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Antimicrobial Potential of *Syzygium aromaticium* (CLOVE) Extracts on Multidrug-Resistant (MDR) Uropathogenic Bacteria Isolated from Clinical Specimens in Bauchi, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author AS collected and analyzed the clinical specimens. Author MYI designed the study, prepare and type the manuscript. Authors IT, SI, RDU and AHI performed the phytochemical analysis. Authors MRS, ZMK, HT and HSM scrutinized the manuscripts, organize the data and perform statistical analysis. Author EBA is the general overseer who supervised the entire work and led the research team. All authors read and approved the final manuscript.

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ABSTRACT

Background: Urinary Tract Infection (UTI) is a common pathogenic inflammatory, distressing, and occasionally life-threatening condition that affects people of all ages and gender, mostly propelled by the emergence of multidrug-resistant bacteria. Cloves are used as spices in food and flavouring agent in drinks it is also used traditionally as a treatment for urinary infections.
Aim: This study was carried out to evaluate the antimicrobial potentials of Clove extracts on multidrug-resistant (MDR) Uropathogenic bacteria.
Design: This is a Clinical and laboratory-based study of patient with cases of UTI
Place and Duration of study: This study was conducted in the Microbiology laboratory of Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH), Bauchi, Nigeria, from January to December, 2021.
Methodology: Two hundred and fourty five (245) clean catch midstream urine samples were collected from patients with suspected cases of urinary tract infection attending GOPD ATBU TH. Bacteria were isolated using standard techniques and antibiotic resistant pattern was tested by Kirby Bauer Disk Diffusion method. Bioactive components of clove was extracted using diethyl ether, ethanolic and water as solvents. Phytochemical analysis of the extracts was also carried out.
Results: Out of the samples analysed, 168 (68.6%) showed significant bacteriuria. UTI was more prevalent in women within the active age group 21-30. The isolates resistant to seven and above commonly used antibiotics are selected from each specie to test for efficacy. The extracts revealed the presence of alkaloids, glycosides, saponins, tannins, flavonoids, sterols and Triterpenes. *In vitro* antimicrobial activity of diethyl ether, ethanolic and aqueous extract of cloves at different concentration of: 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml
and 6.25mg/ml were tested against multidrug resistant isolates. *S. aureus* and *Klebsiella* spp are the most sensitive to all clove extracts while *E. coli* and *P. aeruginosa* are less sensitive. All Three extracts showed a broad spectrum of activities at higher concentrations (200mg/ml) while no or less activity at the lower concentration of the extracts. Diethyl ether extract exerts higher activity than ethanolic and aqueous extract as revealed by the mean diameter of zone of inhibitions, MIC and, MBC values. The MIC values of the extracts were lower than their MBC values suggesting that the extracts inhibited the growth of MDR isolates while being bactericidal at higher concentrations.
Conclusion: *Staphylococcus aureus* and *E. coli* are among the commonest uropathogens clinically encountered in this area and most of the species are resistant to commonly administered antibiotics. Clove extracts had great antimicrobial potential against these bacteria, therefore it can be used in the treatment of UTIs. However, it is necessary to determine its toxicity, pharmacokinetic properties and side effects.

Keywords: Cloves; Multidrug resistant bacteria; phytochemical analysis; Uropathogenic bacteria.

1. INTRODUCTION

"Urinary tract infection (UTI) is the most common and serious Ill-health condition both in the community and hospital settings worldwide" [1]. "It is the second most common infection after respiratory tract infection and an important cause of morbidity in the world affecting all age groups, and usually requiring prompt medical attention" [2]. "Urinary tract infection (UTI) is one of the most common bacterial infections encountered by clinicians in developing countries" [3]. "Uropathogens are group of organisms associated with urinary tract infection, they have specific virulence factors that facilitate their invasion of the urinary tract. Uropathogenic *Escherichia coli* (UPEC) is a causative agent in the vast majority of urinary tract infections (UTIs), including cystitis and pyelonephritis, and infectious complications" [4].

"The emergence of multidrug-resistant bacteria (MDR) has become a major cause of failure for the treatment of various infectious diseases. Inappropriate and irrational use of antibiotics provides favourable conditions for the selection and spread of antibiotic resistance" [5]. "With the recent threat of high multi-drug resistant bacteria, efforts have been intensified by researchers to search for possible alternatives" [6]. "There is a continuous need of producing new drugs as resistance has emerged towards all classes of antibiotics. Multiple drug-resistant strains are resistant to first line of treatment and also the more expensive second and third-line antibiotics" [5]. Thus, there had been a great deal of interest

in developing new antimicrobial substances from natural plants.

Medicinal plants and traditional preparation with antimicrobial activities have been used extensively in many countries [7]. These plants of medical importance have been proven to be very effective even where treatments with antibiotics failed [8]. "Herbal medicine has been widely used all over the world and formed an integral part of primary health care in many individuals" [9, 10]. According to the World Health Organization, 80% of the world's population still relies mainly on plant drugs, the WHO launched a policy on nations to promote and integrate traditional medicine into their national healthcare system [11]. "The use of medicinal plants and herbs for the treatment of pathogenic and non-pathogenic diseases is therefore encouraged. The biological inhibitors by different natural plants such as essential oils and plants extracts have been investigated widely" [12]. Antimicrobial compounds from plants belong to different major groups like Phenolics, quinones, flavonoids, tannins, terpenoids, essential oils and alkaloids. These compounds are known as secondary metabolites.

Clove, *Syzygium aromaticium*, is known as *Kanamfari* in Hausa [10]. It is a perennial tropical plant belonging to the family Myrtaceae, with a genus consisting of about 1200 – 1800 species. Cloves are dried unopened floral buds of an evergreen tree 10 to 12m in height [13]. "It is aromatic dried flower buds of a tree (*Eugenia caryophyllata*) a native to the Maluku islands in Indonesia and used as a spice in cuisines all over the world. It is used in Indian Ayurvedic medicine, Chinese medicine, and western herbalism and dentistry where the essential oil is used as an anodyne (painkiller) for dental emergencies. Cloves are used as a carminative, to increase hydrochloric acid in the stomach and to improve peristalsis" [14] "Cloves are available throughout the year due to different harvest seasons in different countries. It has been used in traditional medicine for the healing of various conditions. The major component of its essential oil, eugenol, has antibacterial, analgesic anaesthetic properties. Viral inhibition property of the extract has been established in hepatitis B, herpes simplex virus 1 and 2" [15].

"The antimicrobial activities of clove have been proved against several bacterial and fungal strains. Clove in particular has attracted attention due to the potent antioxidant and antimicrobial activities standing out among the other spices" [16]. *S. aromaticium* is used as spice in food and flavouring agent in drinks. It also has many therapeutic uses, such as controlling nausea, vomiting, diarrhoea, cough, flatulence, gastrointestinal spasm, analgesic (relieve pain), stimulating nerves and kidney reinforcement [17,18]. In addition, they are used as anti-inflammatory, anti-mutagenic, antioxidant, anti-ulcerogenic, antithrombotic and anti-parasitic [19, 20, 21, 22]. "Traditionally, cloves have been used for the preservation of food products as it has been reported to contain antiseptic and disinfectant properties" [23]. "Clove extract eases nausea or quiets a digestive system in need of calming like with indigestion, hiccups, vomiting, flatulence, diarrhoea and for those who suffer anorexia. It can also reduce the pain that is associated with some of these issues. The extracts of cloves were potent enough to kill microbial pathogens and are also effective against the specific bacteria that causes cholera" [24].

"Bacteria have developed resistance against many antibiotics due to the indiscriminate use of antimicrobial drugs" [25]. "Evolution of highly resistant bacteria strain has compromised the use of new generations of antibiotics, which are sometimes associated with side effects" [26]. "It is known that more than 400, 000 species of tropical flowering plants have medicinal properties and this has made traditional medicine cheaper than modern medicine" [27]. "About 150 million people are diagnosed with UTI each year worldwide, which incurred expenses of billions of dollars on the global economy" [28]. "The extensive uses of antimicrobial agents in the treatment of UTI have invariably resulted in the development of antibiotic resistance, which has become a major problem worldwide" [29].

"Emergence of antimicrobial resistance in the management of UTI is a serious public health issue, particularly in the developing world where apart from high level of poverty, ignorance and poor hygienic practices, there is also a high prevalence of fake and spurious drugs of questionable quality in circulation" [30]. "Detection of UTI causing pathogens and resistance of these pathogens to commonly prescribed antibiotics in clinical setups is essential and helpful in improving the efficacy of empirical treatment [31] constituents from medicinal plants serve as lead compounds in antimicrobial discovery" [32,33]. Hence the needs for plants like clove to be investigated to better understand their pharmacological

properties and efficacy against Uropathogens. The aim of this study is therefore to evaluate the antimicrobial effects of clove extracts on some multidrug resistant uropathogenic bacteria isolated from clinical specimens.

2. MATERIALS AND METHODS

2.1 Area of the Study

The study was carried out in Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH), which serves as a referral centre for general hospitals in the state. It is located in Bauchi local government area of Bauchi state. Geographically, the state occupies about 5.3% of Nigeria's total land mass and located between latitude 10.63 North and longitude 10.08 East.

2.2 Sample Size Determination

A total of 245 samples was collected as determined using the formula recommended by Thrusfield (2007), with 95% confidence interval, at 5% desired absolute precision and a previous prevalence of 20% from a study by [34] was used.

The formula used: $n = z^2p\ (1\text{-}p)/d^2$

Where: n = Sample size

z = Statistic corresponding to the level of confidence
p = Previous prevalence obtained from a similar study by [34].
d = Precision, corresponding to effect size.
$n = (1.96)^2 \times 0.2 \times (1\text{-}0.2)/0.05^2$
$n = 3.8416 \times 0.16/0.0025$
$n = 0.614656/0.0025$
n=245 samples

2.3 Sample Collection and Processing

Urine specimens was collected and processed according to standard microbiological laboratory techniques as described by Cheesbrough [35]. A total of Two hundred and forty-five (245) specimens was collected from in and out-patients. Midstream urine was collected from each patient into a 20 mL calibrated sterile screw-capped universal container which was initially distributed to the patients. The specimen was appropriately labelled, transported to the laboratory, and analysed within two (2) hours after collection. Prior to the collection, all patients were well instructed on how to collect the urine specimen aseptically to avoid contamination.

2.4 Culture Methods

Each urine sample was aseptically inoculated (in triplicates) unto blood agar and Cysteine Lactose Electrolyte Deficient (CLED) and MacConkey agar plates (Oxoid, UK). The plates were incubated aerobically at 37°C for 18–24 hours.

2.5 Morphological Identification of Bacterial Isolates

The bacterial culture isolates were examined for typical colonial characteristics of UTI pathogens, then identified by Gram staining and microscopy.

2.6 Biochemical Identification of Isolates

The isolates were finally identified using the following biochemical tests: catalase, coagulase, Indole, oxidase, urease and citric acid utilization test.

2.7 Antibiotics Susceptibility Testing

Antibiotics susceptibility test was performed using McFarland standard inoculum as described by [35] The following standard discs (Oxoid, UK) was used: Amoxicillin (30µg), Ampiclox (30µg), Ciprofloxacin (10µg), Gentamycin (10µg), Erythromycin (5µg), Ofloxacin (10µg), Cefuroxime (30 µg), Pefloxacin (10µg), Streptomycin (300µg), Augmentin (10µg), Sparfloxacin (5µg), Cotrimoxazole (30µg), Rocephin (30µg) and Chloramphenicol (5µg). The diameter zone of inhibition was measured in millimetres and results were interpreted according to the recommendations of the National committee laboratory standards [36].

2.8 Collection and Identification of Plant Material

Syzygium aromaticium (clove) flower buds were purchased from the Muda Lawal market in the Bauchi metropolis and were identified by a Botanist in the Biological Sciences department Herbarium, ATBU Bauchi.

2.9 Extraction Procedures

The method of [37] and [38] were adopted. The *S. aromaticium* (clove) was washed to remove dust and sand particles. After drying the cloves, it was grounded into powder and extracted using water, ethanol, and Diethyl ether with cold maceration techniques. In order to obtain the

crude extract, traces of solvents were evaporated using a rotary evaporator.

2.10 Aqueous Extraction

Cold maceration method was used as described by Chinwe et al. [38]. After homogenization of the clove into a fine powder, it is weighed by using a sensitive balance. Twenty grams (20 g) of the clove powder was soaked into 100ml of distilled water at room temperature and it was shaken with a rotatory shaker. The distilled water was sufficient enough to cover all the surface of the clove powder in the flask. The flask containing the mixture was corked and left to stand for 24 hours at room temperature. The mixture was filtered with eight layered sterilized Muslin cloth and filter paper No. 1. The crude extract was obtained by evaporating the filtrate with a rotatory evaporator and later transferred to hot air oven set at 40°C for complete drying. After complete drying the extracts were left at room temperature by sealing with aluminium foil and refrigerated until when needed for further analysis.

2.11 Ethanol and Diethyl ether Extraction

Twenty grams (20g) of the crushed clove powder was weighed using a sensitive balance and soaked or macerated into 95% ethanol and 80% diethyl ether respectively. The ethanol and diethyl ether in a separate flask was sufficient enough to cover the clove powder. The flask containing each solvent was corked and left to stand for 24 hours at room temperature and it was shaken with a rotatory shaker. Each solvent was filtered through eight-layered sterile Muslin cloth and then through Whatman No. 1 filter paper. The crude extract was obtained by evaporating the filtrate with a rotatory evaporator and later transferred to hot air oven set at 40°C for complete drying, the extracts were left at room temperature by sealing with aluminium foil and refrigerated until when needed for further analysis.

2.12 Phytochemical Screening

The plant extracts were evaluated for the presence of various phytochemicals using simple qualitative methods described by Trease and Evans [39], and Sofowora [40] in order to identify the phytoconstituents such as: Tannins, Alkaloids, Flavonoids, Saponins, Glycosides, Steroids and Triterpenes.

2.13 Reconstitution of the Clove Extracts

The dried extract was reconstituted by dissolving 0.2g of extract in 1ml of water in order to obtain 200mg/ml of the aqueous extract. For diethyl ether and ethanol extract, a stock concentration of 200mg/ml of each extract were prepared by dissolving 0.4g of the extract in 2ml of dimethyl sulfoxide (DMSO). Subsequently 100, 50, 25, 12.5, 6.25 mg/ml concentrations were prepared from the stock using a 2-fold serial dilution method.

2.14 Sterility Test of the Extracts

Each of the extract (aqueous, ethanolic and Diethyl ether) was tested for sterility after sterilization by inoculating 1ml of each extract on sterilized nutrient agar and incubated at 37°C for 24 hours. The plates were observed for growth. No growth after incubation indicated that the extracts were sterile [41].

2.15 Determination of Multiple Antibiotic Resistance Indices (MARI)

This was obtained by dividing the number of antibiotics to which the isolates were resistant by the total number of antibiotics to which the bacteria was tested. Those with higher multiple antibiotics resistance indices, greater than 0.7 were selected for the antimicrobial efficacy test of the Clove extracts.

2.16 Standardization of the Bacterial Cell Suspension

Inoculums were prepared directly from the MAR isolates where 3-4mls of sterile physiological saline was poured into a test tube for which a loopful of the colonies was taken directly from the plate and emulsified. The suspension was adjusted to match 0.5 McFarland standard which has a similar appearance of an overnight broth culture by adding distilled water [42, 35].

2.17 Preparation of Clove Extracts Susceptibility Discs

Discs were punched using No.1 Whatman filter paper with a diameter of 5mm and were sterilized by dry heat at 121°C for 15 minutes. The disc was allowed to cool, using screw capped bottle, and different concentrations of the clove extract were prepared using Dimethyl sulphur oxide (DMSO) to each of the different weights of the

extract which arrived at the concentration of 6.25,12.5, 25, 50, 100, 200mg/ml. 200 pieces of the paper disc were introduced into 0.5ml of the different concentrations of the extract and allowed to stand until the whole concentration was completely absorbed by the discs, where each disc is capable of absorbing 0.01ml [35].

2.18 Screening for *In vitro* Bacteriocidal activlty of Clove Extracts

The antibacterial activity of aqueous, ethanolic and Diethyl ether extracts of clove were detected using disc diffusion method as described by Mukhtar and Tukur [43]. The growth media were dried in a drier for about 10 minutes to remove excess surface moisture. The plates were aseptically inoculated with the multi-drug resistant test bacteria by streaking method unto the surface of Mueller-Hinton agar. A pair of forceps was used to gently placed the paper disc containing the extracts of *Syzygium aromaticium* (clove) at different concentration, arranged radially and pressed firmly to the inoculated agar surface to ensure even contact. Each disc was sufficiently spaced-out and kept at least 15mm from the edge of the plate to prevent overlapping of zones. This process was repeated for the replicate plates, and the plates were allowed for pre-diffusion time of 15 minutes. Control test were done on the same plate by placing a standard antibiotic disc of Ciprofloxacin (30ug) to serve as positive control. Discs impregnated with DMSO only were placed at the centre of some plates to serve as negative control. The plates were inverted and incubated at 37°C for 24 hours. The degree of sensitivity of the organisms to the extracts was determined by measuring diameter of visible zones of inhibition to the nearest millimetre with respect to each isolate and extracts concentrations [44].

2.19 Determination of Minimum Inhibitory Concentration (MIC)

The MIC is the lowest concentration of the extracts that inhibited or prevented the growth of test microorganisms. Minimum Inhibitory Concentration (MIC) was determined using 2-fold test tube dilution method of [45, 9]. Dilution of the clove extracts was incorporated in nutrient broth in 1:1 ratio initial rough estimates of the MIC values of the clove extracts against the MAR isolates were estimated to determine the range of MIC values. Consequently, the following concentrations were prepared for each extract, using the dilution formula: 100, 50, 25, 12.5 and 6.25 mg/ml. In addition, 0.1ml of standard suspension of the test Organisms was added to each tube. The tubes were incubated at 37°C for 24 hours. A tube containing extract and growth medium without Inoculum was included to serve as the control. The presence of growth (turbid solution) or absence of growth (clear solution) at the end of the incubation period was recorded. The lowest concentration of the extracts showing no growth was regarded as the minimum inhibitory concentration (MIC).

2.20 Determination of Minimum Bacteriocidal Concentration (MBC)

The minimum Bacteriocidal concentration (MBC) of clove extract against the microbes was determined using the method of Baker and Silverton [39], Garba et al. [40]. The tubes of the MIC that showed no growth of the microbes were subcultured by streaking using a sterile wire loop on Nutrient and MacConkey agar media. The plates were incubated at 37°C for 24 hours. The MBC was taken as the lowest concentration of the extract that showed no any colony growth on the agar plates.

2.21 Inclusion and Exclusion Criteria

Only patients that presented and were clinically diagnosed with symptoms of urinary tract infection (UTI), and not on antibiotics treatment for the past 48 hours were included in this study, others were excluded.

2.22 Statistical Analysis

Two-way analysis of variance (ANOVA) randomized complete block design was used to compare the mean differences. SPSS version 23 was used for the analysis. Statistical level ($P<0.05$) was considered significant.

3. RESULTS AND DISCUSSION

In this study (table 1), a total of 168 (68.6%) bacterial isolates, comprising of ten species were recovered from 245 clinical urine specimens, out of which 71(42.3%) were *Staphylococcus aureus*, followed by *E. coli* (21.4%), with *Proteus vulgaris* as the least occurring species.

Infectious diseases are becoming the main cause of mortality and morbidity worldwide. Multidrug-resistant bacteria are the major contributing factors to hugely raised bars of UTI.

Such increase has been attributed to the empirical antibiotic use, undermining the usage of culture and antimicrobial susceptibility profile for patients with UTI. Nowadays, the use of herbal medicines to control infectious diseases has been a source of hope for public health [46].

In the present study, the high-frequency *S. aureus* from cases of UTI can be as a result of sample contamination in the patient hands/skin during collection, since the bacteria is a normal flora of human body. *Staphylococcus saprophyticus*, a Coagulase Negative Staphylococci (CoNS) is commonly encountered in the urine samples of adult women of child-bearing age and has been mistakenly isolated as *S. aureus* [47, 35]. Uncomplicated urinary tract infections (UTI) are caused predominantly by *E. coli,* then by *S. saprophyticus*, *Proteus mirabilis*, and *Klebsiella* species [48]. Perpetual et al. [49], Oladeinde et al. [50] previously reported that *S.aureus* was the predominant isolated uropathogen from patients with signs and symptoms of UTI. This finding agrees with the results of [51, 52] and [53]. Occurrence of Gram positive bacteria, particularly *S.aureus* as the most commonly implicated pathogen in patients with UTI was also reported by Akortha and Ibadin [54].

The high incidence of *S. aureus* could be as a result of its minimal growth requirements, ability to survive long in most unfavourable environment and to find a susceptible host [52]. It could be due to the virulent nature of the organism that gives it the ability to overcome body defence mechanism and resistance to antibiotics [55]. The incidence of *S.aureus* in women could be due to the proximity between the genital tracts and the urethra/anus, which perhaps facilitate auto transmission. However, the relationship between men and women usually favours the transfer of this organism leading to the increasing level of prevalence of UTI among the men [56].

Escherichia coli is the most predominant pathogen causing 80-90% of community-acquired UTIs and more than 30% of Nosocomially acquired UTIs [57, 58]. This bacterium was significantly isolated (21.4%) as the second prevalent isolate in this study, but fails to agree with previous report on uropathogens showing *E. coli* as the most frequently isolated bacteria in patients with UTI [59,1,60,61,62]. Gram negative bacilli (Enterobacteriaceae) were responsible for majority of urinary tract infections and most of the strains were multidrug resistant, while the most common isolated bacteria from UTI was *E. coli* [63].The high incidence of *E.coli* is attributed to the fact that it is a commensal of the bowel and infection is mostly through faecal contamination occasioned by poor hygiene. Improper wiping after urination or defaecation can result in transfer of bacteria from anus to the distal urethra [53].

The prevalence of UTI in this study (68.6%) confirmed the observation that this is one of the common diseases in tropical African countries, as agreed with (67%) found by [30] in a study conducted in Yola, Nigeria. However, some previous studies by [34, 64, 65], reported 20%, 13% and 9.5% respectively. The variation in rates may be partly explained by the differences in study populations and in the criteria used by the centres in selecting urine samples for culture. Some centres exclude samples from patients clinically diagnosed with UTI or previous antibiotic use [66]. The taking of antibiotics prior to presentation at the hospital may be a key factor in bacterial yield [67]. These factors were not considered in this study.

Table 1. Distribution of Uropathogenic bacteria isolated from the study population

Uropathogenic Bacteria	Number of Bacterial isolates (n =168)	Percentage (%) Frequency
Staphylococcus aureus	71	42.3
Escherichia coli	41	24.4
Klebsiella spp	36	21.4
Citrobacter spp	07	4.2
Enterobacter spp	05	2.9
Morganella morganii	03	1.8
Proteus spp	03	1.8
Pseudomonas aeruginosa	02	1.2

3.1 Demographic Characteristics of the Patients in the Study Population

In this study (table 2), a total of 168 bacterial isolates were recovered from 245 clinical urine specimens from various groups of male and female patients aged 0 to above 70 years old. Most of the patients were females (60.1%) and highest frequency (37.5%) of isolates was found in 21-30 years age group. This is followed by 23.8% (31-40 years), with 1.2% as the least in the younger age group (10 years and below).

In this study urinary tract infection span through the active age groups. The age group 21-30 years was the most significant for UTI with females 63 (37.5%) being greatly higher than males (10.1%). The next age group 31-40 was ranked the second (27.4%). The high cases of UTI within these age groups could be attributed to the fact that most of the patients are sexually active adults. Some engage in sexual intercourse more than necessary; engagement of multiple sexual partners and case of female use family planning creams (spermicides) [68]. It has also been documented that in young sexually active females, sexual activity is the cause of 75-90% of bladder infections [69]. Bladder infection (Cystitis) recurs in 25% of healthy women within six months of the first infection, and in 20% of women within 1 year [70].

This study found that females had a high incidence rate (60.1%) of UTI. This might be attributed to anatomical and physiological factors such as the shortness of the urethra and its proximity to the anus [71]. A retrospective population-based study to determine the incidence of first-time symptomatic UTI in children less than 6 years, found that cumulatively, incidence rate was three times greater in girls (6.6%) than boys (1.8%) [72].

3.2 Antimicrobial Resistant Characteristics of the Bacteria Isolates

Antimicrobial resistant pattern of the uropathogenic bacteria isolates against the conventional antibiotics (table 3), revealed high resistance of *S.aureus* to Ampiclox (68%) and Amoxycillin (54%), then *E. coli* (64%) and (44%) respectively. In addition, 55% of *K. oxytoca* and *Citrobacter spp* are resistant to Augmentin, with *E. coli* (53%) and *K. oxytoca* (55%) also resistant to Cotrimoxazole. However, all the *Proteus* (3) and *Pseudomonas* (2) isolates are resistant to 2 to 5 of the antibiotics tested (100%).

The UTI isolates in this study were multidrug-resistant to the most commonly used antibiotics such as Ampiclox, Amoxycillin, Cotrimoxazole, and Augmentin. This was consistent with the findings of [73] where most of the urinary tract bacteria are resistant to aminoglycosides and β-lactams including cephalosporin and fluoroquinolones. Another study in India by Anil et al*.* [74], reported that there is much increase in resistance to antibiotics by urinary bacteria. Alo et al*.* [75] also reported from Sokoto a high prevalence of multidrug resistance to Uropathogenic bacteria to almost all conventional antibiotics. According to Ezeadila et al*.* [67], the resistance exhibited by the isolates against some of the conventional antibiotics could be attributed to the ability of these organisms to acquire some resistance mechanisms which might be genetic or acquired, that make the organisms resist the effects of antibiotics. This phenomenon may be due to genetic changes since antimicrobial resistance occurs naturally over time. It has been shown that highly prescribing habits of physicians are the driving factor for antibiotic resistance to these commonly used antibiotics [76].

However, the irrational use of antibiotics is also playing its role in accelerating antimicrobial resistance. A recent finding which strongly supports the idea is that 80% - 90% of antibiotic prescriptions were found to be made by general practitioners, of that 30% are considered to be completely unnecessary [77] Inappropriate use of antibiotics, such as taking them for viral conditions like flu, or for mild infections that may clear up without treatment is known to fuel resistance. A study in England reported that one in three (34%) of the isolates analyzed were found to be resistant to antibiotic trimethoprim which was once the first choice treatment for UTI [78]. In this study almost half the isolates were found to be multidrug resistant with the majority of them having Multiple Antibiotic Resistant (MAR) index greater than 0.3. Any MAR index greater than 0.2 implies that the strains of such bacteria originate from an environment where several antibiotics are abused or misused [79, 80, 81].This implies that a very large proportion of the bacterial isolates have been exposed to several antibiotics and thus have developed resistance [79, 82].

Table 2. Distribution of Uropathogenic Bacterial isolates according to patients' demographic characteristics

Patients characteristics	No. of Samples collected (n=245)	Number of Uropathogenic Bacterial isolates (n=168)	Percentage (%) Frequency
Age (years)			
0 – 10	07	02	1.2
11 – 20	45	28	16.7
21 – 30	91	63	37.5
31 – 40	43	40	23.8
41 – 50	20	15	8.9
51 – 60	14	09	5.4
61 – 70	05	04	2.4
Above 70	10	07	4.2
Gender			
Male	119	67	39.9
Female	126	101	60.1

Table 3. Distribution of Uropathogenic Bacterial isolates according to Multidrug Resistant pattern

Antibiotics (µg)	Number (%) of Uropathogenic Bacterial isolates (n= 168) and Multidrug Resistant pattern							
	***Staph. aureus* (n=71)**	***E. coli* (n=41)**	***Klebsiella species* (n=36)**	***Citrob. Species* (n=7)**	***Enterob. species* (n=5)**	***Morganella morganii* (n=3)**	***Pseud. aeruginosa* (n=2)**	***Proteus species* (n=3)**
Amoxycillin (30)	38(54)	12(59)	16(44)	01(14)	01(20)	01(34)	01(50)	03(100)
Ampiclox (30)	48(68)	00(0.0)	00(0.0)	00(0.0)	00(0.0)	00(0.0)	00(0.0)	00(0.0)
Augmentin (30)	00(0.0)	15(74)	23(64)	04(55)	02(40)	01(34)	02(100)	02(66)
Ceftriaxone (30)	19(27)	00(0.0)	00(0.0)	00(0.0)	00(0.0)	00(0.0)	00(0.0)	00(0.0)
Cefuroxime (30)	19(27)	00(0.0)	00(0.0)	00(0.0)	00(0.0)	00(0.0)	00(0.0)	00(0.0)
Chloramphenicol (30)	00(0.0)	07(34)	07(19)	01(14)	02(40)	01(34)	02(100)	03(100)
Ciprofloxacin (10)	05(7.0)	06(25)	04(11)	01(14)	00(0.0)	00(0.0)	01(50)	01(34)
Cotrimoxazole (30)	27(38)	16(79)	19(53)	01(14)	01(20)	01(34)	02(100)	02(66)
Erythromycin (10)	20(28)	00(0.0)	00(0.0)	00(0.0)	00(0.0)	00(0.0)	00(0.0)	00(0.0)
Gentamycin (10)	18(25)	03(15)	05(14)	01(14)	01(20)	01(34)	00(0.0)	01(34)
Ofloxacin (10)	00(0.0)	04(20)	06(17)	01(14)	01(20)	01(34)	01(50)	02(66)
Pefloxacin (10)	18(25)	05(25)	11(31)	01(14)	01(20)	01(34)	01(50)	02(66)
Sparfloxacin (10)	00(0.0)	07(34)	10(28)	00(0.0)	01(20)	01(34)	01(50)	03(100)
Streptomycin (30)	05(7.0)	02(10)	02(6.0)	00(0.0)	00(0.0)	00(0.0)	00(0.0)	00(0.0)

Table 4. Phytochemical constituents of Clove extracts

Phytochemicals	Clove extracts and Test inference		
	Diethyl ether	Ethanol	Aqueous
Alkaloids	+	+	+
Glycosides	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Sterols	+	+	+
Triterpenes	+	+	+

Key: + = present & - = absent

3.3 Phytochemical Characteristics of the Clove Extracts

Screening for the phytochemical composition of clove extracts indicated that (table 4) Alkaloids, Glycosides, Saponins, Tannins, Flavonoids, Sterols and Triterpenes are present in the Aqueous, ethanolic and diethyl ether extracts from this study.

Qualitative phytochemical screening of clove (*S. aromaticium)* extracts in this study revealed the presence of the following bioactive constituents; Alkaloids, Flavonoids, Glycosides, Saponins, Sterols, Tannins, and Triterpenes. These bioactive constituents play a significant role in the *in vitro* antibacterial activity of clove extracts. As these phytoconstituents have been reported to contain antibacterial properties [83]. Tannin has been reported to interfere with bacterial cell protein synthesis and is important in the treatment of ulcerated or inflamed tissues and also in the treatment of intestinal disorders [83]. Tannins' antimicrobial activities could be by metal deposition/complexation, hydrogen bonding or specific interactions with viral proteins such as enzymes in microbial cells [84]. Research has shown that polyphenols contribute to the prevention of cardiovascular diseases, cancers, and osteoporosis [85].

Alkaloids are significant phytochemicals accommodating the world's most effective chemical compounds with great biological importance for the protection and survival of plants because they ensure their survival against microorganisms (antibacterial and antifungal) activities [86]. Alkaloids are known to have a powerful effect on animal physiology and play some metabolic roles and control development in living systems [86]. It is also employed in high blood pressure as it dilates the blood-vessels [87]. Alkaloids have many pharmacological activities including antihypertensive effects (indole alkaloids), antiarrhythmic effects (quinidine, spare in), antimalarial activity (quinine), and anticancer actions (dimeric indole, vincristine and vinblastine). Some alkaloids have stimulant properties as caffeine, nicotine and morphine are used as analgesics, and quinine as and antimalarial drug [85].

3.4 Antibacterial Activity of Diethyl Ether Clove Extracts on MDR Isolates

The results of *in vitro* antibacterial activity of Diethyl ether clove extract on multidrug-resistant (MDR) isolates, Table 5 shows that this extract was the most potent compared to ethanolic and aqueous extract. The widest inhibition diameter was observed at 200mg/ml concentration, while 6.25mg/ml has less or no activity. The highest activity was recorded on *S*. *aureus* (19±0.8mm) and *K*. *pneumoniae* (19±0.4mm), while *E. coli* (12±0.8mm) and *P. aeruginosa* (11±0.2) has the least.

3.5 Antibacterial Activity of Ethanolic Extract on the MDR Isolates

The *in vitro* antibacterial activity of Ethanolic extract on the isolates (table 6) showed less activity than diethyl ether extract, but greater than aqueous extract. The highest zone of inhibition was also observed on *S*. *aureus and K. pneumoniae* with 18±0.8mm and 18±0.5mm respectively at 200mg/ml. But at 6.25 to 12.5mg/ml of the ethanol extract concentration, no activity was recorded on *E. coli*, *P*. *aeruginosa*, *K*. *oxytoca* and *Enterobacter spp*.

Table 5. *In vitro* antibacterial activity of diethyl ether Clove extract on Multidrug Resistant (MDR) Uropathogenic isolates

MDR Isolates	Control Ciprofloxacin (10µg)	Concentration (mg/ml) and Zone of Inhibition (mm)					
		200	100	50	25	12.5	6.25
S. aureus	20±0.3	19±0.8	16±0.2	14±0.5	12±0.8	10±0.7	6.0±0.8
E.coli	8.0±0.2	12±0.3	10±0.1	8.0±0.2	5.0±0.3	0.0±0.0	0.0±0.0
Kleb.spp	20±0.6	19±0.4	15±0.3	13±0.2	11±0.6	9.0±0.2	5.0±0.4
Citrob. Spp	20±0.5	17±0.6	16±0.4	14±0.6	12±0.8	10±0.6	6.0±0.2
Enterob. spp	16±0.2	16±0.2	14±0.2	12±0.5	10±0.3	7.0±0.5	0.0±0.0
M. morganii	17±0.8	15±0.4	12±0.8	10±0.8	9.0±0.6	0.0±0.0	0.0±0.0
Proteus spp	15±0.4	14±0.8	12±0.7	10±0.5	7.0±0.3	5.0±0.2	0.0±0.0
P.aeruginosa	8.0±0.1	11±0.2	9.0±0.2	7.0±0.3	5.0±0.4	0.0±0.0	0.0±0.0

Each value is a mean of standard error of three replicate

Table 6. *In vitro* antibacterial activity of ethanolic Clove extract on Multidrug Resistant (MDR) Uropathogenic isolates

MDR Isolates	Control Ciprofloxacin (10µg)	Concentration (mg/ml) and Zone of Inhibition (mm)					
		200	100	50	25	12.5	6.25
S. aureus	20±0.4	18±0.8	16±0.5	12±0.7	8.0±0.9	6.0±0.4	0.0±0.0
E. coli	8.0±0.2	12±0.4	10±0.6	8.0±0.3	5.0±0.7	0.0±0.0	0.0±0.0
Kleb.spp	20±0.3	18±0.5	17±0.2	15±0.5	12±0.5	7.0±0.5	0.0±0.0
Citrob. Spp	20±0.8	17±0.7	15±0.5	12±0.7	9.0±0.4	7.0±0.2	0.0±0.0
Enterob. spp	16±0.6	15±0.2	9.0±0.7	7.0±0.4	5.0±0.6	0.0±0.0	0.0±0.0
M. morganii	17±0.7	15±0.3	10±0.4	8.0±0.6	6.0±0.4	4.0±0.2	0.0±0.0
Proteus spp	15±0.4	12±0.4	10±0.3	8.0±0.7	6.0±0.2	4.0±0.3	0.0±0.0
P.aeruginosa	8.0±0.4	12±0.2	10±0.4	9.0±0.3	7.0±0.5	0.0±0.0	0.0±0.0

Each value is a mean of standard error of three replicate

Table 7. *In vitro* antibacterial activity of aqueous Clove extract on Multidrug Resistant (MDR) Uropathogenic isolates

MDR Isolates	Control Ciprofloxacin (10µg)	Concentration (mg/ml) and Zone of Inhibition (mm)					
		200	100	50	25	12.5	6.25
S. aureus	20±0.4	18±0.9	16±0.5	12±0.8	8.0±0.4	5.0±0.3	0.0±0.0
E. coli	8.0±0.6	9.0±0.2	8.0±0.4	7.0±0.6	5.0±0.3	0.0±0.0	0.0±0.0
Kleb.spp	20±0.4	14±0.9	12±0.8	10±0.4	8.0±0.2	6.0±0.5	0.0±0.0
Citrob. Spp	20±0.8	13±0.3	11±0.2	9.0±0.6	7.0±0.3	0.0±0.0	0.0±0.0
Entrob. Spp	16±0.2	12±0.4	10±0.9	8.0±0.5	6.0±0.7	0.0±0.0	0.0±0.0
M.morganii	17±0.3	13±0.7	10±0.3	8.0±0.3	6.0±0.5	0.0±0.0	0.0±0.0
Proteus spp	15±0.5	10±0.5	9.0±0.7	8.0±0.9	6.0±0.6	0.0±0.0	0.0±0.0
P.aeruginosa	8.0±0.3	9.0±0.5	8.0±0.8	7.0±0.2	6.0±0.2	0.0±0.0	0.0±0.0

Each value is a mean of standard error of three replicate

3.6 Antibacterial Activity of Aqueous Extract on MDR Isolates

The result of *in vitro* antibacterial activity of aqueous extract (table 7), revealed that highest activity was observed at 200mg/ml to all the MDR isolates, followed by 100mg/ml, 50mg/ml and 25mg/ml. No zone of inhibition was observed at 12.5mg/ml and 6.25mg/ml of the clove aqueous extract.

The results antibacterial activity of clove extracts against the ten isolates resistant to seven (7) and above commonly used antibiotics indicated that clove extracts exhibit strong antibacterial activities at different concentrations. This is in conformity with the report of Ifeanyichukwu et al. [60], that the clove possesses antimicrobial activities against uropathogens. Diethyl ether clove extract has broad spectrum activity against the tested MDR isolates was found to be

significantly effective ($p<0.05$) at various treatment.

The mean values revealed that *S. aureus* yield highest zone of inhibition at all the concentration. Aqueous extract servers as a good antibacterial agent it has broad spectrum activity but have less activity compared to diethyl ether extract and ethanolic extract. This implies that the antibacterial activities of all the clove extracts are concentration-dependent. The observations can be justified in terms of the polarity of the compounds being extracted by each solvent. Prakash et al. [61] and Schroeter et al. [84] confirmed that plants differ significantly in their activities against tested microorganisms using different solvents. Yang et al. [22] demonstrated that ethanolic extracts were found to be more effective than aqueous extracts which support the current results.

Garba et al. [9] evaluated seven (7) organic solvents on clove and found that the most effective solvent was diethyl ether. In most cases the organic extracts showed the same or greater activity than the aqueous extracts this may be due to the fact that the most of the bioactive compounds from plant origin are generally soluble in polar solvents. Igbinosa et al. [83] stated that aqueous extracts of plants generally exhibited little or no antimicrobial activities. Wankhede et al. [13] and Sulaiman et al. [14] confirmed that water is not an appropriate solvent to be used for extraction the antibacterial compounds from medicinal plants compared to other solvents.

Clove extracts are reported to demonstrate noticeably high free radical scavenging and peroxide inhibition activity indicating its reducing character, which may somewhat explain the inhibition of bacterial growth. Park et al. [21] studied the antibacterial activity of clove essential oil against five strains of pathogenic bacteria, they found that it could be used as additive constituent in the food and/or pharmaceutical industries field. Mishra et al. [73] explained that the plant substances can affect microbial cells by different antimicrobial mechanisms, including attacking the phospholipid bilayer of cell membrane, disturbing enzyme systems or compromising the genetic material of bacteria. Duane et al. [71] confirmed that increased liberation of intracellular nucleotides and proteinaceous materials from the bacterial cells in the presence of ethanol clove extract containing polyphenols indicates that the most important mode of action is membrane damage, which leads to cell death. They demonstrated that the destructive effect of clove extract on the cell membrane integrity was illustrated as increase in absorbance at 260 and 280nm after incubation of the cells with the extract. An observation from this study was that gram positive bacterial isolates were more susceptible to the clove extracts than Gram-negative isolates. This was in accordance with the previous findings by Ngwa et al. [34] that the susceptibility of Gram-positive bacteria and Gram-negative bacteria depends on their structural composition.

The Gram-positive, particularly *S. aureus* contains only 2% lipid. So the lipid content of the membrane will have an effect on the permeability of bioactive substances in the cloves [88]. Hence this phenomenon may favour the destruction of the cell wall and genetic material of the gram-positive that is the *S. aureus* than that of the gram-negative in particular *E. coli*. A study conducted by Zakaria et al. (2006) reported that the extract was more effective against Gram-positive than Gram-negative bacteria. This may be due to the presence of lipopolysaccharides (LPS) in gram-negative bacteria.

4. CONCLUSION

The result obtained from this study revealed that UTI was more prevalent in women within the active age group 21-30. *Staphylococcus aureus* and *E. coli* are among the commonest uropathogens clinically encountered in this area and most of the species are resistant to commonly administered antibiotics. The clove extracts contain natural bioactive components which can be used in the formulation of drugs against multidrug-resistant bacteria.

CONSENT AND ETHICAL APPROVAL

Ethical approval was obtained from the ATBU teaching hospital research and ethics committee, with written informed consent also sought from all patients, prior to specimen and data collection.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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