Comparison between *in Vitro* Activities of *Solanium Aethiopicum* and *Rauwolfia Vomitoria* Leaves Extract on Urinary Tract Infections (UTI) Isolates from Urine.

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DOI: https://doi.org/10.51584/IJRIAS.2024.907063

Received: 27 June 2024; Revised: 12 July 2024; Accepted: 17 July 2024; Published: 24 August 2024

ABSTRACT

Urinary tract infections (UTI) are treated with antibiotics and untreated case could cause permanent damage of kidneys (pyelonephritis), however, pathogens of cystitis are fast becoming resistant to some antibiotics if not all. This resistance has increased the interest of medical researchers on herbal remedies that can serve as alternatives to conventional antibiotics; in line with this quest , this research x-rayed the effects of both ethanol and aqueous extracts of *Solanium aethiopicum* and *Rauwolfia vomitoria* leaves singly and in combination on hematological parameters, lipid profile, enzymatic antioxidant potentials and renal function test with a view to developing an effective antimicrobial agent for the treatment of urinary tract infection/ kidney diseases. Their efficacy of alternatives were evaluated by agar well diffusion and macro-broth dilution methods on 37 bacterial, and 6 fungal clinical urine isolates of *E. coli*, *S. aureus*, *Psuedomonas aeruginosa*, *Candida albicans*, *Staphylococcus* spp, *Proteus marabilis*, *Candida* and *Klebsiella* species. Activity of the ethanolic extract of the *S. aethiopicum* was significantly higher than the aqueous extract ($P<0.05$). Activities of aqueous and ethanolic extracts of *R. vomitoria* were comparable. All extracts affected 6 of the 8 strains used except ethanolic extract of *S. aethiopicum* that affected 7 strains. Ethanolic extract of *S. aethiopicum* was bactericidal on 2 of the organisms, *E. coli* and *Klebsiella* species with MIC ranging from 5.94 to 6.25 mg/ml and MBC of 6.25 to 9.06 mg/ml. Other single extracts were bacteriostatic on affected organisms. Time-Kill assay of ethanolic extracts of *S. aethiopicum* and *R.vomitoria* against *E. coli* (in $\log_{10}$ cfu/ml) had a viable cell count reduction from 7.14 to 3.28 for *S.aethiopicum* and 7.14 to 5.30 for *R. vomitoria* after 4 h exposure at 2MIC. Action of both plants was synergistic in combined before extraction (CBE) aqueous extract and additive in (CBE) ethanolic extract on *E. coli* but antagonistic to all other strains affected. However, action of both plants in combined after extraction (CAE) aqueous and ethanolic extracts were antagonistic on all affected organisms. The best fractions, fraction E of *R. vomitoria* and 3 of *S. aethiopicum* affected 96.3% and 93.8% of the organisms. Phytochemical analyses of crude extracts revealed the presence of *alkanoids*, glycosides and tannin in all while the fractions had *terpanoids* among others in relative proportions. Also, *chlorogenic* acid responsible for the bactericidal effect on the bacterial was revealed in greater percentage of *S. aethiopicum* than *R. vomitoria* by high-performance liquid chromatography (HPLC). LD_{50} of both plants was > 5000mg of extract/mg body weight of albino rat. Results above justify their folkloric application in the treatment of urinary tract infection.

Keywords: Vomitoria, E.coli, Solanium aethiopicum, Antagonistic, Chlorogenic

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume IX Issue VII July 2024

INTRODUCTION

Urinary tract infections (UTIs) called cystitis are fast on the increase being the most common nosocomial infections as well as community-acquired infections. This increase is thought to be due to increase in antibiotic drug administration which has resulted in increased prevalence of antibiotic-resistant strains that can penetrate the urinary tract (Stamm, 2011). On the other hand, high dependency on packaged and processed foods which might contain better adapting microorganisms due to their survival of the drastic chemical treatment of the food, can lead to these colonizing the gastrointestinal tract and ascending the urinary tract when introduced there to cause UTI (WHO, 2016). Urinary tract infection can be symptomatic or asymptomatic. The recurrent UTI can scar the kidney if it penetrates there, and if left to untreated, can lead to kidney failure to a certain degree (Cheesbrough, 2014).

UTI is an illness caused by colonization of any part of the urinary tract by microorganisms mostly bacteria and fungi. It usually occurs when bacteria that normally live in the bowel travel up the short tube (urethra) that connects the bladder to the outside. Once found within the bladder (*Pseudomonas*, *Enterococcus*, *Escherichia coli* and *Staphylococcus saprophyticus*) grow quickly (Shama *et al*., 2017).

Infections of urinary tract are categorized into two classes based on their locations: lower and upper urinary tract infections. Lower urinary tract infections involve the urethra and the bladder and are referred to as urethritis and cystitis, respectively, while upper urinary tract infections which involve the ureters and kidneys are respectively called ureteritis and pyelonephritis. Sometimes, cystitis due to sexual activity shows no bacteria in urine during urinalysis. Such cases are usually called interstitial cystitis/acute urethral syndrome/frequency-dysuria. Pyelonephritis arises when microorganisms from untreated bladder infections (urinary tract infections) travel up to the kidneys and establish there. This type of disease is life-threatening (Copson, 2018).

UTI may be caused by bacteria, fungi, viruses or a variety of parasites. Most of the bacteria are from a person's own intestine or vagina and are mostly gram-negative bacteria such as *Escherichia coli* (75-90%), *Proteus, Klebsiella, Enterobacter* and *Pseudomonas* species, and the gram-positive *Staphlococcus saprophyticus* (WHO, 2016). Urethritis can be caused by sexual intercourse due to an infection by *Chlamydia trachomatis*. *Candida* species is the highest prevalent fungi that causes UTI, especially *C. albicans,* in those with impaired immune system and patients with catheter in bladder (Shankel, 2017). In rare occasions, other fungi like *Blastomyces* and *Coccidioides* spp can cause UTI. The virus mostly implicated in UTI is Herpes Simplex virus (HSV-2) which affects the penis in males and the vulva, perineum, buttocks, cervix or vagina in females. However, when the urethra is involved, it results in dysuria (painful urination). A number of parasites can infect the urinary tract; the protozoan *Plasmodium* that causes malaria can block small vessels in the kidneys or rapidly damage red blood cells leading to acute kidney failure. *Trichomonas vaginalis,* another protozoan which is sexually transmitted can affect the bladder. The flatworm, *Schistosoma haematobium* affect the kidneys, ureters and the bladder by laying its eggs directly into them from the blood vessels (Baron and Finegold, 2017 and Davis, 2011).

Untreated UTI can lead to acute or chronic kidney infections which could permanently damage the kidney tissues. This is more common in children and older adults whose symptoms are easily overlooked or mistaken for other conditions. Pregnant women who have UTI may have increased risk of delivering low-birth weight or premature infants (Human Biology and Health, 2015).

Kidney diseases (Pyelonephritis) remain one of the major threats to public health and are worldwide problem (Asha and Pushparadan, 2010). They are mainly caused by bacteria and viruses. *Escherichia coli* accounts for close to 80% of cases of kidney and urinary tract infection (UTIs)(CDC, 2016). Other commonly isolated bacteria include *Klebsiella*, *Pseudomonas*, *Enterococcus*, and *Staphylococcus*

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS | Volume IX Issue VII July 2024

saprophyticus.

In spite of the tremendous advances in modern medicine, isolates of UTIs are becoming increasingly resistant to most available antibiotics. There is no effective drug available that stimulates kidney function, offer protection to the kidneys from damage or help to regenerate urethric cells due to multiple drug resistance by microbes (superbug) such as Methicillin Resistance *Staphylococcus aureus* (MRSA) and drug abuse in Nigeria. Many people are now resorting to traditionalists and herbalists, whose herbs are not well quantified, for use. Some observations and oral reports though, show that people with high consumption of garden egg have relief in arthritic pains and swelling. Also, that garden egg cleanses the kidney and helps to solve the problem of dialysis in case of diseased kidney.

This is to determine *In vitro* antagonistic relationship of leaf extracts of *S. aethiopicum* and *Rauwolfia vomitoria* on bacterial isolated from urinary tract infections and kidney induced infection in a view to developing a herbal drug that is capable of replacing resistant antibiotics at a very low cost and available always.

MATERIALS AND METHODS

Study Area

This was carried out in Awka and Nsukka and Lagos Nigeria. Samples (urine) were collected from University Teaching Hospital Itiku Ozalla (UNTH) Enugu, Nnamdi Azikiwe University Teaching Hospital Nnewi and Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Amaku- Awka.

Sample Collection

Plant Material

Collection, authentication and processing of plant material: fresh leaves of *S. aethiopicum* were collected from the Agric farm of Faculty of Agricultural Sciences of the University of Nigeria, Nsukka while *Rauwolfia vomitoria* was collected from a herbalist, Mr. Ugwuodugwu Clement, from Owerri-Ugwu Edem-Aniin Nsukka L.G.A of Enugu state. Both plant materials were identified by Dr. Ugwuozor, a taxonomist of Botany Department of University of Nigeria, Nsukka. Taxomic identity of the plants were achieved by deposited voucher specimen and use of documented literature from Dalziel (2016) in the herbarium unit of Department of Botany, University of Nigeria, Nsukka.

Preparation of Samples Extraction

The leaves of *Solanum aethiopicum* and *Rauwolfia vomitoria* were chopped into tiny bits, air-dried for two (2) weeks under shade at room temperature. The dried samples were pulverized using electric grinder (IKA, Germany), weighed and kept ready for extraction of active ingredients (Nwobu *et al*., 2010).

Extraction procedures

A 20 g portion of the samples each was extracted by maceration in 200 ml ethanol and distilled water for 24 h, the resulting extracts were subsequently filtered using Whatman No 1 filter paper. The extracts were evaporated to dryness using a rotary evaporator (IKA, Germany) at an optimum temperature of 45 ^{0}C. All the extracts (i.e ethanol and aqeous) after evaporating them in a vacuum under reduced pressure were stored in sterile glass bottles at room temperature until use (Nwobu *et al*., 2010).

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Urine specimen

In this study, a total of 350 mid-stream urine spacimens were collected between June and September 2019 from 3 different teaching hospitals which include, University of Nigeria Teaching Hospital Itiku Ozalla (UNTH) Enugu, Nnamdi Azikiwe University Teaching Hospital Nnewind Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Amaku- Awka from patients suffering from urinary tract infections (UTIs) attending treatment. Slovins method of sample collection as described by Ryan, (2014) was fully adopted. They were classified according to name, gender and age of patients. The patients were given sterile bottles and instructed on how to collect clean, mid-stream, voided urine samples. Patients unable to collect their own were aided by medical experts.

Isolation and Characterization of microorganism

A loopful of each fresh urine sample were inoculated onto Cysteine Lactose Electrolyte-Deficient (CLED) agar and incubated for 24 h at 37 ^{0}C. Urine samples, which microscopically exhibited the presence of yeast, was inoculated onto Sabouraud Dextrose Agar (SDA) and incubated at 37 ^{0}C for 24 h. Discrete colonies were inoculated on nutrient agar slants, refrigerated and further tested using microbiological tests (Harley and Prescott, 2012).

Characterization procedures of isolates

The isolates were Gram stained and classified into Gram-negative and Gram-positive strains. The gram-negative bacteria were tested for motility, urease, indole, citrate, oxidase and hydrogen sulphide production while the Gram-positive bacteria were tested for coagulase (i.e, slide-agglutination method) and catalase production. The Gram-negative bacteria was further subcultered onto Eosin Methylene Blue (EMB) agar and the Gram-positive bacteria onto Manitol Salt Agar (MSA) to confirm the identification of isolates. The yeasts were identified based on colour of the colonies when grown on Chromogenic Candida Agar (CCA), and their ability to form germ tube after incubation in human serum for 3 h (serum test) isolated strains in addition with molecular characterization using Polymerase chain reaction-based technique to isolate the DNA and amplify them using universal based primer (Mullis *et al*., 2010).

Isolates were maintained on brain heart infusion agar and stored at 4°C, and were sub-cultured once every two-weeks (Quinn *et al*., 2014). The identified organisms were used for antimicrobial activity.

Gram staining

This was carried out using the modified method of Prescott *et al.* (2008). In this process, a thin smear of the culture was prepared on a clean grease free slide, air dried and heat fixed. The smear was flooded with crystal violet solution for 60 seconds and rinsed with water. It was then covered with Gram iodine for 60 seconds and rinsed with water. Alcohol (95% w/v ethanol) was used to decolorize the slide content for 10 seconds and rinsed with water. The smear was then counter stained with safranin solution for 60 seconds, rinsed with water and air-dried. The stained smears were then observed under the light microscope using oil immersion objective lens.

Motility test

This was carried out using the modified method of Prescott *et al.* (2008). The medium used was semi-solid agar. It was prepared by adding 4 g of bacteriological agar to 15 g of nutrient broth in 1 litre of deionized water. Heat was applied to dissolve the agar and 10 ml amount were dispensed into test tubes and sterilized by autoclaving. The test tubes were allowed to set in a vertical position. Inoculation was done by making a single stab down the center of the test tube to about half the depth of the medium using a sterile stabbing

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rod. The test tubes were incubated at room temperature and growth examined after about 6 hours. Motile bacteria swarm gave a diffused spreading growth that was visible to the naked eye.

Oxidase test

This was carried out using the modified method of Precott *et al.* (2008). Oxidase reagent containing 1.0% (w/v) tetramethyl-p-phenylenedimine dihydrochloride was prepared by dissolving 0.1 g of this compound in 10 ml of deionized water. Strip of filter paper was soaked with this reagent, smears of the isolate from the pure cultures were made on the oxidase paper strips and observed for color change from gray to purple or violet color between 1-5 seconds for oxidase positive organisms. For oxidase-negative bacteria, there was no color change. The change of color was due to the possession of cytochrome.

Methyl-Red Voges-Proskauer (MR-VP) test

Biochemical method was carried out according to Precott *et al.* (2008), 7.5 g of MRVP medium (oxoid) was dissolved in 500 ml of deionized water. Ten millitres (10 ml) of the medium were taken and dispensed into test tubes. The medium was then sterilized and cooled. The test tube was divided into two equal sets, one for methyl red (MR) test and the other for voges proskauer (VP) test. Isolates from the pure stock culture were then inoculated into different sets of test tubes and labeled. The test tubes were incubated at 37°C for 24 h. For methyl red, 2 ml of MR reagent was added to the test tube of the culture and color changes was observed. From yellow to red indicated positive result while the persistence of the yellow color indicated a negative result. Six drops of Barritt reagent (x-naphthol solution) was added to the broth tubes, followed by the addition of 5 drops of 40% (w/v) potassium hydroxide solution. The presence of pink- red coloration showed a positive result while absence of color change was regarded as VP-negative.

Indole test

Biochemical method was carried out according to Prescott *et al.* (2008), Indole is a nitrogen-containing compound formed when the amino acid tryptophan is hydrolyzed by bacteria that have the enzyme tryptophanase. This was detected by using Kovac's reagent. For this test, isolates were grown in peptone water (oxoid) in 500 ml of deionized water. This was dispensed into test tubes in 10 ml amounts and sterilized. The medium was then inoculated with the isolates and incubated at 37°C for 24 h. Five drops of Kovac's reagent were carefully layered onto the top of the 24 hours old culture. The presence of indole was revealed by the formation of red layer colouration on top of the broth culture.

Sugar fermentation

Biochemical method was carried out according to Prescott *et al.* (2008), this test was carried out to know the ability of the isolates to metabolize some sugars (glucose, sucrose, lactose, manitol etc.) with the resultant production of acid and gas or either. Into 1 litre of 1% (w/v) peptone water was added to 3 ml of 0.2% (w/v) bromocresol purple and dispensed in 9 ml amounts, into tests that contained inverted Durham tubes. The medium was then sterilized by autoclaving. The sugar solutions (glucose and sucrose) were each prepared at 10% (w/v) and sterilized. One millitre (1 ml) amount of each sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate isolates and the cultures were incubated at 37 °C for 48 h and was examined for the production of acid and gas. A change in color from purple to yellow indicated acid formation while gas production was assessed by the burbles in the inverted Durham tubes.

Coagulase test

Biochemical method was carried out according to Prescott *et al.* (2008), this test was used to identify those organisms that can produce the enzyme coagulase. A drop of distilled water was placed on each end of a

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slide. This was emulsified by a colony of the test organism. A drop of plasma was added to one of the suspensions and mixed gently. Clumping of the organism within 10 seconds indicated a positive result.

Advanced Characterization of Isolates

Antimicrobial susceptibility testing.

The susceptibilities of the isolates to ten antibiotics were performed by the standard disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2013). The antibiotic discs (Oxoid) used in the present study are shown in Fig. 1

Phytochemical screening

Phytochemical screenings were carried out on the powdered ethanol stalk extract using standard procedures to identify the constituents as described by Sofowara, (2013); Trease and Evans (2012).

Test for Alkaloids:

About 0.5 g of crude powder was defatted with 5% ethyl ether for 15 minutes. The defatted sample was extracted for 20 min with 5 ml of aqueous HCl on a boiling water bath. The resulting mixture was centrifuged for 10 minutes at 3000 rpm. One milliliter (1 ml) of the filtrate was treated with few drops of Mayer's reagent and another 1 ml with Dragendroff's reagent and turbidity was observed.

Test for Tannins

About 0.5 g of the dried powdered samples were boiled in 20 ml 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 coloration.

Test for Terpenoids (Salkowski test)

Five milliliters (5 ml) of the extract was mixed in 2 ml of chloroform, and 3 ml concentrated H_2SO_4 was carefully added to form a layer. A reddish-brown coloration of the interface was formed to show positive results for the presence of terpenoids.

Test for Glycosides (Keller-Killani test)

Five milliliters (5 ml) of the extracts were treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the thin layer.

Test for Steroids

Two ml of acetic anhydride was added to 0.5 g ethanolic extract of the sample with 2ml H_2SO_4. The color changed from violet to blue or green in some samples indicating the presence of steroids.

Test for Saponins

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of an emulsion.

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Test for Flavonoids

Five milliliters (5 ml) of dilute ammonia solution was added to a portion of the aqueous filtrate of the plant extract followed by addition of concentrated H_2S0_4. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing. Few drops of 1% aluminum solution were added to a portion of each filtrate. A yellow coloration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration is positive for flavonoids.

Extraction of phytochemicals

The plant sample (1g) was transferred into a test tube and ethanol (15ml) and 50% m/v potassium hydroxide (10ml) was added. The test tube was left to stand in a water bath maintained at 60 ^{O}c for 60 minutes. The content of the test tube was emptied into a separatory funnel after this time. The reaction product was washed successively with 20ml of ethanol, 10ml of cold water, 10 ml of hot water and 3ml of hexane. The extract was finally washed three times with 10ml of 10% v/v ethanol aqueous solution. The solution was dried with anhydrous sodium sulfate and solubilized with 100 ul of pyridine. Afterward, it was transferred to a vial for analysis

Aqueous Extraction

For aqueous extraction, 200 g of each herb dried powder was placed in 2000 ml de-ionized water in beakers and heated for 10 minutes on a magnetic stirrer hotplate until the temperature reached 95 °C. Subsequently, the mixtures were allowed to cool for 10 minutes to increase extraction of active compound. The pH of the crude extract solution was recorded. Extracts were then filtered through filter paper (Whatmann no. 1) to remove smaller particles using a Buchner funnel. Each final extract was stored in a dark screw-cap sterile container (this is done because phenolics are photo-sensitive) and then stored at 4°C for 24 h before use (Nwobu *et al*., 2010).

Combination Extraction

100g of the grounded herbs were mixed together and soaked in the correct proportion of both distilled water and ethanol to achieve aqueous and ethanol extract respectively of the combined herbs following (Nwobu *et al*.,2010) of preparation. The pH of each crude extract solution was recorded.

Standardization of Inoculum

A 100 ml of 0.5 MacFarland standards was prepared by mixing 99.5 ml of 1 concentrated tetraoxosulphate (vi) acid (H_2SO_4) and 0.5 ml of 1.75 ml barium chloride ($BaCL_2.2H_2O$). It was placed in a brown bottle and stored in the dark and a small quantity of it from the test tube was used to standardize inoculum at any time. Standardization of inoculum involves pouring the overnight inoculum into sterile normal saline (0.85% Nacl) in a test tube and comparing the turbidity to that of MacFarland standards (Cheesbrough *et al*., 2015).

Antibiotic Susceptibility Test

(a) Sterile Mueller-Hinton Agar plates were seeded with 0.1 ml of 0.5 MacFarland (approximately 10^7 cfu/ml) standardized isolates, spread out with a bent glass rod and left to dry on the bench for 30 mins.

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Plates were incubated at 37 ^{O}C for 16-18 h. The diametersof the zones of inhibitions were measured, recorded and interpreted (CLSI *et al.,* 2015) approved standard chart.

(b) Antimicrobial Susceptibility Testing of Crude Extracts

Screening for antimicrobial activity was performed by agar well diffusion method (Shahidi bonjar *et al* .,2010; Saeed and Tariq,2017). Duplicate plates of Mueller-Hinton Agar (MHA) were prepared and 20 ml poured into each sterile Petri dish and allowed to set. Each plate was then seeded with 0.1ml of isolate suspension from overnight broth culture with turbidity adjusted to 0.5 MacFarland standards (containing 1×10^{8} cfu/ml). This was spread out and allowed to dry on the bench for 30 mins. Three holes were bored about 1 cm away from the edges of each plate using a sterile cock borer 6mm diameter.The aqueous extracts was dissolved in distilled water and the ethanol extracts in 6% dimethyl sulfoxide(DMSO). A stock concentration of 100 mg/ml was prepared by dissolving 1g of each plant extract in 10 ml of the diluents. The agar wells were filled with 0.01ml of extract solution at varying concentrations of 50 mg/ml, 25mg/ml and the last with the diluents to serve as the control. This was performed in duplicates. The plates were incubated at 37 ^{O}C for 16 -18 h, after which zones of inhibition was measured with a transparent meter rule and recorded. Culture isolates with holes equal to or greater than 7mm was considered susceptible to the tested extracts (Nwobu *et al.,* 2010).

(d) Determining of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Minimum Inhibitory Concentration (MIC) was determined using two-fold macro-dilution assay for microorganisms that will be susceptible to the various plant extracts. The dilution was performed by dispensing into each tube 1ml of Mueller-Hinton Broth and 1ml of each plant extract starting with a concentration of 50 mg/ml serially diluted two-fold down to 0.780mg/ml with different ranges of micropipettes. This was later inoculated with a loopful of the test organism adjusted to 0.5 MacFarland standards and incubated at 37 ^{O}C for 18 h. Negative control tubes with 1ml of (MHB) and 1ml of each plant extract dilution without any test organism was set up while the positive control tube was set up with 1ml of (MHB) 1ml of the diluents and a loopful of the test organism (containing 4×10^{5} cfu/ml). The lowest concentration of the extract that inhibits the growth of the organism as detected by lack turbidity or the same turbidity as the negative control was designated the MIC (Shadidi-Bunjar, 2010).

The minimum bactericidal concentration (MBC) was defined as the lowest concentration where no bacterial growth was observed. This was determined by sub-culturing onto extract free nutrient agar as described by CLSI (2013).

RESULTS

RESULTS: Isolation and characterization

A total of 350 urine samples (257 from females, 93 from males) were collected from patients suffering from urinary tract infection and attending treatment in these three hospitals: University of Nigeria Teaching Hospital Itiku Ozalla (UNTH), Enugu; Nnamdi Azikiwe University Teaching Hospital,Nnewi and Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Amaku-Awka all in Enugu and Anambra State. Microscopy of centrifuged urine samples showed 16(11 females and 5 males) had significant number of pus cells (pyuria) while 54 (46 females and 8 males) had little pus cells. Calcium oxalate crystals were found in 14 urine samples (4 with growth and 10 without growth). One male،s urine sample contained tyrosine crystals; another contained cysteine crystals with microbial growth on inoculation, while another had schistosoma eggs and a lot of red blood cells. One female،s urine sample had triple phosphate crystals (with no microbial growth); and another 7 had numerous epithelial cells, also without any growth.

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Urinalysis carried out with combi-9 (Medi-Test) showed 17 urine samples (10 with growth and 7 without growth) had protein; 7 urine samples (all with growth) were nitrite positive and 3 urine samples had moderate to high glucose concentration. Fifty-four urine samples (36.2%) from females and twelve urine samples (19.4%) from males showed significant growth. The isolates were identified by Gram stain and biochemical tests as *Escherichia coli* (17), *Staphylococcus* species (12), *Staphylococcus aureus* (7), *Proteus* species (8), *Pseudomonas aeruginosa* (6), *Candida* species (7), *Enterobacter* species (5), *Klebsiella* species (2), and *Candida albicans* (2).

From the above isolates, 43 strains – *Escherichia coli* (12), *Staphylococcous* species (10), *Proteus* species (5), *Staphylococcus aureus* (4), *Candida* species (4), *Klebsiella* species (3), *Pseudomonas aeruginosa* (3), and *Candida albicans* (2) were used in the study.

Activities of the Crude Extracts

Yield of Aqueous and Ethanolic Extracts

Out of 200g of dried and ground plant samples extracted with 2000ml of distilled water (aqueous) or 90% analytical ethanol, *Sonalum aethiopicum* yielded 13.12 g (6.56%) for aqueous extract and 12.56 g (6.28%) for ethanolic extract while *Rauwolfia vomitoria* yielded 10.68g (5.34%) for aqueous extract and 29.20g (14.6%) for ethanolic extract. The powder of the two plant samples combined together before extraction (CBE) in the ratio of 1:1 yielded 20 g (10%) for the aqueous extract and 24 g (12%) for ethanolic extract (Tabl 3).

Table 3: Yield of the Extracts

Extract	Yield (g)	Yield (%)
Aqueous *R. vomitoria*	10.68	5.34
Ethanolic *R. vomitoria*	29.20	14.6
Aqueous *S. aethiopicum*	13.12	6.56
Ethanolic *S. aethiopicum*	12.56	6.28
Aqueous CBE	20	10
Ethanolic CBE	24	12

Key: CBE = Combined Before Extraction

pH of the Plant Extracts.

Table 4 shows the pH of the extract solutions after extraction (before drying) and the reconstituted dry extracts (after drying) used in antimicrobial activity. It showed that the crude extract solutions were more acidic (pH 3.5 to 4.9) before drying and less acidic (pH 3.8 to 5.5) after reconstitution of the dry extracts in distilled water for aqueous extracts and 6% dimethyl sulfoxide (DMSO) for ethanol extracts. The differences were consistently wider for ethanol extracts (> 1) than aqueous extracts (< 0.3)

Antimicrobial Activity of the Single Plant Extracts.

Figure 3: shows the activity of *S. aethiopicum* aqueous extract on the isolates. The average zone of inhibition varied directly with increase in extract concentration. All isolates were inhibited at the highest concentration of 50 mg/ml (effectively 500g of extract) except *Klebsiella* species and *P. aeruginosa.* The mean zone of inhibition on *E. coli* (4.5mm) was significantly lower than those of *C. Albicans*(16.5mm), *S. aureus*(15.5mm) and *Staphylococcus* species (13.8mm). However, the inhibition of the antibiotic

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controls, ciprofloxacin and fluconazole on the bacterial (10.17mm for *E. coli)* and fungal (25.5mm for *C. Albicans)* isolates here were more susceptible than others.

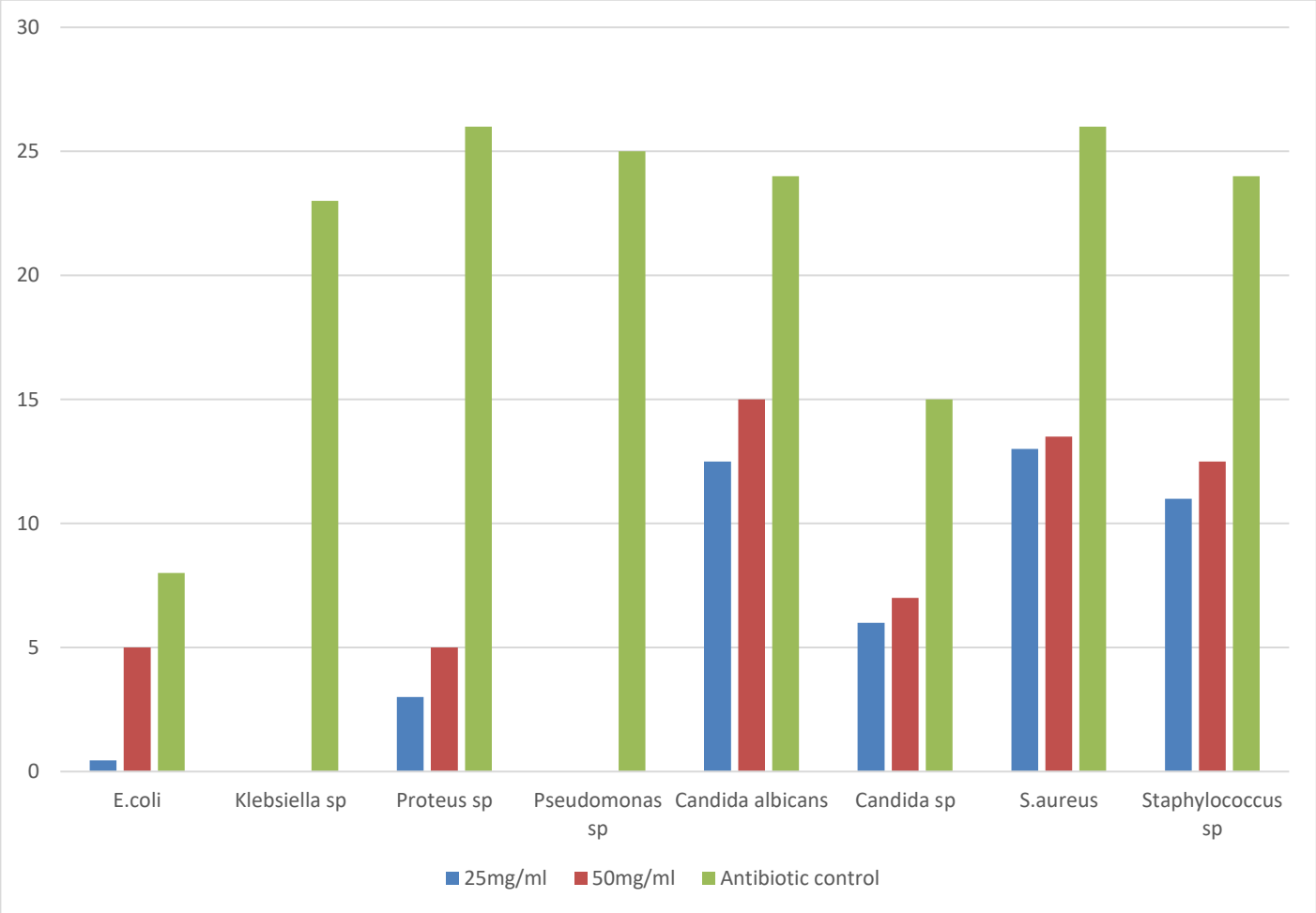

Fig 3: Antimicrobial activity of *S. aethiopicum* aqueous extract on the Urine Isolates.

Figure 4: shows the antimicrobial activity of the ethanolic extract of *S. aethiopicum* on the isolates. The extract exhibited a broad spectrum activity on most of the isolates except *P. aeruginosa. Klebsiella* species not affected by the aqueous extract was inhibited though not significantly. There was no significant difference ($p \geq 0.05$) in the mean zone of inhibition produced against *E.coli* (12mm) and *Klebsiella* species (11.33mm), and against *S. aureus* (I7.5mm) and *Staphylococcus* species (16mm).

Figure 5: presents the antimicrobial activity of *R. vomitoria* on aqueous extract on the isolates. The least inhibited was Proteus species (8mm) while *Klebsiella* species and *P. Aeruginosa* were not inhibited. There was no significant difference ($p \geq 0.05$) between the mean zone of inhibition produced against *E.coli* (8.5mm) and *Proteus* species (8mm), and against *Staphylococcus* species (12.5mm) and *S. aureus* (12.25mm) at 50 mg/ml. However, the mean zone of inhibition of the antibiotic control was significantly higher than that of the extract on all the isolates ($p \leq 0.05$).

Figure 6: presents the antimicrobial activity of *R. vomitoria* on ethanolic extract on the isolates. *Klebsiella* species and *P. Aeruginosa* were not inhibited but others were inhibited at varying degrees. The mean zone of inhibition produced against *E. coli* (10.17mm) was significantly lower ($p \leq 0.05$) than that on *Staphylococcus* species (15.1mm), and *C. albicans* (16mm) but significantly higher than that on *Proteus* species (2.4mm). There was no significant difference ($p \geq 0.05$) in the mean zone of inhibition produced against *E. coli* (10.17mm) and *S. aureus* (11.25mm).

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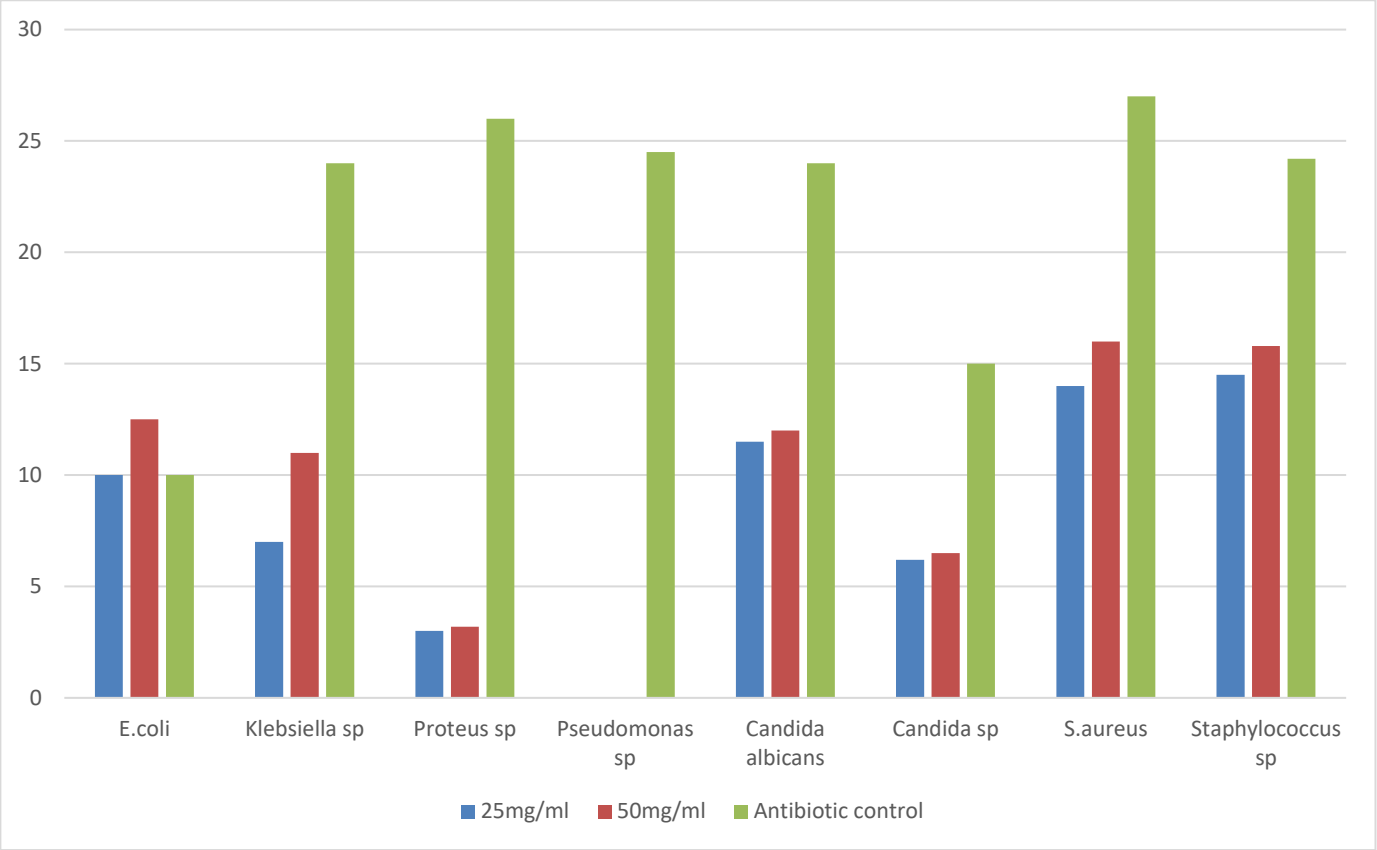

Fig 4: Antimicrobial activity of *S. aethiopicum* ethanolic extract on the Urine Isolates

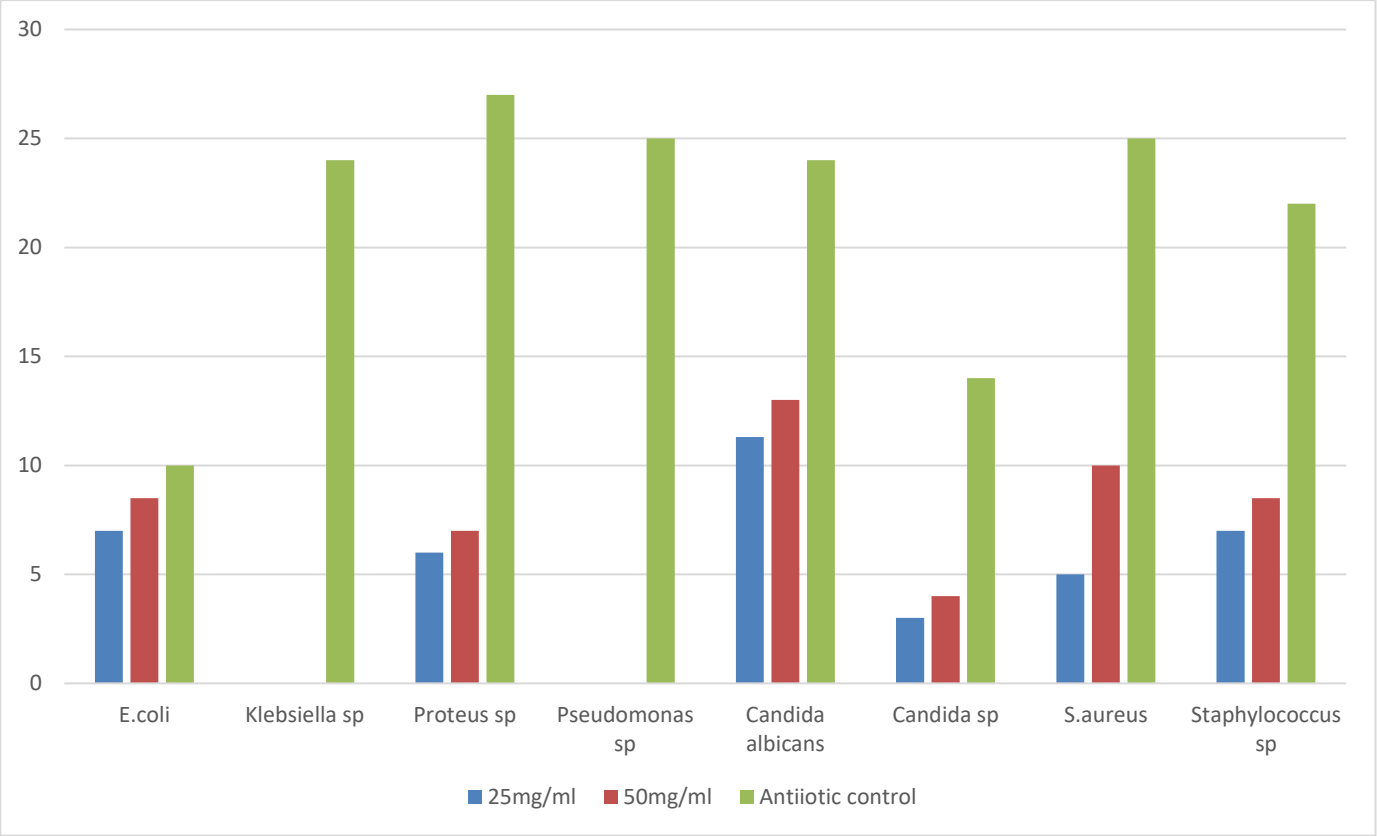

Fig 5: Antimicrobial activity of *R. vomitoria* aqueous extract on the Urine Isolates

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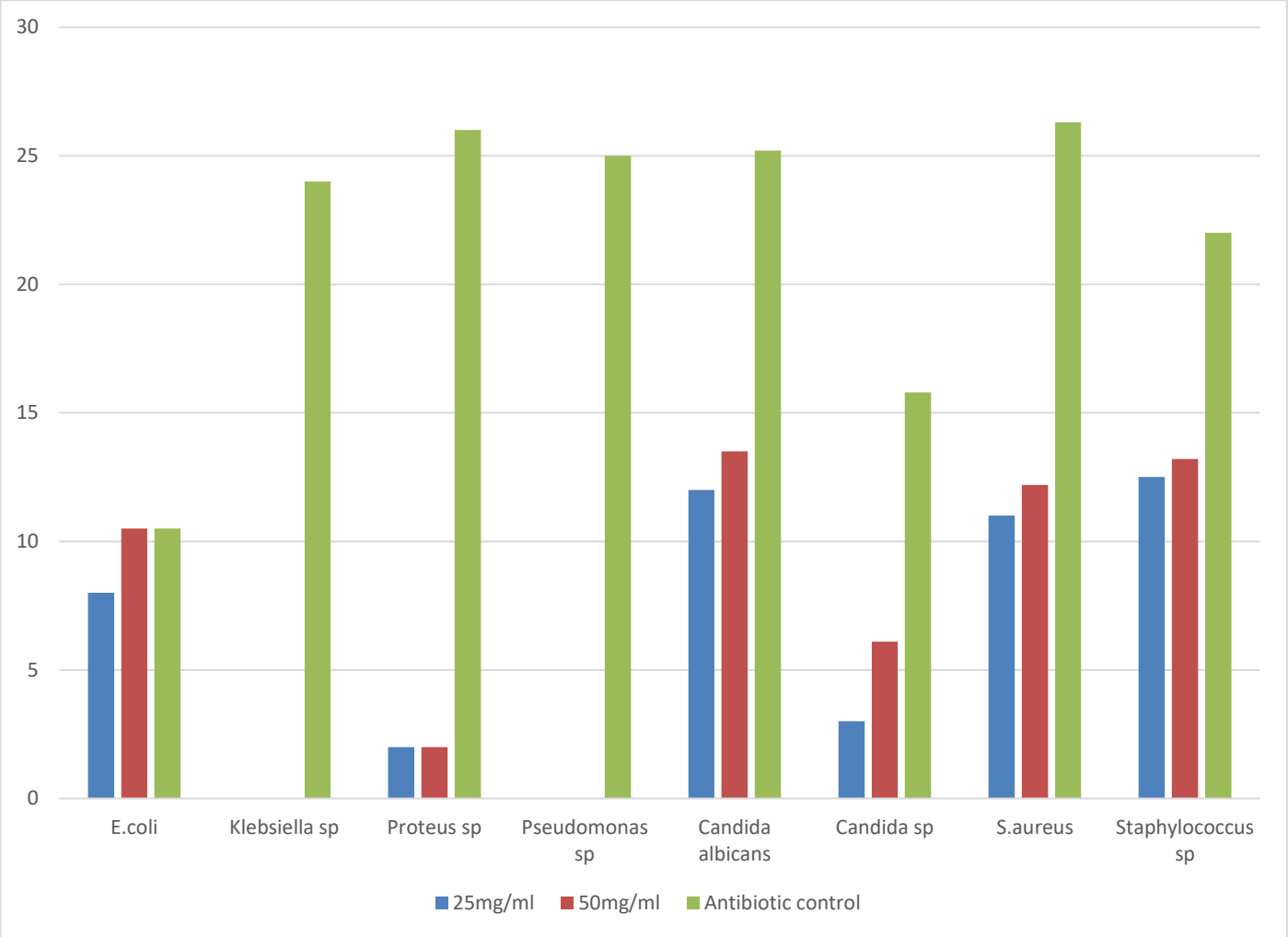

Fig 6: Antimicrobial activity of *R. vomitoria* ethanolic extract on the Urine Isolates.

Antimicrobial Activity of the Combined Extracts

Figure 7: shows the antimicrobial activity of *S. aethiopicum* and *R. vomitoria* combined together before extraction (CBE) aqueous extract on the isolates. The extract had no activity against *Klebsiella* species, *P. aeruginosa* and *Candida* species while the highest mean zone of inhibition was shown against *E. coli* (14.5mm). The mean zone of inhibition against *Proteus* species (2.6mm) was not significantly different ($p \geq 0.05$) from that against *Staphylococcus* species (3.6mm).

Figure 8 presents the antimicrobial activity of *S. aethiopicum* and *R. vomitoria* combined together before extraction (CBE) ethanolic extract on the isolates. There was no inhibition against *Klebsiella* species, *Proteus* species, *P. Aeruginosa*and *Candida* species. The mean zone of inhibition against *E. coli* (14.83mm) was significantly higher ($p \leq 0.05$) than that against *Staphylococcus* species (4.2mm). The extract had moderate activity against *S. aureus* (9.75mm).

Figure 9 shows the antimicrobial activity of *S. aethiopicum* aqueous extract and *R. vomitoria* aqueous extract combined in the ratio 1:1 designated combined after extraction (CAE) on the isolates. This had no activity against *Klebsiella* species, *Proteus* species and *P. aeruginosa.* The activity at 50 mg/ml as indicated by mean zone of inhibition between *Candida* species (4.25mm) and *Staphylococcus* species (4.6mm); and between *C. albicans* (9.5mm) and *S. aureus* (10.75mm) were not significantly different ($p \geq 0.05$). However, activity on *E. coli* (1.25mm) was significantly lower ($p \leq 0.05$) than that against *S. aureus* (10.75mm).

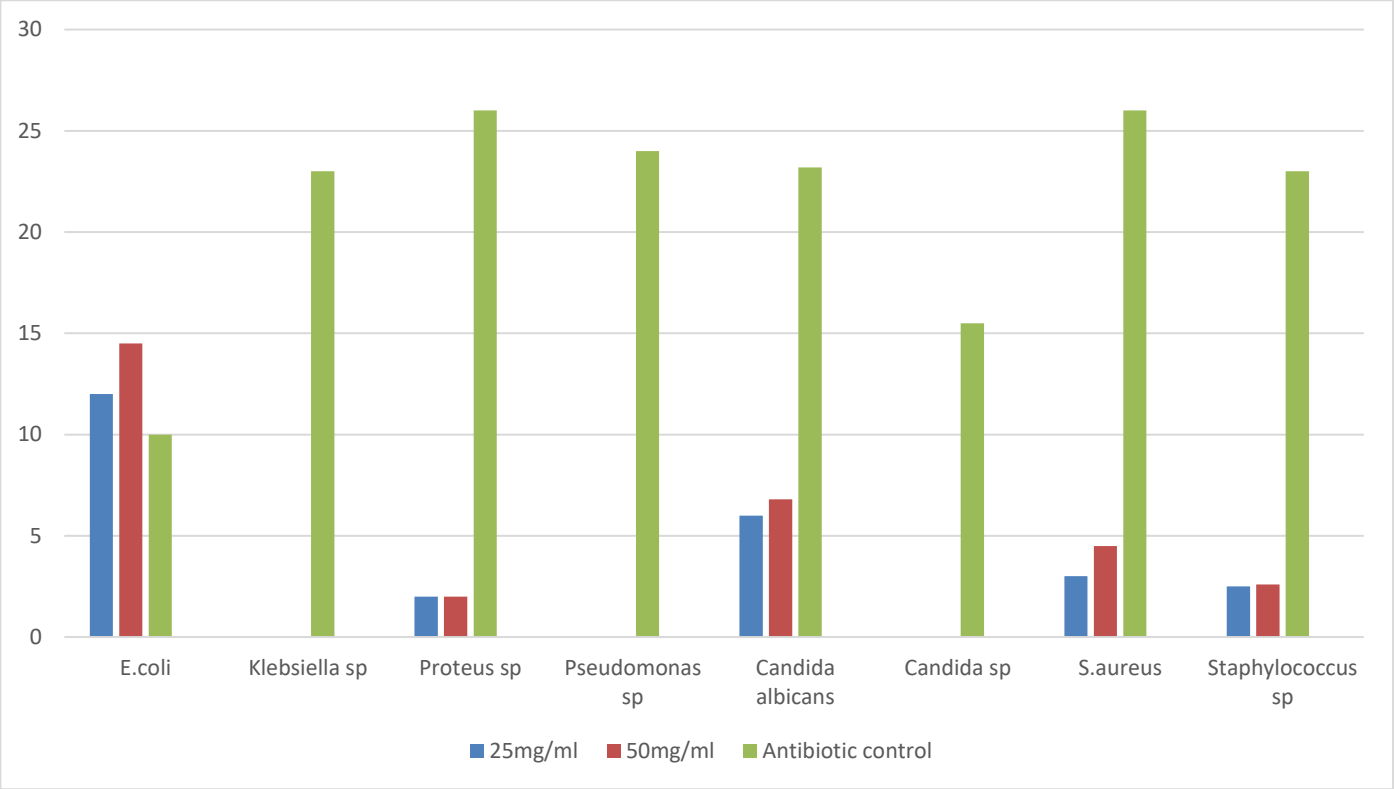

Fig 7: Antimicrobial activity of plants combined together before extraction (CBE) aqueous extract on the Urine Isolates.

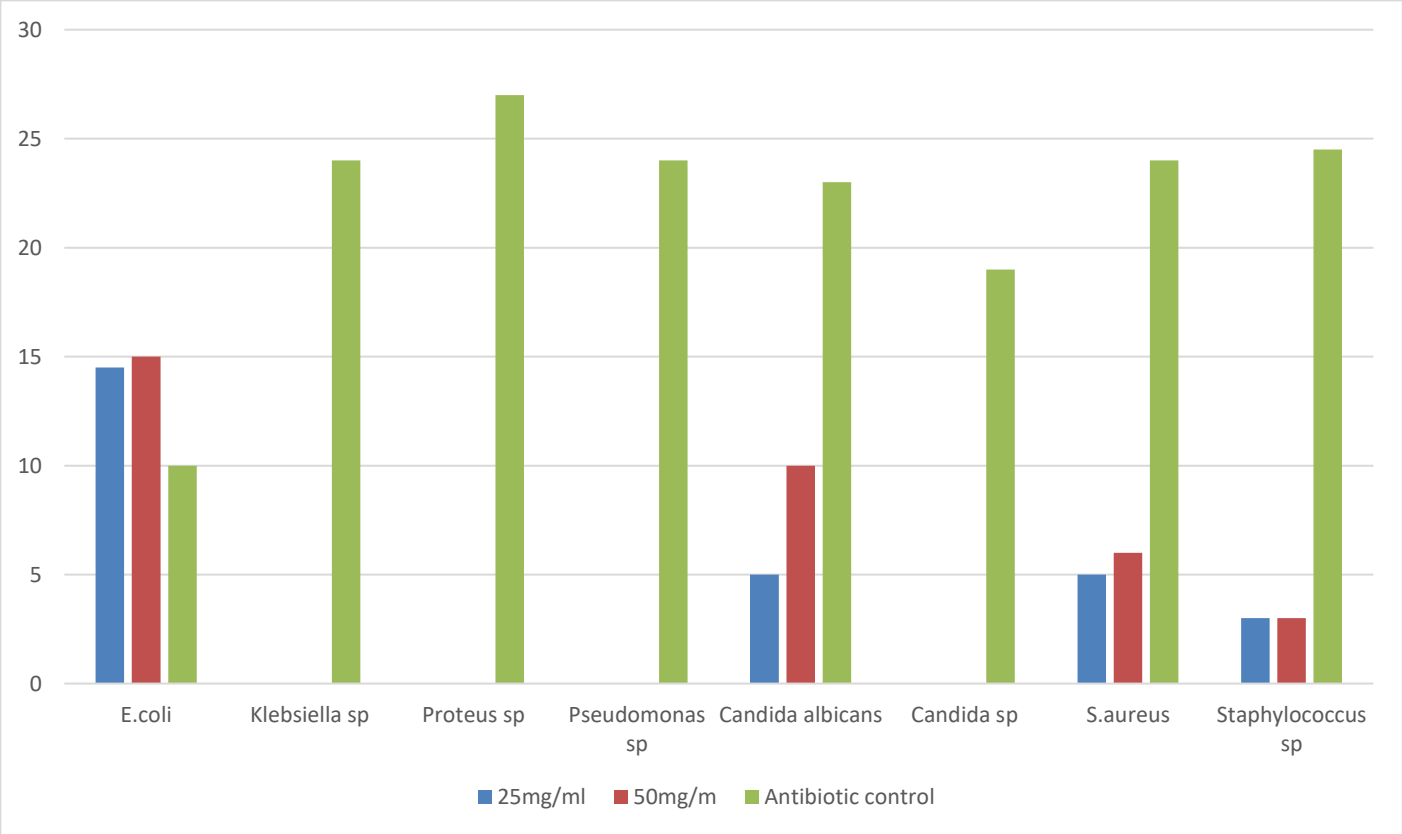

Fig 8: Antimicrobial activity of plants combined together before extraction (CBE) ethanolic extract on the Urine Isolates

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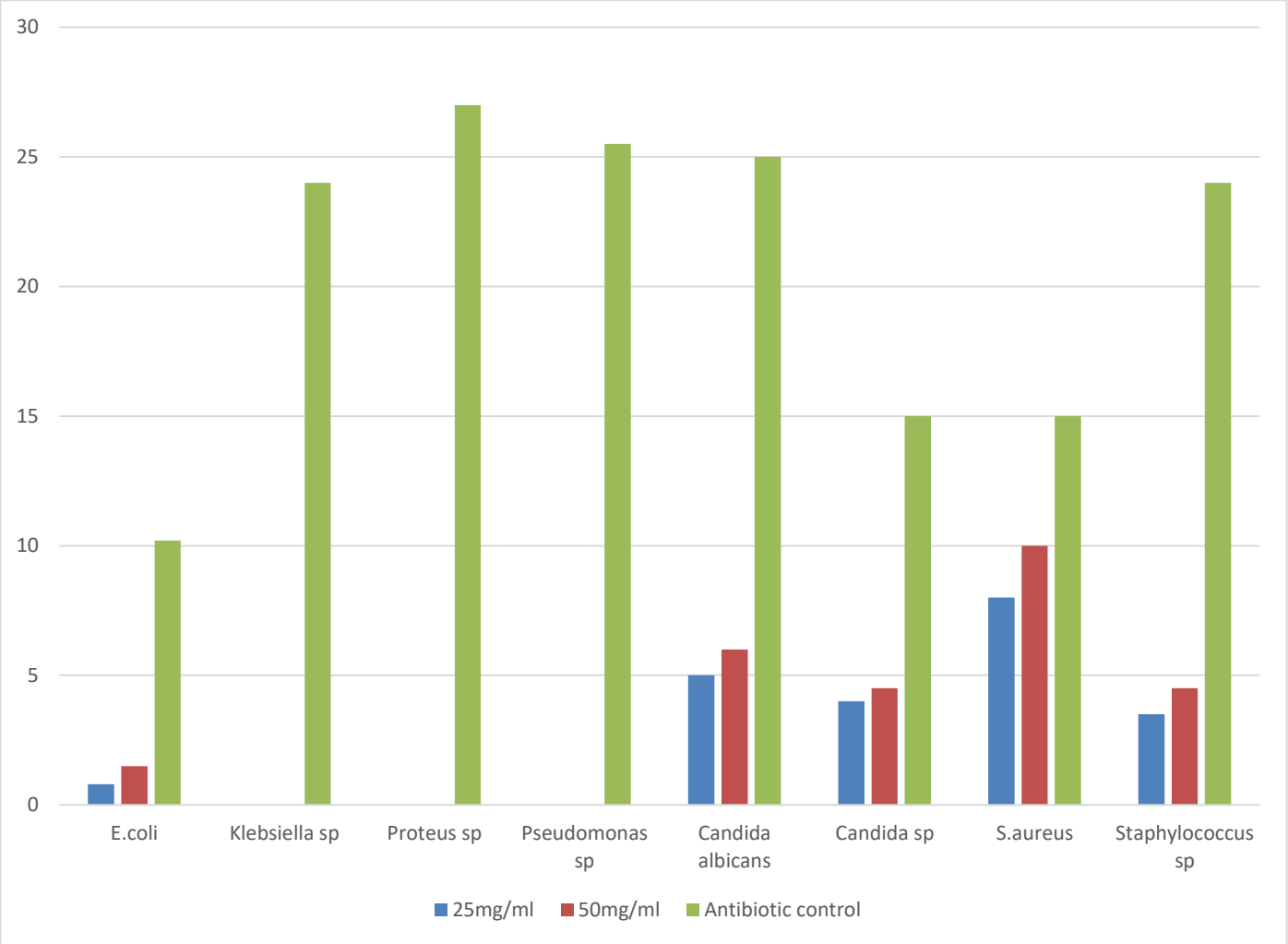

Fig 9: Antimicrobial activity of aqueous extracts of plants combined together after extraction (CAE) on the Urine Isolates.

Figure 10 presents the antimicrobial activity of *S. aethiopicum* ethanolicextractand *R. vomitoria* ethanolic extract combined in the ratio 1:1 (CAE) on the isolates. The mean zone of inhibition against *E. coli* (7.12mm) was significantly ($p \leq 0.05$) higher than that against *Klebsiella* species (2.33mm). There was no significant difference ($p \geq 0.05$) between the zones of inhibition against *E. coli* (7.12mm) and *Staphylococcus* species (7.3mm) and no difference between those of *C. albicans* (12mm) and *S. aureus* (12mm).

Minimum inhibitory concentrations (MIC) and Minimum bactericidal concentrations (MBC).

Table 5: shows that *S. aethiopicum* ethanolic extract exhibited a relatively lower MIC (3.13 mg/ml to 9.38 mg/ml) on most of the isolates than the aqueous extract (6.25 mg/ml to 14.09 mg/ml) except on *Candida* species. Also, the MBC values were low except on Proteus and Candida species.

Ethanolic extract of *R. vomitoria* exhibited the lowest MIC of 2.98 mg/ml against *Staphylococcus* species (Table 6) while the highest MIC of 37.5 mg/ml was exhibited against *Proteus* species by its aqueous extract. The MBC values of the ethanolic extract were generally lower than those of the aqueous extract.

However, the lowest MBC was recorded by *S. aethiopicum* ethanolic extract against *Klebsiella* species (6.25 mg/ml) as shown in table 5

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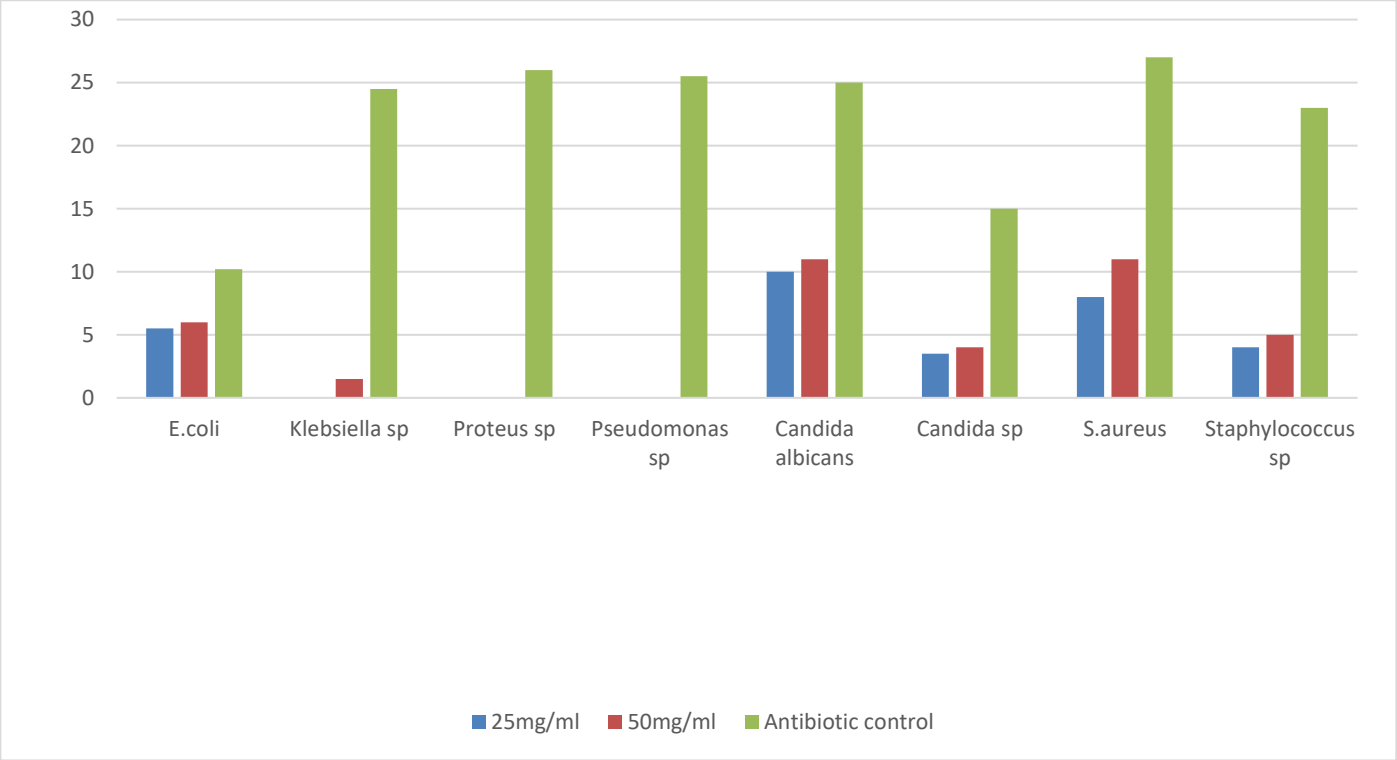

Fig 10: Antimicrobial activity of ethanolic extracts of plants combined together after extraction (CAE) on the Urine Isolates.

Table 10: Phytochemical Analysis of the Crude Extracts

Phytochemicals	ASA	ESA	ARV	ERV	AC	EC	
Alkaloids (Wagner's) (Dragendorf s)	++	++	++	+++	+ +	+++	
Tannins	+	++	++	+++	+	+++	
Anthraquinones	++	–	+	–	–	–	
Flavonoids	–	++	+	+++	++	++	
Terpenoids	+++	++	–	++	++	++	
Saponins	+	–	+	–	+	–	
Phenols Glycosides	+++	+++	+++	+++	+++	+++	+++

Keys: – = absent; + = slightly present; ++ = moderate; +++ = abundant

ASA = *S.aethiopicum*aqueous extract; ESA = *S.aethiopicum*ethanolic extract; ARV = *R. vomitoria* aqueous extract; ERV = *R. vomitoria* ethanolic extract; AC = combined before extraction aqueous extract; EC = combined before extraction ethanolic extract.

DISCUSSION

Microscopy of the centrifuged urine samples showed presence of moderate to many pus cells (white blood cells) in less urine samples that had significant microbial growth on culturing; presence of crystals such as calcium oxalates, tyrosine, cystine and triple phosphate; epithelial cells and schistosoma eggs with

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numerous red blood cells. Presence of pus cells in this quantity called pyuria is an indication of microbial infections since pus cells are white blood cells that have succumbed in defense of the body against pathogens that invaded it. They are excreted few in normal urine singly but in urinary infections in clumps. Pyuria with significant microbial growth ($>10^5$ organisms/ml) in 16 urine samples is a good indication of urinary tract infection while significant microbial growth (bacteriuria) without pyuria in 54 urine samples may be an indication of diabetes, enteric fever, bacterial endocarditis or contaminants from the perineum.

Crystals are formed from chemicals in normal urine and are usually refractive in appearance. Type of crystal found indicates presence or absence of a disease. Calcium oxalate crystals are formed from oxalic acid abundant in diet rich in leafy vegetables or coffee and calcium excreted into urine due to chronic dehydration or hyperparathyroidism. The crystals usually form stones (calculi) in the kidneys, ureters or bladders. Thus presence of calcium oxalate crystals might be an indication of calculi in urinary tract, a predisposing factor to urinary tract infection. Tyrosine crystals presence might indicate a possible severe liver disease while cystine crystals are found in cystinuria, a rare congenital metabolic disorder. Presence of numerous epithelial cells may indicate inflammation of the urinary tract or vaginal contamination of the specimen; triple phosphate crystals in urine is not harmful but indicates the pH is highly alkaline while presence of schistosoma cells and numerous red blood cells indicates the infection, *schistosomiasis* (Cheesbroough, 2015; BMA, 2012).

The percentage yields of crude extract of the plant samples (aqueous and ethanolic) were generally low though yield of the plant combined before extraction (CBE) were higher than the single extracts. The percentage yield of the ethanolic extracts was higher than the aqueous extracts except that of *S.aethiopicum.* Both aqueous and ethanolic extractions of *S.aethiopicum* gave low yield signifying that the plant phytochemicals might not be very polar ones. Low yield of plant extracts has been reported by many researchers.

The low percentage yield is supported by the experiment of Ogbonnia *et al.* (2018), who reported that out of 400 g coarse powder of *Treculia Africans* stein bark soaked in 1600 ml of 70% ethanol for 72 h with constant stirring, the filtrate dried and evaporated yielded a constant dry weight extract of 22.24 g (5.56%). Hassan *et al.* (2005), on their own reported that 300 g of air-dried powdered leaves of *Asparagus aficants* exhaustively extracted with petroleum ether (60-80°C) with a soxhlet extractor for 24 h, yielded 4.55 g (1.52%) of extract after drying under reduced pressure. When pulverized dried stem leaf of *Croton zamhesicus* was defated, successively extracted with ethyl acetate and finally methanol using soxhlet extractor, 52.023 g (12.64%) extract was produced out of 400 g of the dry powder (Reuben *et al.*, 2018). However, in the experiment carried out by Okoli and Iregbu (2012), cold aqueous extract of *Synclisa scabrida* whole root gave a percentage yield of 11.27% while its ethanolic extract gave a yield of 4.53%, thus supporting the fact that yield of ethanolic extraction can be lower than that of the aqueous extraction as was exhibited by *S.aethiopicum.* The lower yield of aqueous extractions in our experiment could also be attributed to lesser time for extraction (24 h) allowed, unlike (that of the ethanolic extraction (48 h), in order to prevent hydrolysis and possible loss of activity of the extracts. Thus extract yield of any plant is a factor of the soluble chemicals present, solvent used and method of extraction.

Mean zone of inhibition diameter (MID) in millilitres was used to show antimicrobial activities of the plant extracts and was a factor of both percentage of each organism affected and diameter of zone of inhibition. The ethanolic extract of *S.aethiopicum* (fig. 3) had better activity on most of the isolates including *Klebsiella* species unlike its aqueous extract (fig. 2) and thus can be said to have a better broad spectrum activity, since both gram-negative and gram-positive organisms were affected (Matu and Van Staden, 2003). Ethanolic extract of a related species, *S .menognena* worked on by Adeshina *et al.* (2010), showed a better activity at a concentration of 20 mg/ml on *E .coli* and *S. aureus* with inhibition diameter of 23 mm and 20 mm respectively. Activity of aqueous and ethanolic extracts of *S.aethiopicum* leaves on *Candida* species

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including *C. albicans* as recorded by Ogunshe *et al* (2018), ranged from 15 mm to 25 mm in inhibition diameter (at 500 µl and 1000 µ l). This is similar to that observed in our research where activity on our strains ranged from 12 mm to 19 mm (at 250 µl and 500 µl). Experiments of Sonibare *et al.* (2011), supports the non- activity of the ethanolic extract of *S.aethiopicum* leaves on *P .aeruginosa.*

R.vomitoria aqueous and ethanolic extracts are also broad spectrum in action though both effects were comparable except on *Proteus* species where the aqueous extract had activity on 80% *Proteus* species as against the 20% of the ethanolic extract. Ethanolic extracts of other plants of the genera *Rauwalfia caffra* have been stated to have a broad spectrum action (Adeniyi *et al.*, 2010). In our work *Proteus* species, a gram negative organism was affected more by aqueous extract of *R.vomitoria* (8.0 mm) at 500 µg/ml of extract than the ethanolic extract (2.4mm) though the activity was not significantly different ($p \geq 0.05$) statistically. This is in line with activity of aqueous extract of *Helichrysum pedunculatum* leaves which was reported to have more activity on *Proteus vulgaris* ATCC6830 than its ethanolic extract (Aiyegoro *et al*., 2018). However, its activity on the organism is lower than the activity shown by *Hybanthus ennaespermus* Muell aqueous extract (at 300 µg/ml) which was 15.2mm though they tested one urine isolate of the organism.

Aqueous extract of plants combined together before extraction (CBE) in fig. 6 showed the activity of *S.aethiopicum* was synergistic to that of *Rauwolfla vomitoria* on *Escherichia coli* but antagonistic on other susceptible organisms. This was because CBE activity on *E. coli* was significantly higher than aqueous extract of *S.aethiopicum* or *R. vomitoria* alone. On the other hand, their activities were additive in the ethanolic extract of CBE on *E. coli* and antagonistic on the other organisms, since CBE activity was not significantly ($p \geq 0.05$) higher than any of the single extract. However, activities of both aqueous and ethanolic extracts were comparable. The result of CBE extracts on *E. coli* validates its use by our herbalist to cure urinary tract infection since the organism is the predominant cause of the infection.

Aqueous and ethanolic extracts of each plant combined in equal ratio designated combined after extraction (CAE) in figures 8 and 9 respectively were found to be antagonistic to each other on all the organisms, some incompletely and on some, completely in comparison to the single extracts. However, ethanolic extracts of (CAE) had better activity than the aqueous extracts. Antagonistic activity of different extracts in combination on *S. aureus* was shown in experiment of Eja *et al.* (2011), where activity of *Gongronema latifolia* aqueous extract (16 mm) was antagonistic to *Allium sativum* aqueous extract (18mm) when they were combined in the ratio 1:1 on the same organism (13mm).

Comparison of the aqueous and ethanolic extracts activity on *E.coli* showed CBE activity to be significantly higher than those of (CAE). CBE aqueous extract had little activity on *Proteus* species though no activity on *Klebsiella* species while (CAE) ethanolic extract showed little activity against *Klebsiella* species only. On the other hand, aqueous and ethanolic extracts of the CAE had greater mean zones of inhibition diameter on the gram- positive organisms than the CBE extracts, though not significantly ($p \geq 0.05$) different. Thus, the result of the effect of CBE on *E.coli* in comparison to CAE supports our herbalists' practice of cutting and mixing different plant samples together before extraction and administration. The better activity of CBE on *E.coli* in comparison to CAE might be as a result of the fact that possible inhibitors were prevented from isolation in the combination unlike in the single extracts combined together after extraction.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of each plant in tables 5 and 6 determined the concentration of the antimicrobial agent that would prevent growth, and kill the microorganism(s) respectively. Generally, the ethanolic extracts of *S.aethiopicum* and *R. vomitoria* had lower MICs on most of the organisms than the aqueous extracts especially on *E .coli*- the aetiologic agent of urinary tract infection (5.94 and 5.31 mg/ml respectively). The MBCs were 9.06 and 20.63 µg/ml, respectively. *Solanum aethiopicum* ethanolic extract is presumed to be bactericidal on *E.coli* since its MIC and MBC (5.94 and 9.06 respeclively) values are near, while *Rauwolfia vomitoria* ethanolic extract is bacteriostatic to it since its MIC (5.31) was more than three times the MBC (20.63) values. The MIC and

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MBC recorded for the extracts in this research work was in direct contrast to that recorded by Doughari and Obidah (2008), on *S.aureus* (SA 0.2 MBFTY) and *E .coli* (Ec 002 MBFTY) by ethanolic extract of *Leptadenia lancifolia* stem bark which were 0.08 and 0.06 mg/ml for MIC and 0.1 and 0.06 mg/ml for MBC, respectively. Thus the higher MICs and MBCs of our research plants showed that higher concentration of our plant extracts are needed to inhibit growth and kill the organisms, respectively. The MBC of the aqueous and ethanolic extracts of our plant samples against most of the isolates were generally high, with MBC against *Proteus* species by three of the extracts being above 50 mg/ml.

CONCLUSION

The findings of this study serve as the basis for making the following conclusions:

Urinary tract infection in the under studied places is found more prevalent in females than males. *E.coli* is still the most predominant isolate though incidences of other isolates are fast on the increase. Crude ethanolic extract of *S.aethiopicum* and *R.vomitoria* have broad – spectrum antimicrobial activity but ethanolic extract of *S.aethiopicum* is presumed bactericidal while that of *R.vomitoria* is bacteriostatic against *E. coli.*

RECOMMENDATION

We recommend that an *in vivio* aspect of this research should be done to ascertain the healing effect on human.

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