	—
PHC 17 × 10 ⁻³ A	+	—	—	+	+	+	—	+
PHC 17 × 10 ⁻³ B	+	—	—	+	+	+	—	+

Fig. 1. Shows the antibiotic susceptibility test of Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples. The identified Gram negative organisms. It was observed that *Proteus mirabilis*, *Proteus vulgaris*, *Enterobacter cloacae*, *Serratia mercesien*, *Escherichia coli* has the highest zone of inhibition to streptomycin (20mm), while, *Klebsiella pneumoniae* has the lowest zone of inhibition to streptomycin (15mm). *Escherichia coli* have the highest zone of inhibition to Ampicillin (20mm), while *Klebsiella pneumoniae* have the lowest zone of inhibition to Ampicillin(11mm). *Enterobacter aerogens* has the highest zone of inhibition to Ceporex(20mm),, while *Klebsiella pneumoniae* has the lowest zone of inhibition to ceporex(9mm),*Proteus mirabilis*, *Proteus vulgaris*, *Escherichia coli* has the highest zone of inhibition to Tarivid (20mm),while *Klebsiella pneumoniae* has the lowest zone of inhibition to Tarivid (13mm),... *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogens*, *Enterobacter cloacae*(20mm), have the highest zone of inhibition to Nalixadic acid(20mm),, while *Klebsiella pneumoniae* has the lowest zone of inhibition to Nalixadic acid. *Enterobacter aerogens*, *Enterobacter cloacae*, *Escherichia coli*, *Serratia mercesiens*, *Proteus mirabilis*, *Proteus vulgaris* all have high zone of inhibition to Reflacine, Gentamycin, Augmentin, Ciporoflox and Septrin (20mm), while, *Klebsiella pneumoniae* has the lowest zone of inhibition to Ciproflox and Septrin.

Fig. 2. Shows the Percentage Inhibition Ratio of Streptomycin (10µg) against Asymptomatic

Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples. *Escherichia coli* 8%, *Serratia mercesiens* 8%, *Escherichia coli* 8%, *Klebsiella pneumoniae* 8%, *Klebsiella pneumoniae* 7%, *Enterobacter cloacae* 8%, *Enterobacter aerogens* 8%, *Enterobacter cloacae*7%, *Pseudomonas aeruginosa* 8%, *Proteus mirabilis* 8%, *Klebsiella pneumoniae* 6%.

Fig. 3. Shows the Percentage Inhibition Ratio of Ampicillin, (10µg) against Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples *Escherichia coli* 10%, *Serratia mercesiens* 7%, *Escherichia coli* 8%, *Klebsiella Pneumoniae* 7%, *Klebsiella Pneumoniae* 6%, *Enterobacter cloacae* 10%, *Proteus vulgaris* 6%, *Proteus mirabilis* 7%, *Klebsiella pneumoniae* 7%, *Pseudomonas aeruginosa* 7%, *Enterobacter aerogens* 9%, *Enterobacter cloacae* 9%, *Escherichia coli* 7%.

Fig 4; Shows the Percentage Inhibition Ratio of Ceporex (10µg) against Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples *Enterobacter cloacae* 9%, *Pseudomonas aeruginosa* 10%, *Proteus mirabilis* 7%, *Klebsiella pneumonia* 5%, *Proteus vulgaris* 8%, *Enterobacter cloacae* 8%, *Klebsiella pneumoniae* 4%, *Klebsiella pneumoniae* 6% ,*Escherichia coli* 9%, *Serratia mercesiens* 8%, *Escherichia coli* 8%, *Escherichia coli* 8%, *Enterobacter aerogens* 10%.

Table 7. Biochemical Tests for Identification of Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples

Isolates	Oxidase	Catalase	Hemolysis	Organisms identified
PHC 1 × 10 ⁻³	–	–	Gamma	<i>Klebsiella pneumoniae</i>
PHC 2 × 10 ⁻³	+	–	Beta	<i>Proteus mirabilis</i>
PHC 3 × 10 ⁻³ A	–	+	Beta	<i>Proteus vulgaris</i>
PHC 3 × 10 ⁻³ B	–	+	Beta	<i>Bacillus subtilis</i>
PHC 4 × 10 ⁻³	–	+	Beta	<i>Pseudomonas aeruginosa</i>
PHC 5 × 10 ⁻⁵	–	–	Beta	<i>Enterobacter cloacae</i>
PHC 7 × 10 ⁻⁵	–	+	Gamma	<i>Klebsiella pneumoniae</i>
PHC 8 × 10 ⁻³	–	–	Gamma	<i>Klebsiella pneumoniae</i>
PHC 9 × 10 ⁻⁵	+	–	Beta	<i>Escherichia coli</i>
PHC 11 × 10 ⁻³	–	+	Beta	<i>Bacillus cereus</i>
PHC 14 × 10 ⁻⁵ A	–	–	Beta	<i>Bacillus subtilis</i>
PHC 14 × 10 ⁻⁵ B	+	–	Beta	<i>Serratia marcescens</i>
PHC 16 × 10 ⁻³ A	+	–	Beta	<i>Escherichia coli</i>
PHC 16 × 10 ⁻³ B	+	–	Beta	<i>Escherichia coli</i>
PHC 17 × 10 ⁻³ A	+	–	Beta	<i>Enterobacter aerogen</i>
PHC 17 × 10 ⁻³ B	–	–	Beta	<i>Enterobacter cloacae</i>

Table 8. Secondary metabolite (Phytochemical) Constituents of Seeds *Afr. Melegueta*

Constituents	Results
Tannin	+
Saponin	+
Flavonoid	+
Terpernoids	+
Cardiac glycoside	+
Alkaloid	+

+ = Present - = Absent

Fig 5; Percentage Inhibition Ratio of Tarivid (10µg) against Asymptomatic Bacteriuria Isolates Enterobacter cloacae 8%,*Pseudomonas aeruginosa* 8%,*Klebsiella pneumonia* 6%,*Proteus mirabilis* 9%,*Proteus vulgaris* 9%,*Enterobacter cloacae* 8%,*Klebsiella pneumonia* 6%,*Klebsiella pneumonia* 6%,*Escherichia coli* 8%,*Serratia mercesiens* 8%,*Escherichia coli* 8%,*Escherichia coli* 8%,*Escherichia coli* 8%,*Escherichia coli* 8%,*Enterobacter aerogens* 8%.

Fig 6; Percentage Inhibition Ratio of Nalidixic (30µg) against Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples,Shows the *Enterobacter cloacae*8%, *Pseudomonas aeruginosa* 10%, *Klebsiella pneumoniae* 5%, *Proteus mirabilis* 6%, *Proteus vulgaris* 6%, *Enterobacter cloacae*7%, *Klebsiella pneumonia* 7%, *Klebsiella pneumoniae* 7%,, *Escherichia coli* 8%,*Serratia mercesiens* 9%, *Serratia mercesiens* 9%, *Escherichia coli* 8%, *Escherichia coli* 9%, *Enterobacter aerogens* 9%.

Fig 7; Shows the Percentage Inhibition Ratio of Reflacin (10µg) against Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples. *Enterobacter cloacae*7%, *Enterobacter aerogens*7%, *Escherichia coli* 8%, *Escherichia coli* 7%, *Serratia mercesiens* 9%, *Escherichia coli* 8%, *Klebsiella pneumonia* 5%, *Klebsiella pneumoniae* 8% ,*Enterobacter cloacae* 8%, *Proteus vulgaris* 8%, *Proteus mirabilis* 8%, *Klebsiella pneumoniae* 7%,*Pseudomonas aeruginosa* 8%.

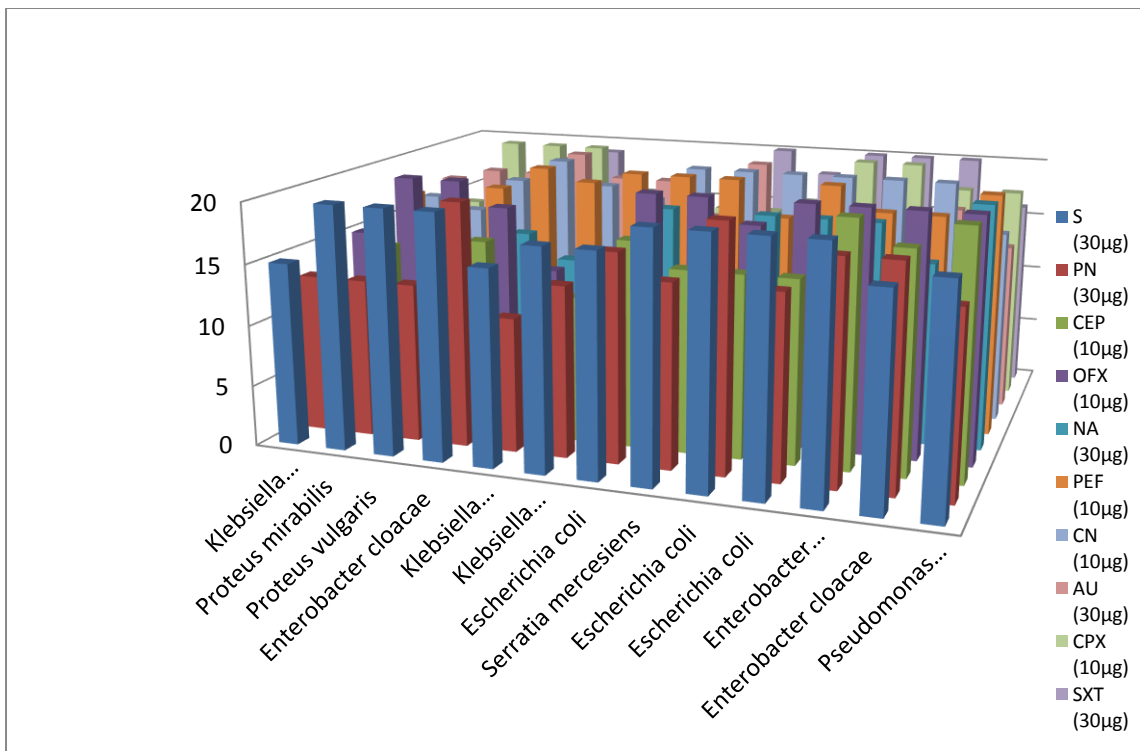


Fig. 1. Antibiotic Susceptibility Test of Asymptomatic Bacteriuria isolated(Identified Gram Negative)from pregnant women attending antenatal clinic Urine samples (Zones of Inhibition Unit (Milimetre (Mm)

KEY: S- Streptomycin, PN- Ampicilin, CEP- Ceporex, OFX- Tarivid, NA- Nalidixic, PEF- Reflacin, CN- Gentamycin, AU- Augmentin, CPX- Ciproflox, SXT- Septrin UNIT: Milimetre (mm)

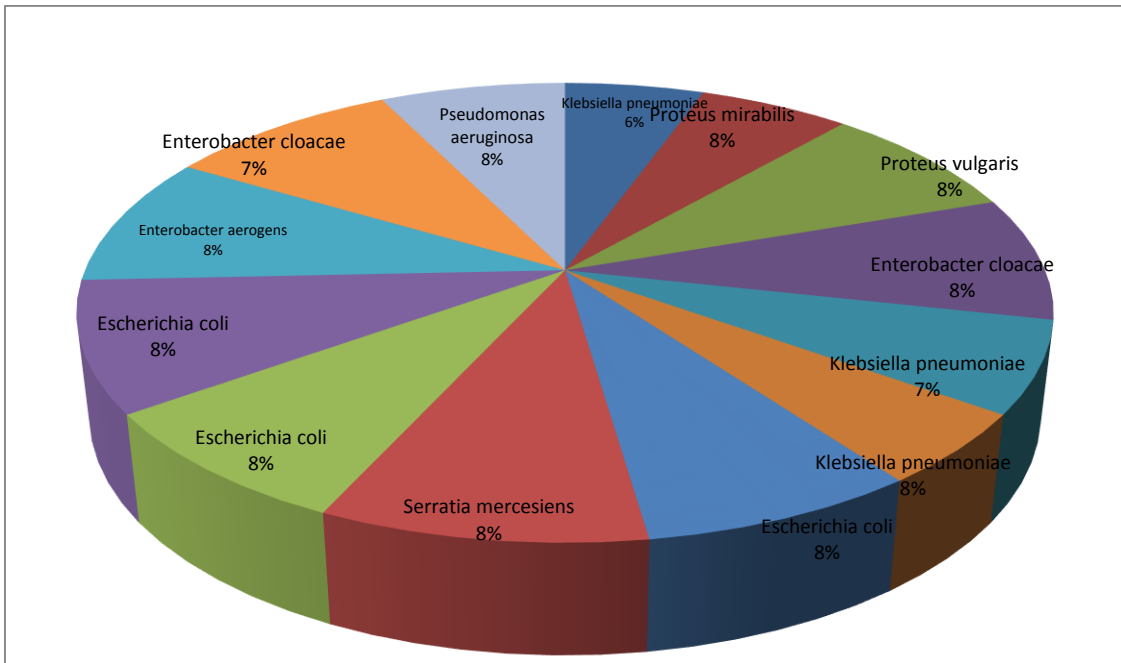


Fig. 2. Percentage inhibition ratio of Streptomycin (30 ug against isolated organism)

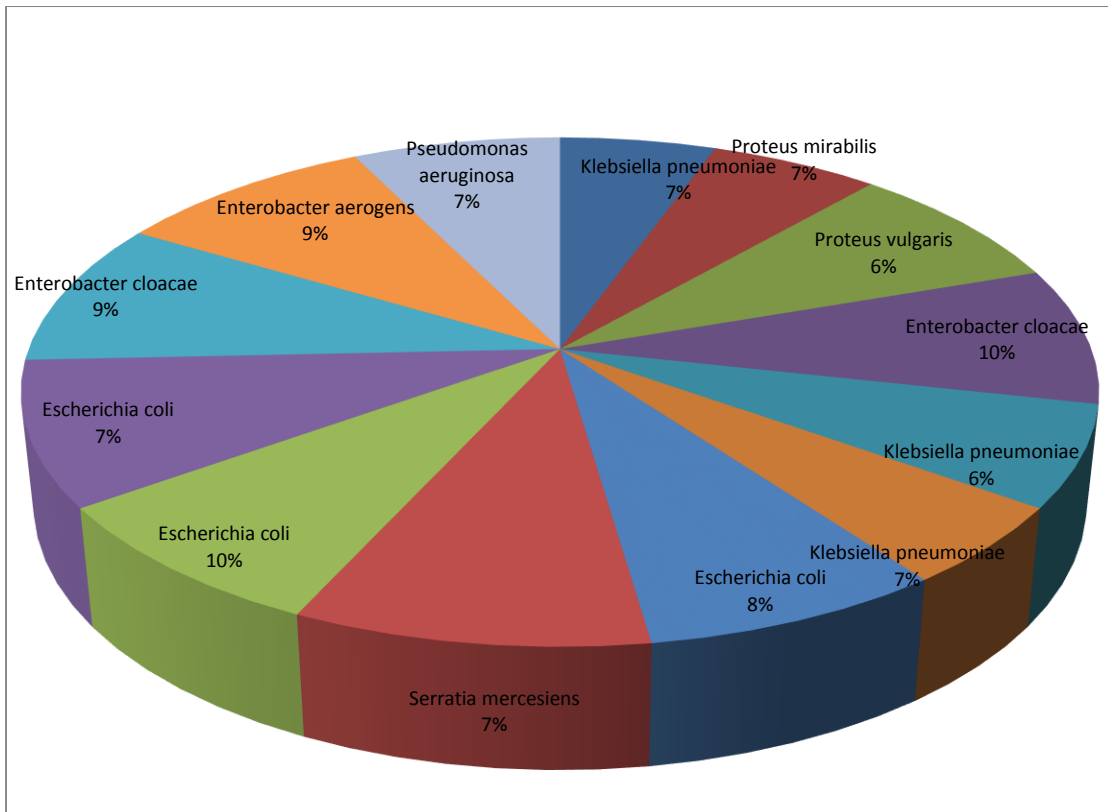


Fig. 3. Percentage inhibition ratio of Ampicilin (30 ug against isolated organism)

Fig 8; Shows the Percentage Inhibition Ratio of Gentamycin(10µg) against Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples *Pseudomonas aeruginosa* 7%, *Klebsiella pneumoniae*7%,*Proteus mirabilis* 6%,*Proteus*

vulgaris 8%, *Enterobacter cloacae* 9%, *Klebsiella pneumoniae* 8%, *Klebsiella pneumoniae* 7%, *Escherichia coli* 8%, *Serratia mercesiens* 9%, *Escherichia coli* 8%, *Escherichia coli* 9%, *Enterobacter aerogens* 8%, *Enterobacter cloacae* 8%.

Fig 9; Shows the Percentage Inhibition Ratio of Augmentin (30µg) against Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples *Enterobacter cloacae* 8%, *Enterobacter aerogens* 7%, *Escherichia coli* 7%, *Escherichia coli* 7%, *Serratia mercesiens* 9%, *Klebsiella pneumonia* 8%, *Pseudomonas aeruginosa* 6%, *Klebsiella pneumonia* 8%, *Proteus mirabilis* 8%, *Proteus vulgaris* 8%, *Enterobacter cloacae* 9%, *Escherichia coli* 7%, *Klebsiella pneumonia* 8%.

Fig 10; Shows the Percentage Inhibition Ratio of Ciproflox (10µg) against Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples. *Enterobacter cloacae* 8%, *Pseudomonas aeruginosa* 8%, *Klebsiella pneumoniae* 7%, *Proteus mirabilis* 9%, *Proteus vulgaris* 8%, *Enterobacter cloacae* 9%, *Klebsiella pneumoniae* 6%, *Klebsiella pneumoniae* 5%, *Escherichia coli* 8%, *Serratia mercesiens* 9%, *Escherichia coli* 8%, *Escherichia coli* 9%, *Enterobacter aerogens* 9%.

Fig 11; Shows the Percentage Inhibition Ratio of Septrin (30µg) against Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples. *Enterobacter cloacae* 9%, *Pseudomonas aeruginosa* 7%, *Klebsiella pneumoniae* 6%, *Proteus mirabilis* 6%, *Enterobacter aerogens* 9%, *Escherichia coli* 9%, *Escherichia coli* 8%, *Serratia mercesiens* 9%, *Escherichia coli* 8%, *Klebsiella pneumoniae* 5%, *Klebsiella pneumoniae* 7%, *Enterobacter cloacae* 9%, *Proteus vulgaris* 8%..

Fig 12; Shows the antimicrobial screening of ethanol of *Aframomum melegueta* seed at 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml against Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples. It was observed in this table, all the isolates has high zone of inhibition against ethanol seed extract of *Aframomum melegueta* at 100mg/ml and 50mg/ml, at 25mg/ml and 12.5mg/ml. The

diameter of the zone of inhibition reduced, At 6.25mg/ml there was no visible zone of inhibition. It was observed that at 100mg/ml, *Klebsiella pneumoniae* has the highest zone of inhibition with diameter 25mm while *Enterobacter cloacae* has the lowest zone of inhibition with 16mm. At 50mg/ml, *Klebsiella pneumoniae* has the highest zone of inhibition with diameter 17mm while *Enterobacter cloacae* has the lowest zone of inhibition with 13mm. At 25mg/ml, *Escherichia coli* has the highest zone of inhibition with diameter 14mm while *Bacillus subtilis* has the lowest zone of inhibition with diameter 10mm. At 12.5mg/ml, *Escherichia coli* and *Proteus mirabilis* have the highest zone of inhibition with diameter 9mm. At 6.25mg/ml, there was no visible zone of inhibition on most of the organisms.

Fig 13; Shows the antibiotic susceptibility test against Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples. Identified Gram positive organisms isolates in diameter of zones of inhibition. It was observed in this table, *Bacillus subtilis* and *Bacillus cereus* has high zone of inhibitions to all the antibiotics used with diameter 20mm, 18mm and 17mm respectively.

Fig. 14. Percentage Inhibition Ratio of *Afr. melegueta* ethanol Seed extract against Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples at 100mg/ml. *Escherichia coli* 6%, *Serratia mercesiens* 7%, *Escherichia coli* 7%, *Klebsiella pneumoniae* 6%, *Klebsiella pneumoniae* 8%, *Enterobacter aerogens* 6%, *Enterobacter cloacae* 5%, *Pseudomonas aeruginosa* 6%, *Bacillus subtilis* 7%, *Bacillus cereus* 6%, *Enterobacter cloacae* 6%, *Proteus vulgaris* 5%, *Proteus mirabilis* 6%, *Klebsiella pneumoniae* 7%, *Bacillus subtilis* 6%.

Fig. 15. Percentage Inhibition Ratio of *Afr. melegueta* ethanol Seed extract against Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples at 50mg/ml. *Bacillus cereus* 6%, *Bacillus subtilis* 6%, *Pseudomonas aeruginosa* 6%, *Enterobacter aerogens* 6%, *Enterobacter cloacae* 5%, *Escherichia coli* 7%, *Bacillus subtilis* 6%, *Escherichia coli* 7%, *Serratia mercesiens* 7%, *Klebsiella pneumonia* 7%, *Klebsiella pneumoniae* 6%, *Escherichia coli* 7%, *Enterobacter cloacae* 6%, *Proteus vulgaris* 5%, *Proteus mirabilis* 6%.

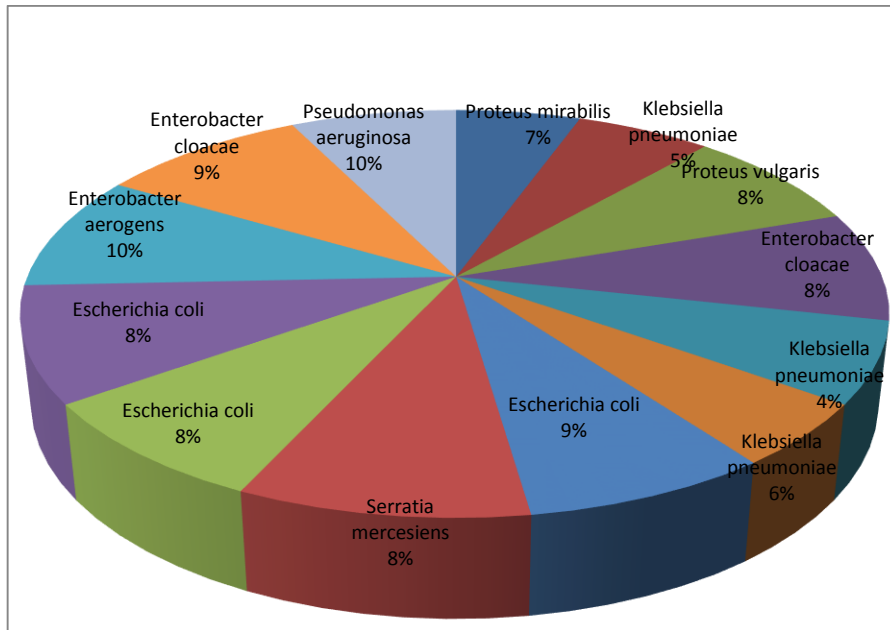


Fig. 4. Percentage Inhibition Ratio of Ceporex (10µg) against Asymptomatic Bacteriuria Isolates

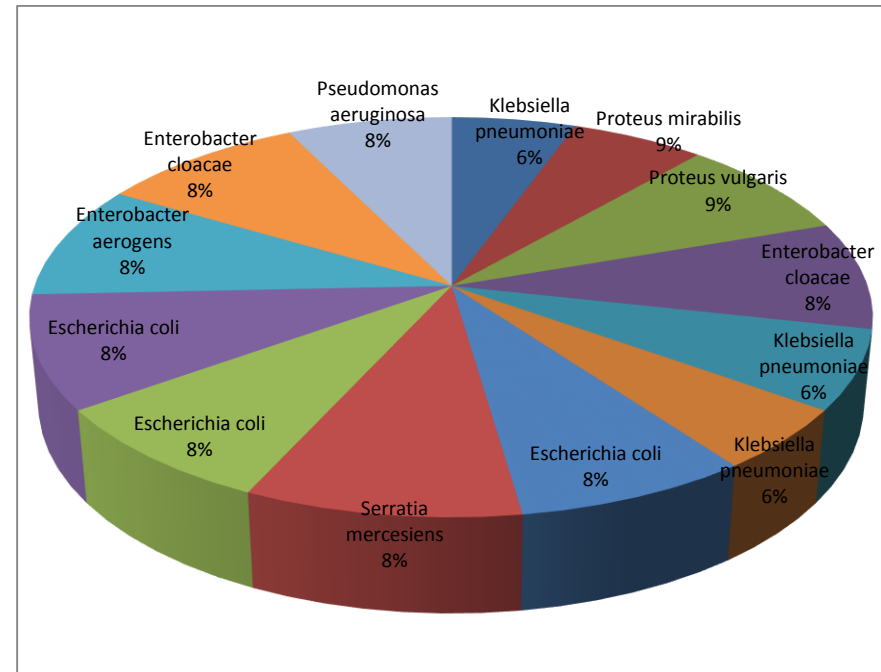


Fig. 5. Percentage Inhibition Ratio of Tarivid (10µg) against Asymptomatic Bacteriuria Isolates

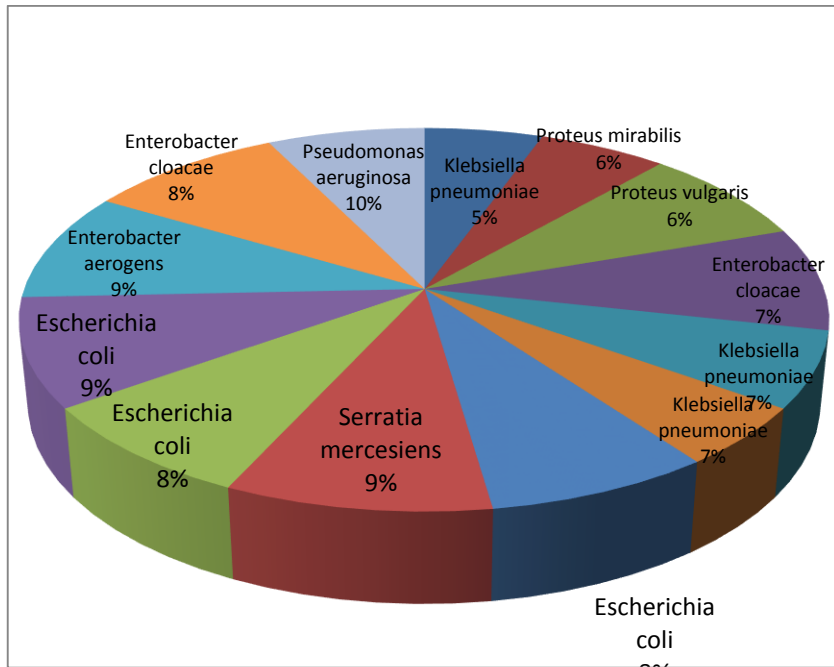


Fig. 6. Percentage Inhibition Ratio of Nalidixic (30µg) against Asymptomatic Bacteriuria Isolates

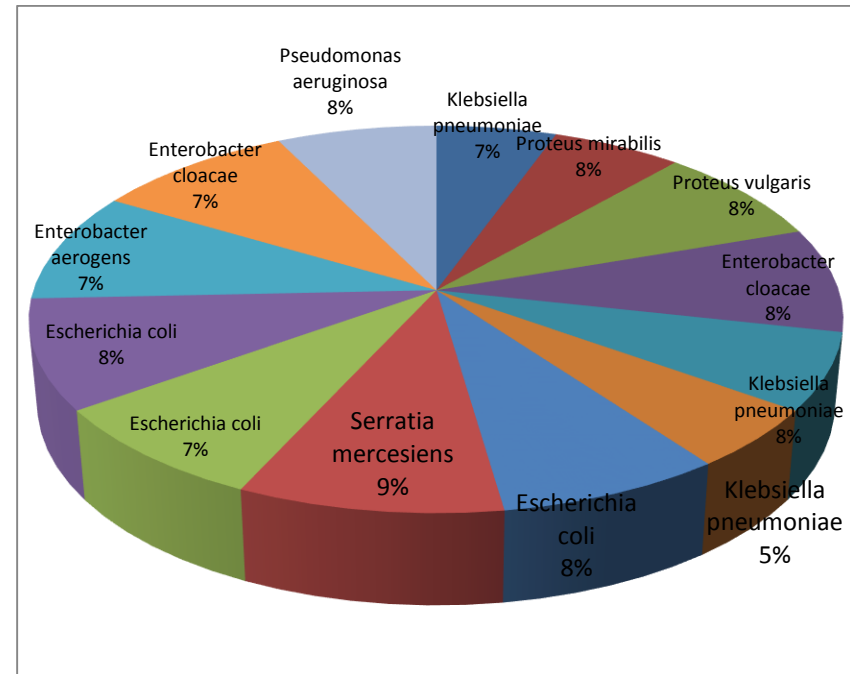


Fig. 7. Percentage Inhibition Ratio of Reflacine(10µg) against Asymptomatic Bacteriuria Isolates

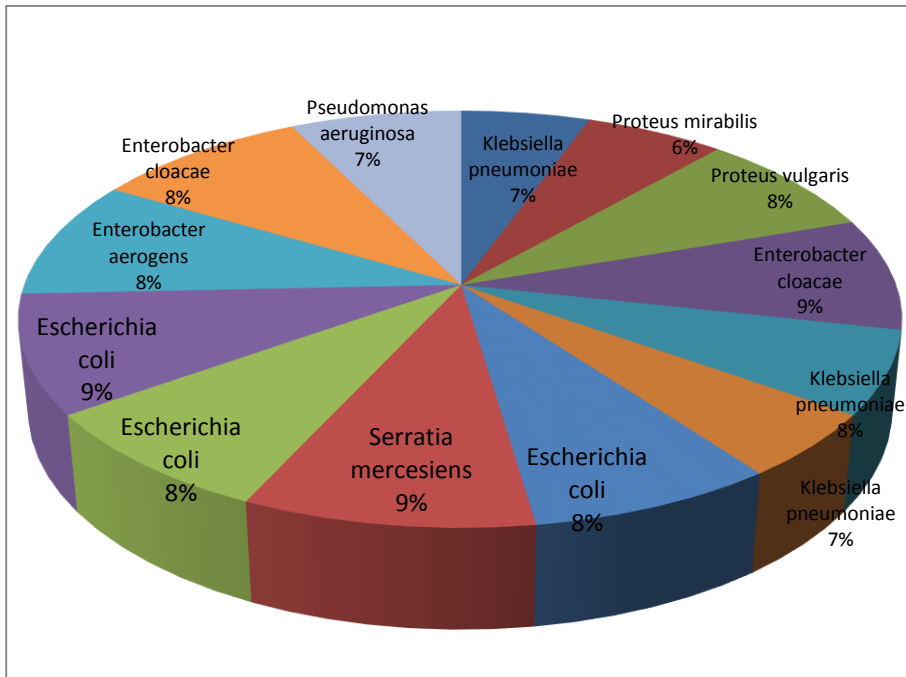


Fig. 8. Percentage Inhibition Ratio of Gentamycin(10µg) against Asymptomatic Bacteriuria Isolates

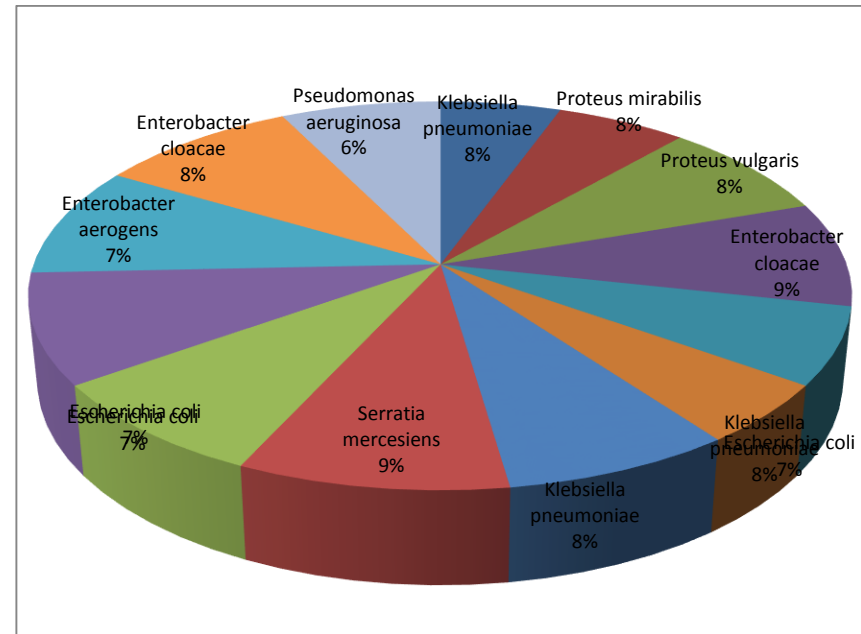


Fig. 9. Percentage Inhibition Ratio of Augmentin (30µg) against Asymptomatic Bacteriuria Isolates

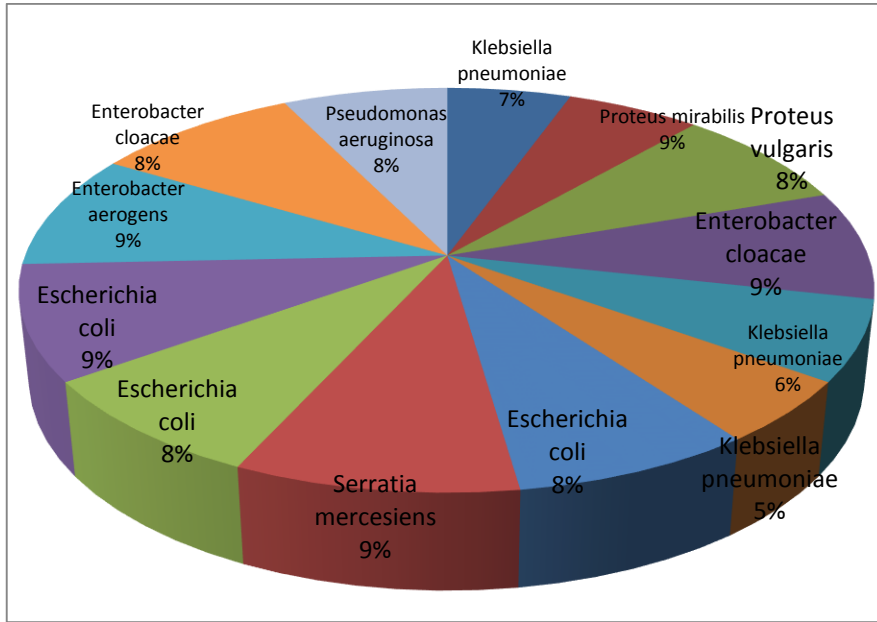


Fig. 10. Percentage Inhibition Ratio of Ciproflox (10µg) against Asymptomatic Bacteriuria Isolates

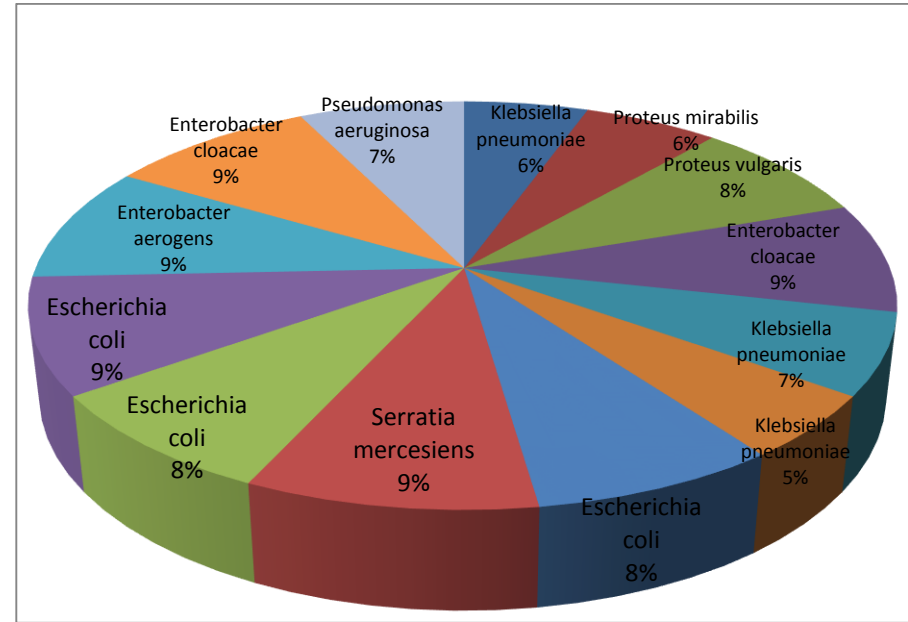


Fig. 11. Percentage Inhibition Ratio of Septrin (30µg) against Asymptomatic Bacteriuria Isolates

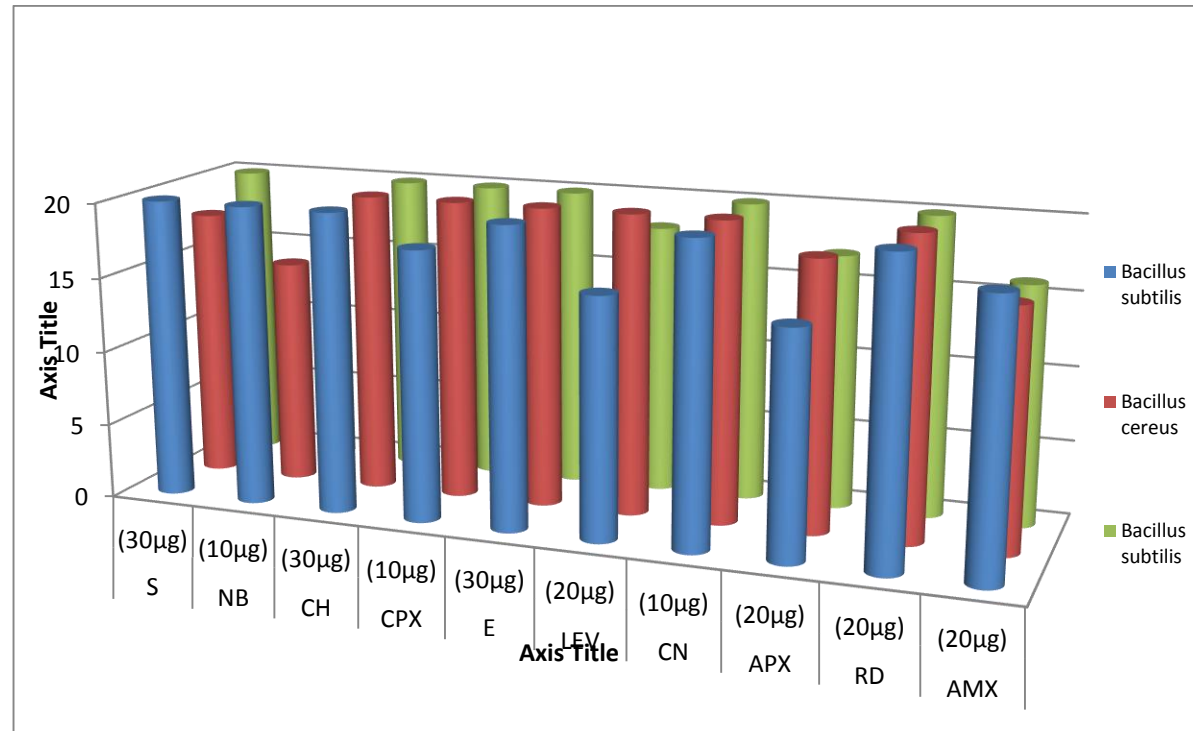


Fig. 12. Antibiotic Susceptibility Test of Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples (Gram Positive Bacterial)

KEY : S - Streptomycin, NB – Norflaxacin, CH- Chloramphenicol, CPX- Ciproflo, E- Erythromycin, LEV- Levofloxacin, CN- Gentamycin, APX- Ampiclox, RD- Rifampicin, AMX- Amoxil. UNIT: Milimetre(mm)

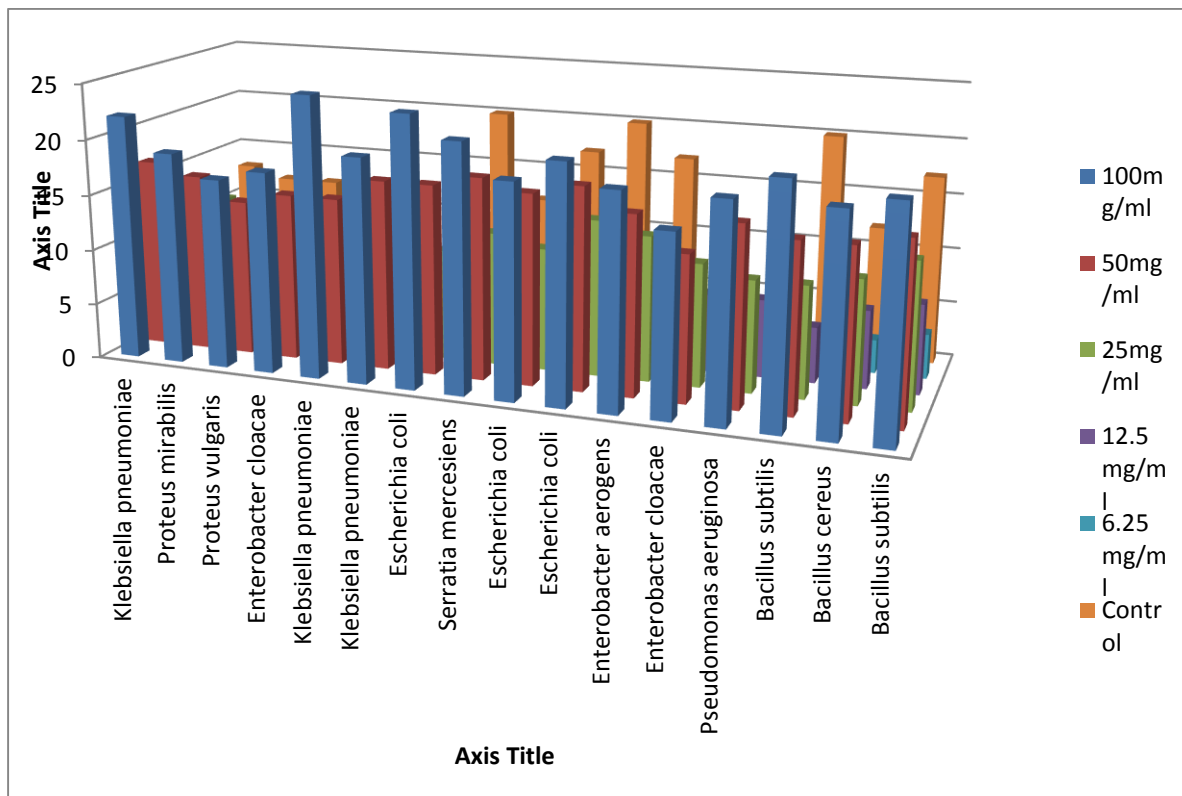


Fig. 13. Antimicrobial Screening of *Afr. melegueta* ethanol Seed extract against Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples

Fig. 16. Percentage Inhibition Ratio of *Afr. melegueta* ethanol Seed extract against Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples at 25mg/ml, *Bacillus cereus* 6%, *Bacillus subtilis* 5%, *Pseudomonas aeruginosa* 5%, *Enterobacter cloacae* 6%, *Enterobacter aerogens* 7%, *Escherichia coli* 7%, *Escherichia coli* 6%, *Serratia mercesiens* 6%, *Bacillus subtilis* 7%, *Klebsiella pneumoniae* 6%, *Proteus mirabilis* 7%, *Escherichia coli* 5%, *Klebsiella pneumoniae* 7%, *Klebsiella pneumoniae* 6%, *Enterobacter cloacae* 6%, *Proteus vulgaris* 6%.

Fig. 17. Percentage Inhibition Ratio of *Afr. melegueta* ethanol Seed extract against Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples at 12.5mg/ml. *Bacillus subtilis* 8%, *Bacillus cereus* 7%, *Pseudomonas aeruginosa* 7%, *Bacillus subtilis* 5%, *Enterobacter cloacae* 7%, *Enterobacter aerogens* 4%, *Escherichia coli* 7%, *Escherichia coli* 9%, *Serratia mercesiens* 5%, *Klebsiella pneumoniae* 5%, *Proteus mirabilis* 9%, *Proteus vulgaris* 8%, *Escherichia coli* 4%, *Enterobacter cloacae* 7%, *Klebsiella pneumoniae* 4%, *Klebsiella pneumoniae* 4%.

Fig. 18. Percentage Inhibition Ratio of *Afr. melegueta* ethanol Seed extract against Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples at 6.25mg/ml. *Bacillus subtilis* 11%, *Bacillus cereus* 8%, *Bacillus subtilis* 8%, *Pseudomonas aeruginosa* 14%, *Enterobacter cloacae* 14%, *Proteus mirabilis* 14%, *Proteus vulgaris* 11%, *Escherichia coli* 14%, *Enterobacter aerogens* 0%, *Escherichia coli* .8%.

Fig. 19. Growth Dynamic of Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples Using Ultraviolet Spectrophotometer With Wavelength 480λ. It was observed that at 0hour, *Proteus mirabilis* has the highest growth rate of 0.297λ and *Serratia mercesiens* has the lowest growth rate of -0.083λ. At 84th hour, *Bacillus subtilis* has the lowest death rate of 0.254λ and *Escherichia coli* have the highest death rate of 0.167λ.

Fig. 20. Growth Dynamic and Killing Time of Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples with Addition of Ciprofloxacin Antibiotic At 48th Hour Using Ultraviolet Spectrophotometer With

Wavelength 480λ. It was observed, At 0 hour, *Proteus mirabilis* has the highest growth rate of 0.253λ and *Escherichia coli* has the lowest growth rate of 0.083λ. At 84th hour, *Serratia mercesiens* has the lowest death rate of 0.087λ and *Escherichia coli* has the highest death rate of 0.020λ.

Fig 21: Growth Dynamic and Killing Time of Asymptomatic Bacteriuria isolated from pregnant women attending antenatal clinic Urine samples With Addition of *Aframomum melegueta* Ethanol Seed Extract Antibiotic At 48th Hour Using Ultraviolet Spectrophotometer With Wavelength 480λ.

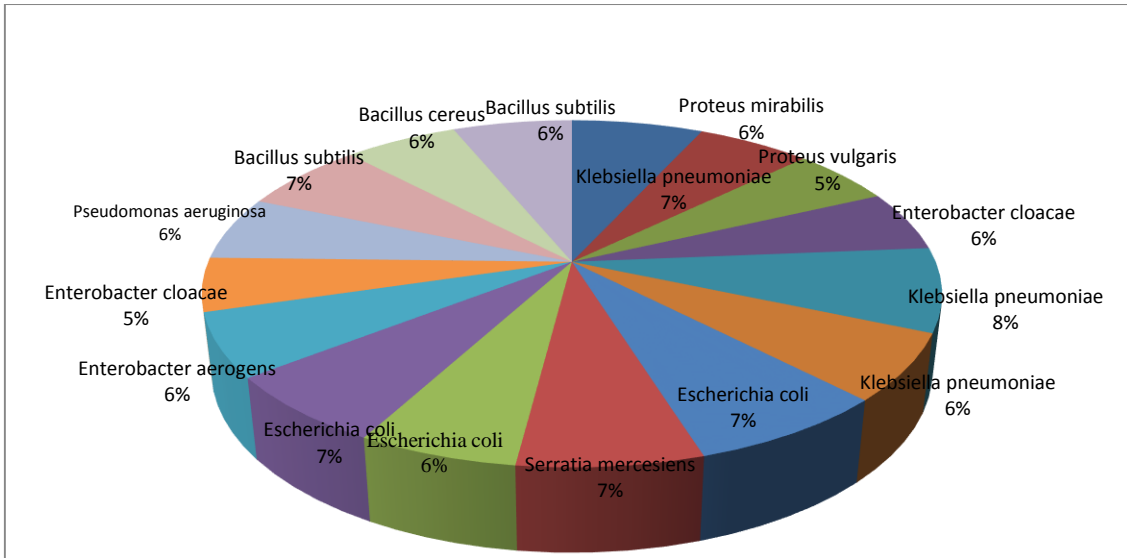


Fig. 14. Percentage Inhibition Ratio of Ethanol Extract of *Afr. melegueta* Seed against Asymptomatic Bacteriuria Isolates at 100mg/ml

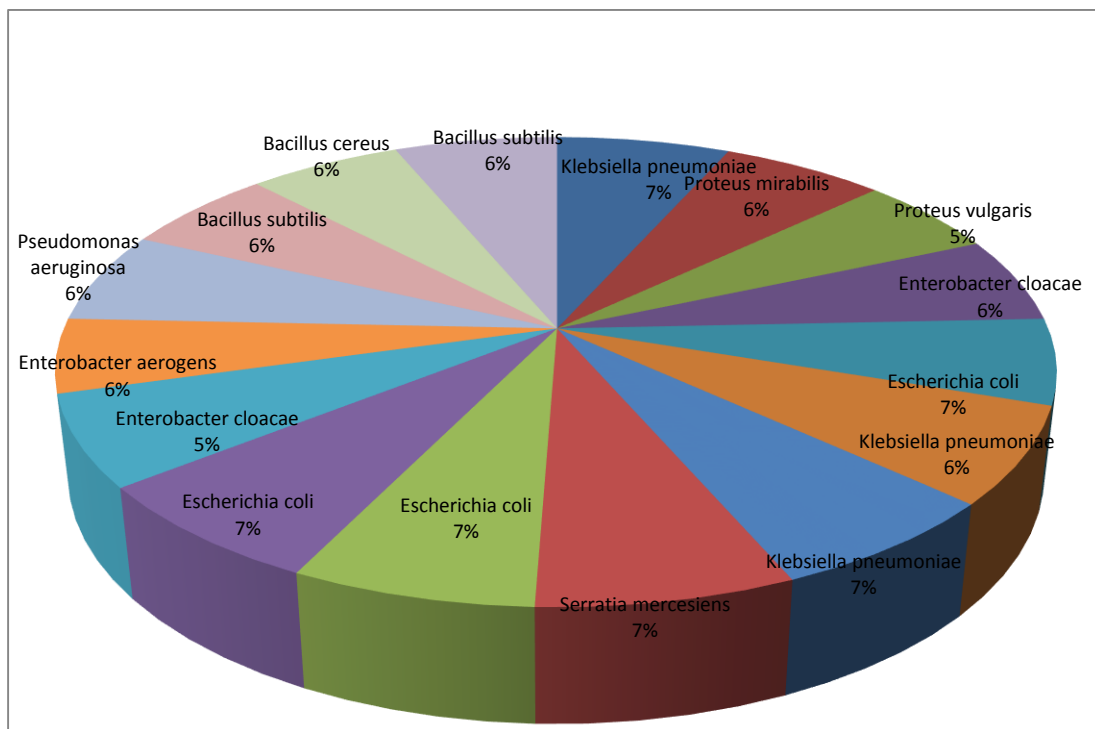


Fig. 15. Percentage Inhibition Ratio of Ethanol Extract of *Afr. melegueta* Seed against Asymptomatic Bacteriuria Isolates at 50mg/ml

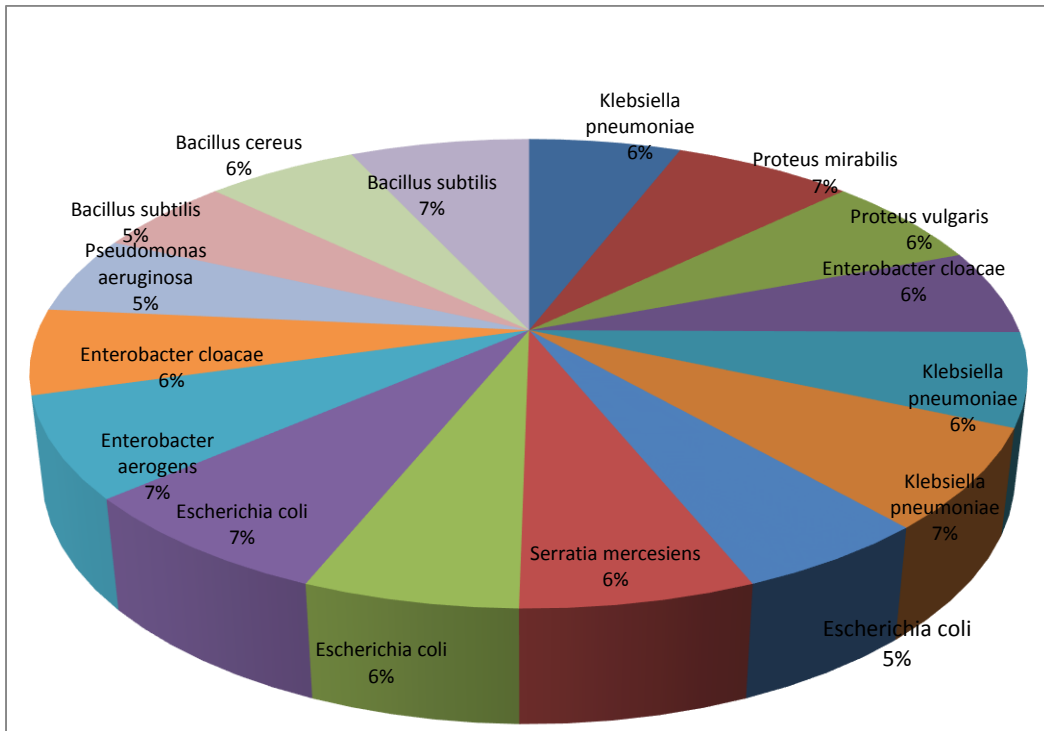


Fig. 16. Percentage Inhibition Ratio of Ethanol Extract of *Afr. melegueta* Seed against Asymptomatic Bacteriuria Isolates at 25mg/ml

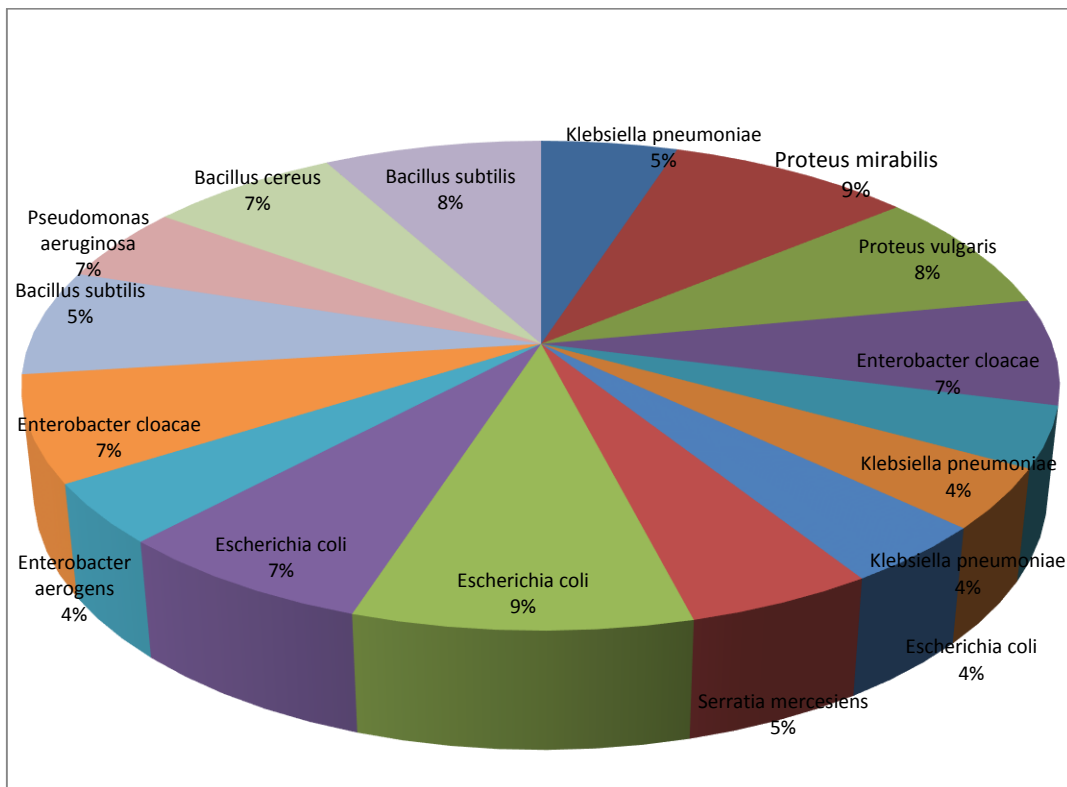


Fig. 17. Percentage Inhibition Ratio of Ethanol Extract of *Afr. melegueta* Seed against Asymptomatic Bacteriuria Isolates at 12.5mg/ml

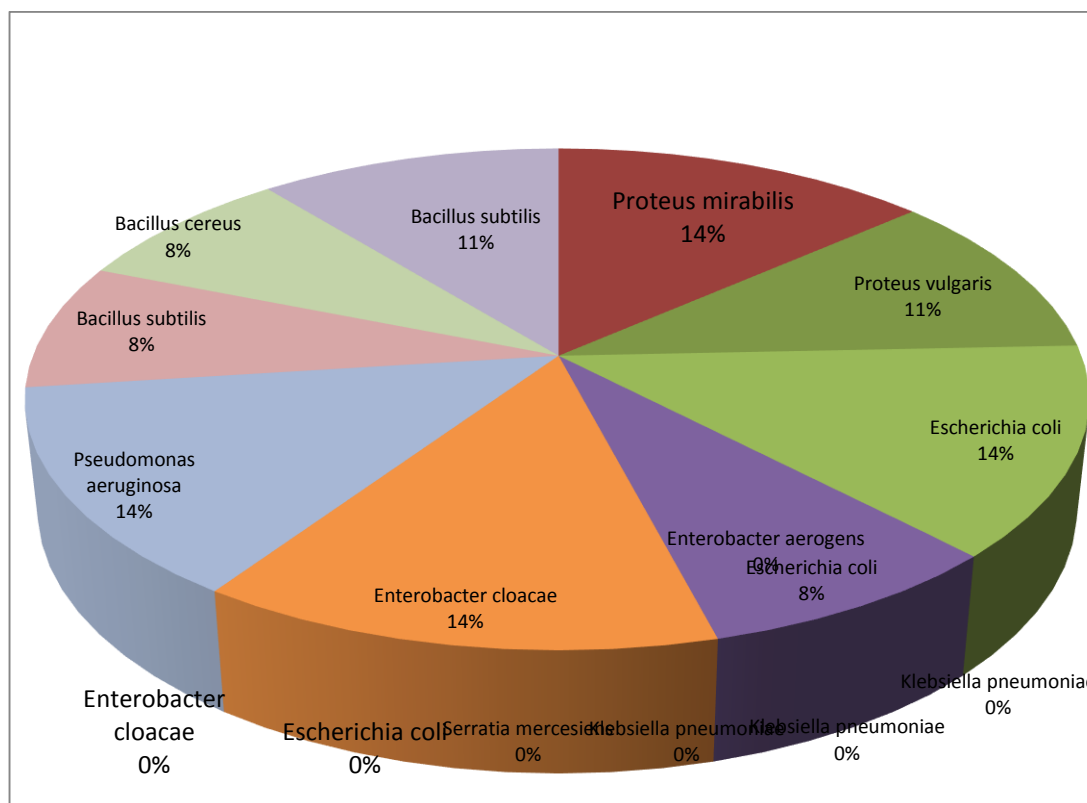


Fig. 18. Percentage Inhibition Ratio of Ethanol Extract of *Afr. melegueta* Seed against Asymptomatic Bacteriuria Isolates at 6.25mg/ml

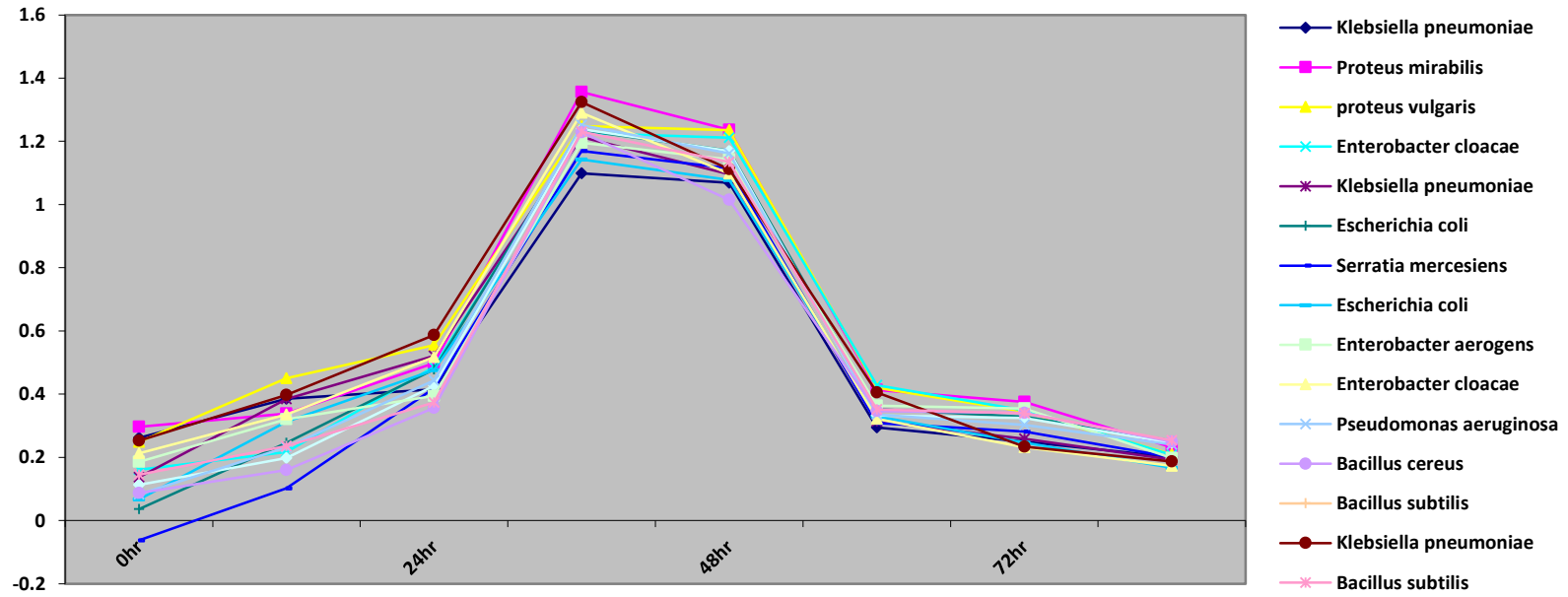


Fig. 19. Growth Dynamic of Bacterial Isolates Using Ultraviolet Spectrophotometer with Wavelength 480Å

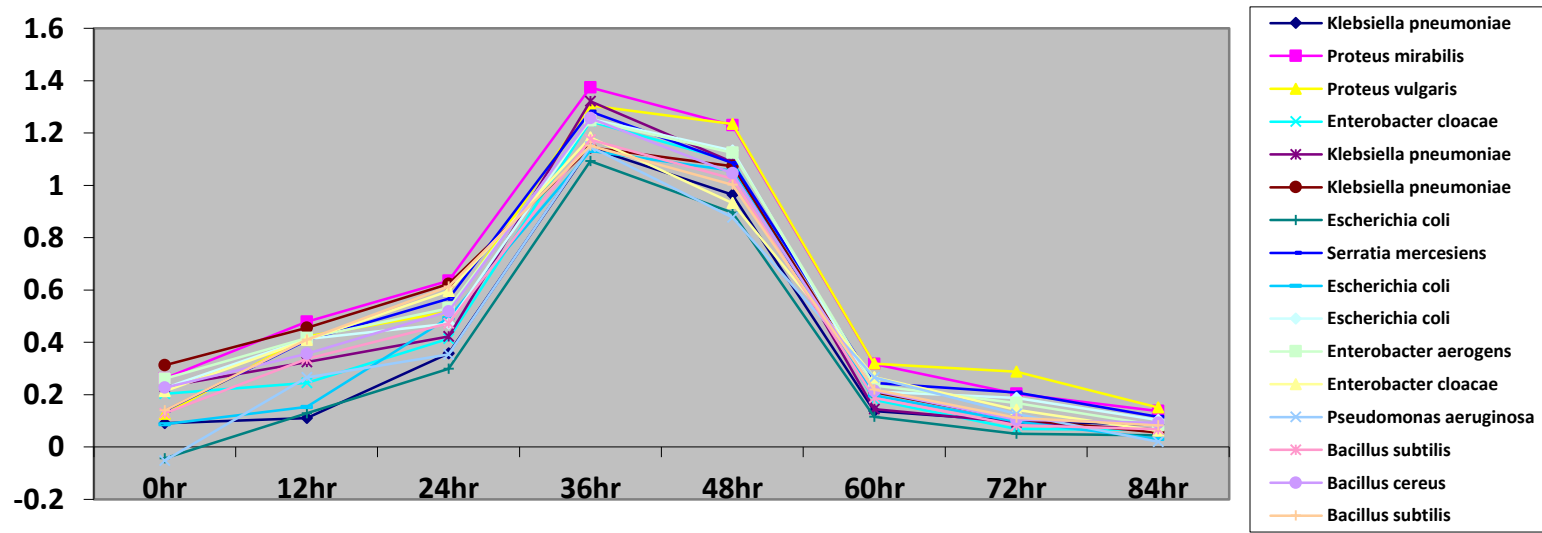


Fig. 20. Growth Dynamic and Killing Time of Bacterial Isolates With Addition of Ciprofloxacin Antibiotic At 48th Hour Using Ultraviolet Spectrophotometer With Wavelength 480λ

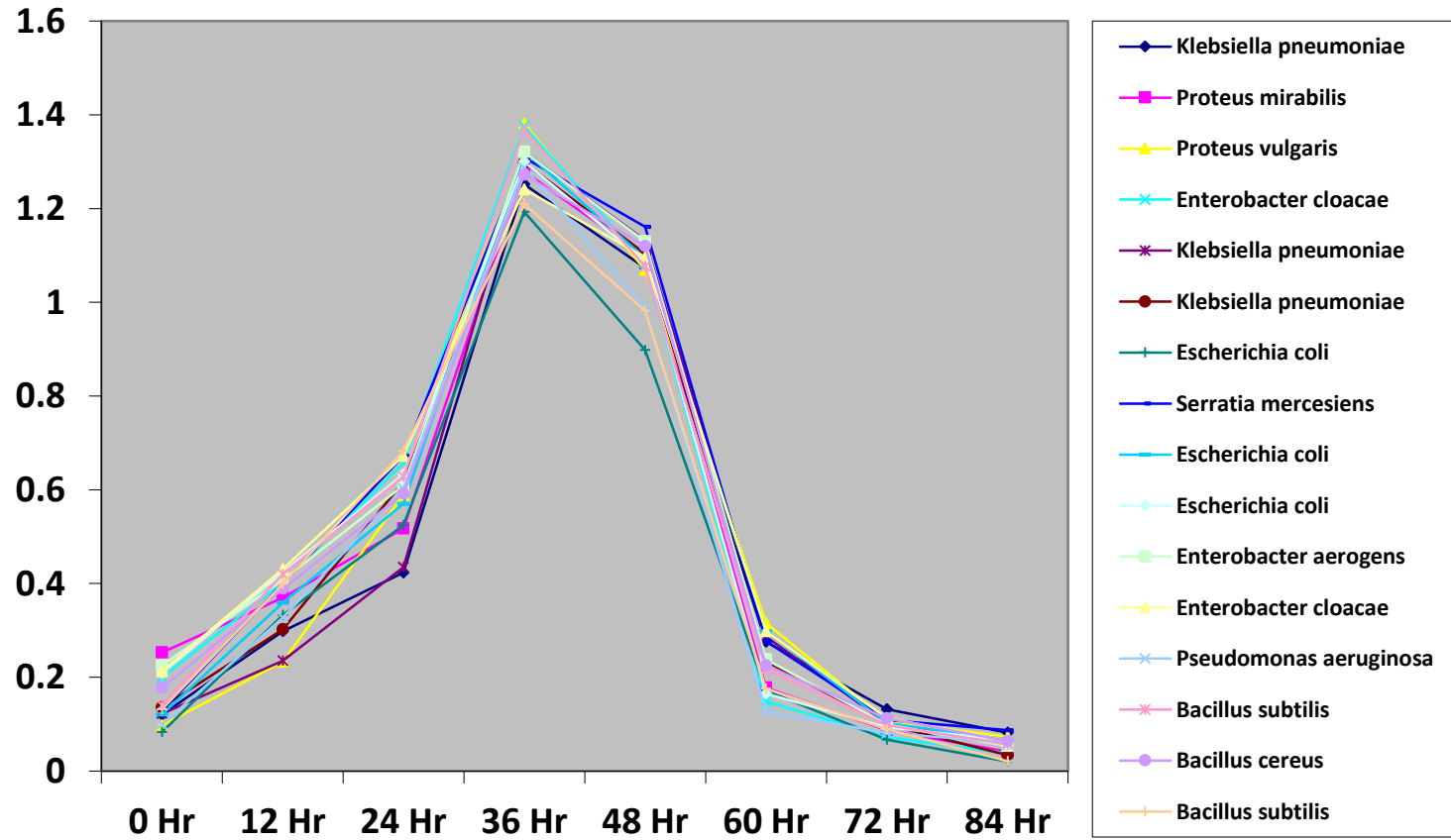


Fig. 21. Growth Dynamic and Killing Time of Bacterial Isolates with Addition of *Aframomum melegueta* Ethanol Seed Extract Antibiotic At 48th Hour Using Ultraviolet Spectrophotometer With Wavelength 480 λ

4. DISCUSSION

The purpose of this research work is to compare the activity of medicinal plant (*Aframomum melegueta*) and conventional antibiotic against Asymptomatic Bacteriuria isolates from pregnant women attending ante-natal clinic in a major primary health center in Akoko, south, Ondo state Nigeria. The target Asymptomatic Bacteriuria isolates which is inherent in pregnant women with no observable features. The rate of growth/ death of Asymptomatic Bacteriuria Isolates were x-rayed.

Pregnant women are at an increased risk of acquiring urinary tract infection due to functional and anatomical changes in pregnancy. In most cases the urinary tract infection is asymptomatic. If asymptomatic urinary tract infection in pregnancy is not treated, it is associated with 30% risk of developing pyelonephritis [32] with subsequent low birth weight and or preterm delivery. Asymptomatic urinary tract infection in pregnancy is common because the short urethra in women makes the urinary tract to be easily contaminated with fecal flora [33]. In this study, *E. coli* and *Klebsiella pneumoniae* were the most common isolated organism. *E. coli* is a common microorganism in the perineum and failure to maintain personal hygiene may increase the risk infection with *E. coli*. [33]. In addition, Gram negative bacteria have a distinct structure which enables the organism to attach, grow and invade the Uro-epithelium. This may result in invasive infection and pyelonephritis [32]. The second most common isolated organism was *Enterobacter cloacae*.

In this study, some of the Gram negative isolates were resistant to commonly used antibiotics. *Klebsiella pneumoniae* was resistant to Ampicillin, Ceporex, Nalixadic acid and Septrin. *Proteus mirabilis* was resistant (45%) to Ampicillin, Nalixadic acid and Septrin. *Proteus vulgaris* was resistant (50%) to Ampicillin. *E.coli*, the most common isolate was sensitive to Gentamycin and most of the antibiotics used. This is similar to what has been found in other studies [34,2,35], in which the Gram negative isolates are resistant to commonly used antibiotics.

The use of plant derived drugs for the treatment of different diseases has long been exploited

traditionally for decades by herb practice with a good knowledge of local flora. In the treatment of diseases or infections, medicinal plants are considered as the best alternative to synthetic drugs. Consumed either as a preventive measure or as a viable treatment solution, phytodrugs are not prone to certain side effects commonly associated with the use of synthetic drugs. The presence of bioactive compounds plants are being studied to help elucidate which of them individually or collectively are responsible for their antimicrobial activities thereby confirming the belief that local plants are the platform for traditional African medicine [36].

In Nigeria, native plants are used either in whole or in combination with other plants as herbal medicine to develop new cures to diseases or physical ailment. Like in other developing countries, native Nigerian plants are consumed as 'plants for medicine and plants for food' [37]. The therapeutically and dietary needs that specific native plant species meet has been underscored in many developing countries (Osuntokun, et al., 2015) .

Aframomum melegueta is widely spread across tropical Africa including Nigeria, Liberia, Sierra Leone, Ghana, Cameroon, Cote D'ivoire and Togo. The phytochemicals obtained from the seed of *Aframomum melegueta* has been used for years in the treatment of infectious diseases. The grains of *Aframomum melegueta* possess active ingredients that may be exploited for local development of antimicrobials [38,39].

The result of this work showed that the ethanol seed extract of *Aframomum melegueta* inhibites the growth of all the asymptomatic bacteria tested. This suggests that the plant extract has a broad spectrum in activity. Higher antimicrobial activity of the *Aframomum melegueta* extracts was observed on *E.coli*, *Klebsiella spp*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterobacter cloacae*, *Enterobacter aerogens*, *serratia marcescens*, *Bacillus subtilis*, and *Bacillus cereus* has moderate activity at high concentration of 100mg/ml and 50 mg/ml. The zones of inhibition on all of the isolates became lower as the concentration of the extract is reduces.. The antimicrobial effect of *Aframomum melegueta* is due to the secondary metabolite i.e phytochemical constituents, present in it. *Aframomum melegueta* seeds are rich in phytonutrient such as Flavonoids, Phenolic,

Tannins, Saponin, Terpenoids, Cardiac glycosides and Alkaloids. The biological function of flavonoids includes protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors [38]. This may be the reason behind the use of the extracts of this plant in the treatment of intestinal troubles in herbal medicine [39].

The presence of phenolic compounds in the seed of *Aframomum melegueta* indicates that this plant might be an antimicrobial agent. This is because phenols and phenolic compounds have been extensively used in disinfection and remain the standard with which other bactericides are compared. Extracts from *Aframomum melegueta* therefore have potent antiseptic or bactericidal properties. (26). This finding supports the use of extracts from *Aframomum melegueta* in treating wounds that not only heals fast but also prevents the formation of infections [25].

The presence of phenol further indicated that the seed of this plant could act as anti-inflammatory, anti-clotting, antioxidant, immune enhancers and hormone modulators. This plant also has quantity of saponin content. Some of the general characteristics of saponin include formation of foam in aqueous solutions, hemolytic activity cholesterol binding properties and bitterness [25]. Apart from saponin other metabolite constituents of *Aframomum melegueta* detected include the alkaloids and tannin. Alkaloids ranked the most efficient therapeutically significant plant substance. Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects. They exhibit marked physiological activity when administered to animals. The high tannin content could be partly responsible for the hot, bitter and pungent taste of *Aframomum melegueta* seed. Tannin has a stringent property; it hastens the healing of wounds and inflamed mucous membrane [25]. The presence of tannin in this plant strongly supports its use in treating wounds, burns and hemorrhoids in herbal medicine. The aqueous extract was found to be bacteriostatic. While ethanolic extract was bactericidal at higher concentrations. The presence of phytochemical supports its uses as antimicrobial agent.

Ultraviolet spectrophotometer was also used to determine the growth dynamic as well as the

death rate of the isolates, the addition of antibiotics to the organism at the 48th hour speed up the death rate of the organisms and also, the addition of ethanol seed extract at the 48th hour also speed up the death rate of the organisms isolated from the urine samples. Bacterial growth is proliferation of bacterium into two daughter cells, in a process called binary fission. Providing no event occurs, the resulting daughter cells are genetically identical to the original cell. Hence, bacterial growth occurs. Both daughter cells from the division do not necessarily survive. However, if the number surviving exceeds unity on average, the bacterial population undergoes exponential growth [27].

The measurement of an exponential bacterial growth curve in batch culture was traditionally a part of the training of all microbiologists; the basic means requires bacterial enumeration (cell counting) by direct and individual (microscopic, flow cytometry) (Skarstad, et al., 2007), direct and bulk (Biomass), indirect and individual (colony counting), or indirect and bulk (most probable number, turbidity, nutrient uptake) methods. In the study, Ultraviolet spectrophotometer is used to determine the lag phase, log phase or exponential phase, stationary phase, and death phase. Ultraviolet spectrophotometer was used to determine the death rate of the organisms with addition of antibiotics and ethanol seed extract of *Aframomum melegueta*. During lag phase, bacteria adapt themselves to growth conditions for active growth and rapid cell multiplication [40].

During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs. During the lag phase cells change very little because the cells do not immediately reproduce in a new medium [40]. The log phase is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period. At the exponential phase, Ciprofloxacin was added to speed up the rate of death of the organisms. This helps us to understand that antibiotics can be used to control the death rate of the organisms, in the study, it was observed that at 0 hour, *Proteus mirabilis* has the highest growth rate of 0.262λ and *Escherichia coli* have the lowest

growth rate of -0.043λ . At 84th hour, *Proteus vulgaris* has the lowest death rate of 0.151λ and *Pseudomonas aeruginosa* have the highest death rate of 0.020λ with the addition of ciprofloxacin. This shows the effect of antibiotics on the organism every 12 hours. In this study, it was observed that at 0 hour, *Proteus mirabilis* has the highest growth rate of 0.253λ and *Escherichia coli* have the lowest growth rate of 0.083λ . At 84th hour, *Serratia mercesiens* has the lowest death rate of 0.087λ and *Escherichia coli* have the highest death rate of 0.020λ with the addition of seed extract of *Aframomum melegueta* [22].

Stationary phase is often due to a growth-limiting factor such as the depletion of an essential nutrient, and/or the formation of an inhibitory product such as an organic acid. Stationary phase results from a situation in which growth rate and death rate are equal. The number of new cells created is limited by the growth factor and as a result the rate of cell growth matches the rate of cell death. At death phase (decline phase), bacteria die. This could be caused by lack of nutrients, environmental temperature above or below the tolerance band for the species, or other injurious conditions, and also the action of the phytochemicals on the bacteria [40].

5. CONCLUSION

This study serves as an indication to the varied possible applications of indigenous medicinal plants like *Aframomum melegueta* towards the treatment of different arrays infections especially the Asymptomatic Bacteriuria in pregnant women. it validates traditional knowledge and adds to the growing literature on herbal sources of emerging nutraceuticals. The information on the therapeutic effect of this plant (*Aframomum melegueta*) in the treatment of urinary tract infections seems to be sparsely known throughout Nigerian cultures. The findings in this study have hence provided scientific support for ethanomedical antimicrobial activity of *Aframomum melegueta* seed extract. Moreover, the mechanism of action of the extracts of *Aframomum melegueta* seed extract contains secondary metabolite which validates its antimicrobial activity.

6. RECOMMENDATION

From the analysis observed from the ethanolic antimicrobial activity, *Aframomum melegueta*

seed extract contains phenolic, alkaloids and other secondary metabolites which validates its antimicrobial activity against asymptomatic bacteriuria in pregnant women, it is advisable for pregnant women to use *Aframomum melegueta* at their second trimester, to eradicate opportunistic infections. I thereby recommend that *Aframomum melegueta* seed should be encouraged in modern day medicine to cure bacterial infections and to improve the women immunity in pregnancy.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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