Comparative Study between Conventional Antibiotics and Medicinal Plant on Urinary Tract Organisms

Osuntokun Oludaretemitope, Olufakuyeoluwatosin.

Department of Microbiology, Faculty of Science, AdekunleAjasin University, AkungbaAkoko, P.M.B 001, Ondo State, Nigeria

Abstract— *This study reports the bacteriological aetiology of Urinary Tract Infections (UTIs), the effect of antibiotics, antimicrobial potency of Alstoniaboonei leaf extract and also compare the activity of antibiotics and the leaves extract of Alstoniaboonei on the isolated organisms among pregnant women attending the Antenatal clinics at the Federal Medical Centre Owo, Ondo State, Nigeria for a period of two weeks. A total of 50 mid-stream urine samples were collected from pregnant women between the ages of 15-40years. The urine samples from patients were cultured on MacConkey agar. The isolates were identified based on colonial morphology and biochemical tests using API 20 NE kits. This results showed that 22 urine samples had significant bacterial growths while 28 had no significant growth. The highest isolate was Escherichia coli (50.0%). This was followed by Klebsiellapneumoniae (22.7%), Pseudomonas aeruginosa(9.1%), Enterobacteraerogens (9.1%), Citrobacterfreundii (4.5%) and Proteus mirabilis (4.5%). The most effective antibiotics in this study were Gentamycin, Ofloxacin, Nitrofuratoin and Ciprofloxacin whileCefixime, Ceftazidime, Cefuroxime, Erytromycin, Cloxacillin, Augmentin and Ceftriaxone were resistant. The leaves extract of Alstoniaboonei produced the highest zone of inhibition at (100%) concentration and the zone of inhibition were 24.0mm, 20.0mm, 18.0mm, 16.0mm, 14.5mm, and 12.0mm respectively for Escherichia coli, Proteus mirabilis, Klebsiellapneumoniae, Pseudomonas aeruginosa, Enterobacter spp. andCitrobacter spp. to antibiotics used. The high effect of Alstoniaboonei leave reported in this study should be of great concern, and good personal hygiene should be practiced to avoid Urinary tract infections.The purpose of this research work is to isolate and identify Gram negative organisms found in the Urinary Tract,determine the in-vitro antibiotics sensitivity patterns of the bacterial isolates,determine the in-vitroAlstoniaboonei leaves extract sensitivity patterns of the bacterial isolates organisms and to compare the activity of conventional antibiotics and Alstoniaboonei leaves extract of the bacterial isolates.*

Keyword -Alstoniaboonei,Urinary Tract Infections (UTIs), Conventional antibiotics.

I. INTRODUCTION

A urinary tract infection (UTI) is an infection that affects part of the urinary tract. When it affects the lower urinary tract which involve the bladder infection (cystitis), urethra infection (urethritis), prostate (prostatitis) and when it affects the upper urinary tract it is known as kidney infection (pyelonephritis). Most cystitis and pyelonephritis are caused by bacteria. The most common cause of infection is *Escherichia coli*, though other bacteria. The most common nonbacterial pathogens are fungi (usually *Candidal species*), and less common, Mycobacteria, Viruses, and Parasites. The predominant parasitic causes of urinary tract infections are filariasis, trichomoniasis, malaria, and schistosomiasis(Takhar*et al.*, 2011).

Uncomplicated urinary tract infection is usually considered to be cystitis or pyelonephritis that occurs in premenopausal adult women with no structural or functional abnormality of the urinary tract and who are not pregnant and have no significant comorbidity that could lead to more serious outcomes. Also, some experts consider urinary tract infections (to be uncomplicated even if they affect postmenopausal women or patients with well-controlled diabetes. In men, most urinary tract infections (occur in children or elderly patients, are due to anatomic abnormalities or instrumentation, and are considered complicated (Hooton *et al.*, 2013).

The rare urinary tract infections that occur in men aged 15 to 50 yrs are usually in men who have unprotected anal intercourse or in those who have an uncircumcised penis, and they are generally considered uncomplicated. Complicated urinary tract infections can involve either sex at any age. It is usually considered to be pyelonephritis or

cystitis that does not fulfill criteria to be considered uncomplicated. A urinary tract infections is considered complicated if the patient is a child, is pregnant, or has any of the following: A structural or functional urinary tract abnormality and obstruction of urine flow, a comorbidity that increases risk of acquiring infection or resistance to treatment, such as poorly controlled diabetes, chronic kidney disease, or immunocompromise and recent instrumentation or surgery of the urinary tract (Roberts*et al*., 2013). In complicated cases or if treatment fails, a urine culture may be useful. In uncomplicated cases, urinary tract infections are treated with a short course of antibiotics such as nitrofurantoin or trimethoprim/sulfamethoxazole.Resistance too many of the antibiotics used to treat this condition is increasing (Salvatore *et al*., 2011). In complicated cases, a longer course or intravenous antibiotics may be needed. If symptoms do not improved in two or three days, further diagnostic testing may be needed. It is serious health problem affecting millions of people each year and the leading cause of Gram-negative bacteriaemia. urinary tract infections are also the leading cause of morbidity and health care expenditures in person of all ages. In the united States, it is estimated from surveys of office practices, hospital-based clinics and emergency departments that these infection account for over eight million cases of urinary tract infection annually and more than 1 million hospitalizations, for an overall annual cost in excess of $1billion (Kunin, 1994). The pathogens producing urinary tract infection have been said to be mostly derived from the hospital (Ebie*et al.*, 2001).

Urinary tract infection has become the most common hospital- acquired infection, accouting for as many as 35% of nosocomial infections, and it is the second most common cause of bacteriamia in hospitalized patients (Weinstein *et al.*,2000). Urinary tract infection also accounts for a significant part of the work load in clinical microbiology laboratories and enteric bacteria (in particular, *Escherchia coli*) remain the most frequent cause of urinary tract infection, although the distribution of pathogens cause urinary tract infection is changing. Untreated Upper urinary tract infection in pregnancy carries well documented risks of morbidity, and rarely, mortality to the pregnancy women. Sexually active young women are disproportionally affected. An estimated 40% of women reports having had a urinary tract infection at some point in their lives (Kuin, 2001). Recently published studies have added to the body of knowledge concerning the pathogenesis, diagnosis and management of urinary tract infections.

Urinary tract infections typically occur when bacteria enter the urinary tract through the urethra and begin to multiply in the bladder. Usually, a urinary tract infection is caused by bacteria that can also live in the digestive tract, in the vagina, or around the urethra, which is at the entrance to the urinary tract.(Duerden*et al*., 1990;Ebie*et al.*, 2001).

Alstoniaboonei, a large evergreen tree is one of the widely used medicinal plants in Africa and beyond. The important plants of genus *Alstonia* includes *Alstoniascholaris*, *Alstoniaboonei*, *Alstoniacongensis* and *Alstoniamacrophylla*which have proved to be useful in various diseases (Opoku and Akoto, 2015). Almost all plant parts viz. leaves, stem bark; root and inflorescences have been used and are further under investigative study. It is not edible as food but possess roots, stems, barks, leaves fruits, seeds, flowers, and latex which are claimed to have medicinal properties in some cultures. *Alstoniaboonei*is a very large, deciduous, tropical forest tree belonging to the dogbane family *Apocynaceae.* It is native to tropical West Africa, with a range extending to Ethiopia and Tanzania. Its common name in the English timber trade is **cheese wood, pattern wood, or stool wood,** while its common name in the French timbe trade is 'emiem'. The wood is fine-grained, lending itself to detailed carving; the tree also finds many uses in folk medicine. Like many other members of the apocynaceae (a family rich in toxic and medicinal species), *A. boonei* contains alkaloids and yields latex (Fakae*et al*., 2000).

The leave of Alstonia tree is one of the effective analgesic herbs available in nature. All the parts of the plant are very useful but the leave cut from the matured tree is the part that is most commonly used for therapeutic purposes. The bark of the tree is highly effective when it is used in its fresh form; however, the dried one could equally be used. Therapeutically, the bark has been found to possess antirheumatic, anti-inflammatory, analgesic/pain-killing, antimalaria/antipyretic, antidiabetic (mild hypoglycaemic), antihelminthic, antimicrobial and antibiotic properties (Haidi and Bremner, 2001). A decoction could be sweetened with pure honey and be taken up to 4 times daily as an effective painkiller for the following conditions; Painful menstruation (dysmenorrhoea), when associated with uterine fibroid or ovarian cysts in women; lower abdominal and pelvic congestion associated with gynaecological problems such as pelvic inflammatory diseases; to relieve the painful urethritis common with gonococcus or other microbial infections in men(Fakae*et al*., 2000).

Alstonia decoction also exerts a mild antibacterial effect in this case, relieving the aches and pains associated with malaria fever. Alstonia is taken in the form of preparations that exhibits antipyrexia and anti-malaria effects, to combat rheumatic and arthritic pains. The decoction of Alstonia bark could be taken alone as an effective pain-killing agent (Fakae*et al*., 2000). It is also useful in expelling retained products of conception and afterbirth when given to women. Asthma can be treated with a drink prepared from parts of Tremaorientalis and decoction of the bark of *Alstoniaboonei*mixed with theroots and bark of cola and fruits ofXylopiaparviflorawithhard potash. The bark decoction of *Alstoniaboonei*isused with other preparations in the treatment of fracturesor dislocation, jaundice, and for inducing breast milk. Itslatex is taken as a purgative. The hardened latex is used forthe treatment of yaws (Odugbaemi*et al*., 2007).

Fig.1.1: Alstoniabooneileave with stems

II. MATERIALS AND METHODS

2.1 STUDY POPULATON AND DATA COLLECTION

Clinical samples were collected from fifty patients and samples were collected over a period of 2weeks from pregnant women attending Ante-natal clinic at Federal Medical Centre Owo, Ondo State. The inclusion criteria include pregnant women in the age range of 15 to 45 years, volunteering to participate. Excluded from this research are menstruating or non-pregnant women.

2.2 COLLECTION OF SAMPLES FROM PATIENTS

Each subject was required to produce about 5-10ml of early morning mid-stream urine catch into sterile universal bottle and placed into the transport medium (Amies) and transported to the laboratory (Olajubu 2015).

2.3 METHOD OF ISOLATION

Urine culture

With the aid of a sterile inoculating loop, a loopfulof each mid-stream urine provided by subject was streaked on MacConkey plate. The plates of media were then incubated aerobically at 37^{O}C overnight. After incubation, the cultural and morphological characteristics of distinct, well isolated colonies were studied. These include the shape, size, elevation, edge, surface and color. Stock cultures of pure isolates were labeled accordingly and stored (Prescott *et al.*, 2008). Colony counts of <10.000 cfu/mL were considered not indicative of bacteriuria; colony counts in the range of 10.000 – 100.000 cfu/mL were probably due to contamination; while colony counts of >100.000 cfu/mL definitely indicated bacteriuria (Thomson *et al.*, 2003).

2.4 ISOLATION AND IDENTIFICATION OF ORGANISMS

Well distinct colonies from the plates were isolated and streaked on fresh nutrient agar, and incubated at 37^{O}C for 24hours and stored in the refrigerator until required for further tests.

Preliminary characterization of bacterial isolates was based on colonial characteristics; further characterization was carried out with various biochemical tests using analytical profile index (API 20E) test kit (Tankeshwar*et al.*, 2015).

2.5 BIOCHEMICAL TESTS USING API 20NE KITS

The Analytical Profile Index-20NE test strip (from BioMerieux, Inc.) is series of couples containing various freeze dried reagents and color indicators designed for biochemical tests. This API is used to identify the enteric gram negative rods (although API makes a variety of other test strips for yeast, Staph, anaerobes, etc.) 20 separate test compartments are on the strip, all dehydrated. A bacterial suspension is used to rehydrate each of the wells. Some of the wells will have color changes due to pH differences: others produce end products that have to be identified with reagents. A profile number is determined from the sequence of + and - test results, and then looked up in a codebook having a correlation between numbers and bacterial species.

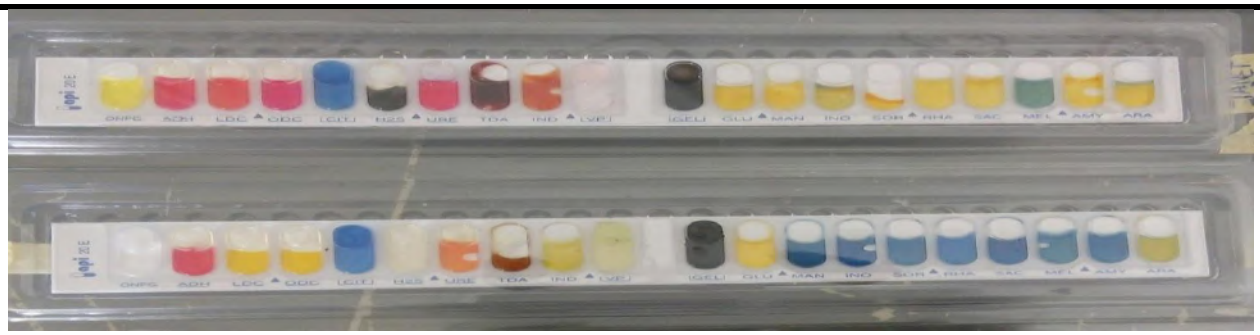

Fig.2: BioMerieux, API 20NE

ANTIBIOTICS SUBSCEPTIBILITY TESTING

The antibiotics susceptibility of the isolates was determined by the disc agar diffusion method. The antibiotics employed for Gram-positive were: ceftazidime (Caz); cefuroxime (Crx); gentamicin (Gen); ceftriaxone (Ctr); erythromycin (Ery); cloxacillin (Cxc); ofloxacin (ofl); augmentin (Aug). These employed for gram-negative were: ceftazidime (Caz); cefuroxime (Crx); gentamicin (Gen); cefixime (Cxm); Ofloxacin (Ofl); augmentin (Aug); nitrofuratoin (Nit); ciprofloxacin (Cpr). Resistance (R) (Moyo*et al.*, 2010).

Placement of disc

Antibiotics disc were applied to the surface of the inoculated plates using a pair of sterile forceps which had been flamed and cooled. The disc was placed equidistance from each other on the plates and then pressed firmly into agar with the sterile forceps to ensure complete contact with the agar. The plates were inverted and placed in the refrigerator 30minutes for diffusion of the antibiotics into the agar, after which they are removed and incubated at 37^{O}C for 16-18hours under aerobic condition (Nicolle *et al.*, 2005).

Reading of results

After 18hours of incubation, the related susceptibility of each isolates to each drug was indicated by a clear zone of growth of inhibition around the disc. The diameter of the zone was measured using a calibrated ruler from the other side of the plate. Isolates are then scored as either sensitive or resistant depending on the size of the clear zone (Rizvi*et al.*, 2011).

PLANT SAMPLE

2.9.2.1 Source and collection of plant samples

The leaves *of Alstoniaboonei* used in this study were collected from fresh water swamp forest in ore, Odigbo local government area of Ondo State, Nigeria.

2.9.2.2 Authentication of plant samples

The plants were authenticated at the Department of Plant Science and Biotechnology, AdekunleAjasin University, Akungba-Akoko, Ondo state, Nigeria.

2.9.2.3 Preparation of plant samples

The leaves of *A. boonei* after collection were first washed thoroughly with sterile distilled water and appropriately air dried at room temperature for two weeks to ensure the samples lose most of their moisture content. The leaves of *A. boonei*after being air dried, was powdered and milled at the department of Microbiology, AdekunleAjasin University, Akungba-Akoko, Ondo State, Nigeria.

2.9.2.4 Extraction of plants

For the extraction of plant part, 500g of powdered plant sample was weighed into corked containers containing 1500ml each of dichloromethane, the mixtures were initially shaken rigorously and left for 7 days. The mixtures were filtered using sterile whatman filter papers, and the filtrates were collected directly into sterile crucibles. The filtrate was extracted using rotary evaporator, and the residues obtained were kept at room temperature (Osuntokun, 2015).

2.9.5 Standardization of plants extracts

At aseptic condition, the extracts are reconstituted by adding 1g of extracts to 2.5ml of Dimethylsulphoxide (DMSO) and 7.5ml of sterile distilled water, making it 100mg/ml. For extracts, 5ml of distilled water is measured into four sterile bijou bottles. In bijou bottle A, 5ml from the 100mg/ml bijou bottle was drawn and added, making it 50mg/ml. The serial concentration was prepared to get concentrations of 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml respectively (Osuntokun, 2014).

2.9.3 Preparation of Innoculum

Slants of the various organisms are reconstituted using an aseptic condition. Using a sterile wire loop, approximately one isolated colony of each pure culture was transferred into 5ml of sterile nutrient broth and incubated for 24 hours. After incubation, transfer 0.1ml of the isolated colony using a sterile needle and syringe into 9.9ml of sterile distilled water contained in each test tube and then mixed properly. The liquid now serve as a source of inoculum containing approximately 10^{6}cfu/ml of bacterial suspension.

2.9.3.1 Source of test organisms

The test organisms were obtained from the stock culture of organisms isolated from urine culture fron pregnant women attending ante-natal clinic at Feredal Medical Centre Owoondo State.

2.9.3.2 Standardization of test organisms

Slants of the various organisms were reconstituted using aseptic condition, using a sterile wire loop, approximately one isolated colony of each pure culture was transferred into 5ml of sterile nutrient broth and incubated for 24 hours. After incubation, 0.1ml of the isolated colony was transferred into 9.9ml of sterile distilled water contained in each test tubes using a sterile needle and syringe, and then mixed properly. The liquid now serve as a source of inoculum containing approximately 10^6cfu/ml of bacterial suspension (El Astal, 2005).

2.9.4 Antimicrobial screening of the extracts

Agar well diffusion method was employed for the antimicrobial testing using the scheme of Osuntokun, 2014, with slight modifications.

2.9.4.1 Antibacterial screening of the extract

All the test bacteria, were sub-cultured onto sterile Mueller Hinton agar plates, and incubated at 37^0c for 18-24 hours. Five distinct colonies for each organism were inoculated onto sterile Mueller Hinton broth and incubated for 3-4hours. All inocula were standardized accordingly to match the 0.5 McFarland standard, and this standard was used for all susceptibility tests. All the extracts were reconstituted accordingly into the following concentrations; 100, 50, 25, 12.5mg/ml, using Dimethylsulphoxide (DMSO). The susceptibility testing was investigated by the agar well diffusion method. A 0.1ml of 1: 10,000 dillution (equivalent to 10^6cfu/ml) of fresh overnight culture of the clinical isolates grown in Mueller Hinton agar and Sabouraud dextrose agar was seeded into 40ml of Mueller Hinton agar, and properly mixed in universal bottles. The mixture was aseptically poured into sterile Petri dishes and allowed to set. Using a sterile cork borer of 6mm diameter, equidistant wells were made in the agar. Drops of the re suspended, (2ml per well) extracts with concentrations between 100mg/ml to 12.5mg/ml were introduced into the wells till it was filled. Levofloxacin 50mg/ml was used as the control experiment for bacteria, while fluconazole 50mg/ml was used as the positive control for fungi. The plates were allowed to stand on the bench for an hour, to allow pre diffusion of the extracts before incubation at 37^0c for 24 hours for the bacterial isolates and 24^0c for 48 hours for the fungal isolates. The zones of inhibition were measured to the nearest millimetre (mm) using a standard transparent metre rule. All experiments were performed in duplicates (Osuntokun and Oladele, 2014).

III. RESULTS

In this study, cultures that showed 10^5 bacterial colonies per ml of urine were said to have significant growth. Twenty-two (22%) bacterial pathogens were isolated in 50 specimens examined in this study.

Table 2 shows the incidence of UTIs in relation to age of the subjects. A higher percentage of pregnant women [7(31.8%)] with UTIs were found within the age brackets of 26-30 years, followed by age groups 15-20years and 21-25years having a total of 5(22.7) and 4(18.2) bacterial isolates respectively.

Table 3 also shows the frequency of isolation of bacterial pathogens of the subjects.Ofthe 22 bacterial pathogens obtained, *E. coli* [11 (50.0%)] was the commonest offending bacterial pathogen isolated.Other bacterial pathogen incriminated in this study were *Klebsiella spp.*[5 (22.7%)], *Proteus mirablis* [1(4.5%)], *Enterobacter spp*. [2(9.1%)], *Pseudomonasaeruginosa* [2 (9.1%)], *Citrobacterfreundii* [1(4.5%)].

Total 4 shows the overall prevalence with age distribution of bacterial pathogens in UTIs among pregnant women. This indicates that the highest number of bacterial isolates was obtained from pregnant women within the age brackets of 26-30 years followed by 15-20years and 21-25years.

Table.2: Incidence of UTIs in relation to age distributions of pregnant women.

Age group (yrs)	No tested (%)	No. positive (%)	No. negative (%)
15-20	12(24.0)	5(22.7)	7(25.0)
21-25	11(22.0)	4(18.2)	7(25.0)
26-30	10(20.0)	7(31.8)	3(10.7)
31-35	7(14.0)	3(13.6)	4(14.3)
36-40	10(20.0)	3(13.6)	7(25.0)
Total	50(100)	22(44.0)	28(56.0)

Table.3: Percentage occurrence and distribution of bacterial pathogen in UTIs among pregnant women.

Isolate	Frequency (%)
Escherichia coli	11(50.0)
Klebsiella pneumonia	5(22.7)
Proteus mirablis	1(4.5)
Enterobacteraerogenes	2(9.1)
Pseudomonas aeruginosa	2(9.1)
Citrobacterfreundii	1(4.5)
Total	22(100)

Total.4: Overall prevalence with age distribution of bacterial pathogens in UTIs among pregnant women.

	No. of isolate (%)	15-20	21-25	26-30	31-34	35-40
E. coli	11(50.0)	4	2	1	2	2
Klebsiellasp	5(22.7)	Nil	1	3	Nil	1
Proteus sp	1(4.5)	Nil	Nil	Nil	1	Nil
E. aerogens	2(9.1)	Nil	Nil	2	Nil	Nil
P.aeruginosa	2(9.1)	Nil	1	1	Nil	Nil
C. freundii	1(4.5)	1	Nil	Nil	Nil	Nil
Total	22(100)	5(22.7)	4(18.2)	7(31.8)	3(13.6)	3(13.6)

Table 5 shows the appearance of urinary pathogens on MacConkey agar. Identification of isolates based on colonial morphological and characteristics. A total of twenty-two isolates were cultured from all the fifty urine samples from the respondents.

Table 8 shows the zone of inhibition of organism isolated in millimeter. This indicates that Ofloxacin has the highest zone of inhibition against *Escherichia coli* whileCeftriaxone had the least zone of inhibition against *Pseudomonas sp.*

Table 9shows the diameter (in mm) of the zones of inhibition of bacterial growth at different concentrations (100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml) of *Alstoniaboonei* leaf extract. *Escherichia coli* was shown to be the most susceptible organism out of all the test organisms, showing its highest susceptibility at a concentration of 100mg/ml with 24mm in diameter and its lowest susceptibility at a concentration of 12.5mg/ml with 16mm in diameter. *P. aeruginosa and Proteus mirabilis* also showed intermediate susceptibility to the leaf extract with 16mm and 20mm zones of inhibition respectively at 100mg/ml and 10mm and 12mm diameter respectively at 12.5mg/ml. from this study, *Citrobacterfreundii*was observed to be the least susceptible organism to the *Alstoniaboonei* leaf extract among the test organisms used, with 12mm in diameter zone of inhibition at 100mg/ml and 8.0mm in diameter at 12.5mg/ml.

Table.5: Colonial Morphological and Characterization of the isolates.

Isolate	Cultural morphology on MacConkey Agar	Size	Identification
1	Smooth pinkish colony	2-3 mm in diameter	*Escherichia coli*
2	Moist, raise and mucoid	Large	*Klebsiella sp.*
3	Pink translucent entire smooth	2 mm in diameter	*Escherichia coli*
4	Large	3-4mm diameter	*Proteus sp*
5	Smooth pinkish colony	2-3 mm in diameter	*Escherichia coli*
6	Rough colonies	Large	*Pseudomonas sp.*
7	Smooth pinkish colony	2-3 mm in diameter	*Escherichia coli*
8	Moist, raise and mucoid	Large	*Klebsiella sp.*
9	Rough colonies	Large	*Pseudomonas sp.*
10	Smooth pinkish colony	2-3 mm in diameter	*Escherichia coli*

11	Pink,round edge	1-3	*Enterobacter sp.*
12	Smooth pinkish colony	2-3 mm in diameter	*Escherichia coli*
13	Pink,round edge	1-3	*Enterobactersp*
14	Moist, raise and mucoid	Large	*Klebsiella sp.*
15	Smooth, shiny surface,	1mm in diameter	*Citrobacter sp.*
16	Smooth pinkish colony	2-3 mm in diameter	*Escherichia coli*
17	Pink translucent entire smooth	2 mm in diameter	*Escherichia coli*
18	Moist, raise and mucoid	Large	*Klebsiella sp.*
19	Pink translucent entire smooth	2 mm in diameter	*Escherichia coli*
20	Moist, raise and mucoid	Large	*Klebsiella sp.*
21	Pink translucent entire smooth	2 mm in diameter	*Escherichia coli*
22	Pink translucent entire smooth	2 mm in diameter	*Escherichia coli*

Biochemical Test

Table.6: Biochemical Test result on Analytical Profile Index (API NE)

TESTS	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6
ONPG	-	-	+	-	+	+
ADH	-	-	-	+	-	+
LDC	-	-	+	-	+	-
ODC	-	+	-	-	+	-
CIT	-	_	+	+	-	-
H2S	-	+	-	-	-	+
URE	-	+	+	+	-	-
TDA	-	+	-	-	-	-
IND	-	-	-	-	-	-
VP	-	-	-	-	+	-
GEL	-	+	-	+	-	-
GLU	+	-	+	+	+	+
MAN	+	-	+	-	+	+
INO	-	-	+	-	+	-
SOR	+	-	-	-	+	+
RHA	+	-	+	-	+	+
SAC	-	-	+	-	+	+
MEL	+	-	+	+	+	+
AMY	-	-	+	-	-	-
ARA	+	-	+	+	+	+

Probable organism	*Escherichia coli*	*Proteus mirabilis*	*Klebsiellapneumoniae*	*Pseudomonas aeruginosa*	*Enterbacteraerogenes*	*Citrobacterfreundii*

Table.7: sugar formation of organisms isolate

Isolate	Glu	Man	Ino	sor	Rna	Sac	Mel	Amy	Ara
Escherichia coli	+	+	_	+	+	_	+	_	+
Klebsiella pneumonia	+	+	+	_	+	+	+	+	+
Proteus mirabilis	_	_	_	_	_	_	_	_	_
Enterobacteraerogenes	+	+	+	+	+	+	+	_	+
Pseudomonas aeruginosa	+	_	_	_	_	_	+	_	+
Citrobacterfreundii	+	+	_	+	+	+	+	_	+

Table.8: ZONES OF INHIBITION OF ORGANISM ISOLATED (MM) ANTIBIOTICS

Isolates	**Caz**	**Crx**	**Gen**	**Cxm**	**Ofl**	**Aug**	**Nit**	**Cpr**	**Ctr**	**Ery**
E. coli	10	13	18	12.0	20	14	14	6	15	12
Proteus sp	7.0	13	8.0	11.0	14.0	15	12.0	10	12.0	15.0
Klebsiellasp	12.0	10.0	14.0	17.0	16.0	14.0	16.0	14.0	13.0	16.0
P. aeruginosa	13	6.0	4.0	4.0	12.0	10.0	13.0	6.0	4,0	6.0
E. aerogenes	7.0	6.0	10/0	7.0	8.0	4.0	12.0	10.0	3.0	6.0
C. freundii	6.0	8.0	5.0	6.0	5.0	4.0	4.0	7.0	9.0	6.0

Caz–Ceftazidime,Crx–Cefuroxime, Gen –Gentamycin, Ofl–Ofloxacin, Aug –Augmentin,
Ctr– Ceftriaxone, Ery–Erythromycin, Cxc–Cloxacillin, Cxm–Cefixime, Nit –Nitrofurantoin,
Cpr–Ciprofloxacin.

Table.9: Antimicrobial activities of dichloromethane extract of Alstoniabooneileaf against the clinical isolates.

Test Organisms — **Dichloromethane extract of *A. boonei*leaf Concentration (mg/ml)**

	100	**50**	**25**	**12.5**
Isolate	100	50	25	12.5
Echerichia coli	*24.0*	22.0	18.0	16.0
Proteus mirabilis	*20.0*	14.0	12.0	10.0
Pseudomonas aeruginosa	*16.0*	14.0	10.4	10.0
Klebsiellapneumoniae	18.0	16.0	14.0	12.0
Enterbacteraerogenes	14.5	14.0	12.0	10.0
Citrobacterfreundii	*12.0*	10.0	8.0	8.0

IV. DISCUSSION

This study reports the microbiological examination of urine samples of 50 pregnant women that fall within the age groups 15-40years. This investigation has shown that the incidence of UTIs in this population was 44.0%. The result of this current study also compare favourably with 58% incidence of UTIs reported by Onifade et al., (2005) in a similar study among pregnant women in Southwestern Nigeria, This high incidence of UTIs may be due to hormonal effects observed in pregnancy, which reduces the tone of the ureteric musculature aided perhaps by mechanical pressure from the gravid uterus leading to urinary statis thus encourages bacterial proliferation in urine, which is an excellent culture media (Obiogbolu, 2004). It may also be due to genuine population susceptibility since it is known that such factor as low socio-economic status, sexual intercourse, pregnancy among other factors are common among Nigeria women too (Andriole, 1985; Ebie*et al*., 2001).

Results in this current study showed that *E. coli* (50.0%) predominated over *Klebsiella spp*. (22.7%). This result is contrary to findings of OMonigbo*et al.,* (2001) whose *Klebsiella spp.* is more prevalent than *E. coli* in utis. This result compares favourably with the findings of other workers who found E coli more predominant over *klebsiella spp.* In similar studies in UTIs Ebie*et.al.,* (2001). The 50.0% recorded for E coli also agrees with the findings reported by Delzell (2000) who reported that this pathogen is the most common pathogens of UTIs during pregnancy. This high incidence of the *E coli* could be attributed to the fact that it is a commensal of the bowel and that infection is mostly by feacal contamination due to poor hygiene.This is owed to the fact that commensals of the intestines are more involved in the UTIs because of the anatomy proximity to the genitourinary area (Obiogbolu 2004).

Other pathogens isolated in order of prevalence include *Klebsiella spp. Proteus mirabilis, P aeruginosa, Enterobacteraerogenes, Citrobacterfreundii.* This also revealed that the isolated pathogens in this study were coliforms which are index organism of safety, good hygiene and sanitary quality. This conforms to the report of (Anyamene*et al.,*2002) that the dominant etiologic agents accounting for more than 85% of cases of UTIs are the Gram-negative Bacilli which are normal flora of the intestinal tract.*Escherichia coli, Klebsiellapneumoniae, Pseudomonas aeruginosa, Enterobacter , Citrobacterfreundii* and *Proteus mirabilis* isolated from the urine samples were susceptible to Gentamycin, Ofloxacin, Nitrofuratoin and Ciprofloxacin while*the isolated list above*were resistant toCefixime, Ceftazidime, Cefuroxime, Erytromycin, Cloxacillin, Augmentin and Ceftriaxone. This is agree to Nicolle *et al.,* (2000), who reported that opportunistic and systemic pathogens that cause UTI are resistant to most antibiotics and makes them difficult to treat.

An observation was made which showed less zone of inhibition at 12.5% concentration of the leaves extract of Alstoniaboonei. From the result represented on table 9, it was observed that the zone of inhibition increases with the concentration of the leaves extract of *Alstoniaboonei* i.e. an increase in the leaves extract concentration lead to an increased zone of inhibition. This observation agrees with Wilix*et al*., (2009). From table 9, it was observed that the highest zone of inhibition was gotten from 100% leaves extract of *Alstoniaboonei* against *Escherichia coli* (24.0mm) and the lowest zone of inhibition was recorded against *Citrobacter spp.* at 12.5% concentration of extract (8.0mm). Therefore, *Citrobacter spp.* is the least susceptible and *Escherichia coli*is the most susceptible to the leaves extract of *Alstoniaboonei* as indicated with clear zones of inhibition. This study also showed that 100% leaves extract of Alstoniaboonei inhibited growth of all the test bacteria. It was observed that the leaves extract of Alstoniaboonei produced the highest zone of inhibition at (100%) concentration and the zone of inhibition were 24.0mm, 20.0mm, 18.0mm, 16.0mm, 14.0mm, and 12 respectively for *Escherichia coli, Proteus mirabilis, Klebsiellapneumoniae, Pseudomonas aeruginosa, Enterobacter spp. and Citrobacter spp.* (Table 9) compare to antibiotics used (Table 8). Which the highest zone of inhibition were 20.0mm in Ofloxacin against E. coli. Followed by 16.0mm, 14.0mm,12.0mm,8.0mm, 5.0mm *in OfloxacinagainstKlebsiellapneumoniae, Proteus mirabilis,Pseudomonas aeruginosa,Enterobacterspp, andCitrobacter respectively.*

In comparison with the antibiotics used for this study, the leaves extract of *Alstoniaboonei* in its undiluted state has a higher antibacterial activity against *Escherichia coli, Proteus mirabilis,Klebsiellapneumoniae, Pseudomonas aeruginosa, Enterobacter spp. and Citrobacter spp.* (Table 8).The demonstration of activity of the*Alstoniaboonei* leaves extract against Gram negative bacteria is an indication of narrow spectrum of activity and thus can be used to source antibiotic substances for drug development that can be used in the control of these bacterial infections.

RECOMMENDATION

It is thereby recommended that crude extract of *Alstoniaboonei* which is a medicinal plant should be encouraged in modern day medicine for the cure of urinary tract infections and bacterial infection due to thehighest zone of inhibition which indicates the most susceptible to organisms isolated.

REFERENCES

[1] Adair CE, Kowalsky L, Quon H, (2003). Risk factors for early-onset group B streptococcal disease in neonates: a population based case-control study. CMAJ;169:198–203.

[2] Adekunle, A.S. &Adekunle, O.C. (2009). Preliminary assessment of antimicrobial properties of aqueous extract of plants against infectious diseases. Biology and Medicine. 1(3): 20-24.

[3] Adesokan AA, Yakubu MT, Owoyele BV, Akanji MA, Soladoye A, Lawal OK (2008). Effect of administration of aqueous and ethanolic extracts of Enantiachlorantha stem bark on brewer's yeast-

induced pyresis in rats. Africal Journal of Biochemical Research. 2(7): 165-169

[4] Alexander, D., Reith, B., Ourreshi, S. and Twaddle, S. (2006).Mamagement of suspected Bacterial Urinary Tract Infections in Adults. A national clinical Guidelines, SlottishIntercollegiate Guidelines Networks (SIGN) p.46.

[5] Amole OO, Ilori OO (2010). Antimicrobial activity of the aqueous and ethanolic extracts of the stem bark of Alstoniaboonei . International Journal of Phytopharmacology. 1(2):119– 123. :987–994.

[6] Andriole, V.T. (1985). The role of Tamm-Horsfall protein in the pathogenesis mof reflux nephropathy and chronic pyelonephritis. Yale Journal of Biological Medicine. 58:91-100.

[7] Anonymous (2007a). Medicinal, Culinary and Aromatic Plants in the near East-Medic… http://www.faO.Org/dorep/x5402e16.htm (cited 4 August, 2007).

[8] Anyamene, C.O., Stellamaris, N., Muoneke and Umerie, G.N.C. (2002). Bacterial isolates associated with Urinary tract infections in Akwa and Environs. Journal of Applied Science. 5(4): 3092-30

[9] Beerepoot MA, Geerlings SE, van Haarst EP, (2013). Nonantibiotic prophylaxis for recurrent urinary tract infections: a systematic review and meta- analysis of randomized controlled trials. Journal of Urology. 190(6) :1981-9.

[10] Cheesebrough M (2004). District laboratory practice in tropical countries. Part 2. Cambridge University Press, p. 357

[11] Colgan R, Nicolle LE, Mcglone A, (2006). Asymptomatic Bacteriuria in Adults. Am Fam Physician. 74: 985-990.

[12] Colgan R, Williams M, Johnson JR (2011). "Diagnosis and treatment of acute pyelonephritis in women.". American family physician. 84 (5): 519–26.

[13] Cubeddu, Richard Finkel, Michelle, A. Clark and Luigi, X. (2009). Pharmacology (4th Ed). Philadelphia: Lippincott Williams & Wilkins. Pp.397.

[14] Doughari, J.H.; Human, I.S, Bennade, S. &Ndakidemi, P.A. (2009). Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. Journal of Medicinal Plants Research. 3(11): 839-848.

[15] Duerden, B.I., Reid, T.M.S., Jewsbury, J.M. and Turk, D.C (1990). A new shortbook of medical parasitic Infection. ELBS Publishers pp. 576-581.

[16] Ebie, M.Y., Kandakai-Olukemi, Y.T., Ayanbadejo, J. and Tanyigna, K.B. (2001). Urinary Tract Infections in a Nigerian Military Hospital. Nigerian Journal of Microbiology.15 (1): 31-37.

[17] Enayat K, Fariba F, Bahram N. (2008). Asymptomatic bacteriuria among pregnant women referred to outpatient clinics in Sanandaj, Iran. International Brazilian Journal of Urology. 34: 699-707.

[18] Engleberg, N.C., DiRita, V. and Dermody, T.s. (2007) "63" Schaechter's mechanism of Microbial Disease (4Ed.) Baltimore: Lippincott Williams & Wilkins pp. 618

[19] Eves, FJ; Rivera, N (2010). "Prevention of urinary tract infections in persons with spinal cord injury in home health care.". Home healthcare nurse. 28 (4): 230–41.

[20] Fakae B.B., A. M. Campbell, J. Barrett (2000). "Inhibition of glutathione S-transferases (GSTs) from parasitic nematodes by extracts from traditional Nigerian medicinal plants," PhytotherapyResearch , vol. 14, no. 8, pp. 630–634,.

[21] Gosse B. K., T. A. Bryson, and T. Gokou (2000) "Study of triterpenoids from Alstoniaboonei ," SociétéOuest-Africaine de Chimie , vol. 5, no. 8, pp. 123–128.

[22] HadiS.and J. B. Bremner (2001)."Initial studies on alkaloids from Lombok medicinal plants," Molecules, vol. 6, no. 2, pp. 117–129.

[23] Hooton TM, Roberts PL, Cox ME, Stapleton AE (2013). Voided midstream urine culture and acute cystitis in premenopausal women. New England Journal of Medicine. 369(20): 1883-1891

[24] Hooton, T. M. (2000). A prospective study of asymptomatic bacteriuria in sexuallyactive young women. New England Journal of Medicine. 343(14): 92-97

[25] Jablonski D (2004). Extinction: past and present. Nature, 427: 589.

[26] Kala CP (2000). Status and conservation of rare and endangered medicinal plants in the Indian trans-Himalaya. Biological Conservation. 93(3):371-379.

[27] Kala, C.P., 2005. Current status of medicinal plants used by traditional Vaidyas in Uttaranchal state of India. Ethnobotanical Research Applied, 3: 267-278.

[28] Karlowsky JA, Hoban DJ, Decorby MR, Laing NM, Zhanel GG (2006). Fluoroquinolone-resistant urinary isolates of Escherichia coli from outpatients are frequently multi-drug: Results from the North American urinary tract infection collaborative

alliance-quinolone resistance study. Antimicrobial Agents Chemotherapy. 50(6): 2251-2254.

[29] Lucy H, Edgar JD (1999). Medicinal Plants: A reemerging Health aid. Electronic. J. Biotechnol. 2(2): 1-15.Medicina-Tropical. 37 (2), 186 – 188.

[30] Lucy H, Edgar JD (2001). Medicinal Plants: A reemerging Health aid. Electronic. Journal of. Biotechnology. 2(2): 1-15. Medicina-Tropical. 37 (2), 186 – 188.

[31] Moyo SJ, et al. Tanzan J Health Research (2010). Bacterial isolates and drug susceptibility patterns of urinary tract infection among pregnant women at Muhimbili National Hospital in Tanzania.

[32] Nascimento, G.G.F., J. Locatelli, P.C. Freitas and G.L.Silva, 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Brazilian JournalofMicrobiologigy. 31: 247-256.

[33] Nautiyal S, Rao KS, Maikhuri RK, Negi KS and Kala CP (2002). Status of medicinal plants on way to Vashuki Tal in Mandakini Valley, Garhwal, Uttaranchal. Journal of Non-Timber Forest Products. 9: 124-131

[34] Nicolle LE (2001). "The chronic indwelling catheter and urinary infection in long-term- care facility residents". Infection Control Hospital Epidemiology. 22 (5): 316–21.

[35] Nicolle, L. E., Bentley, D. and Garibaldi, R. (2000).Antimicrobial use in long-term care facilities.Infect Control Hospital Epidemiology. 21:537-545.

[36] Nicolle, L. E., Bradley, S., Colgan, R., Rice, J. C., Schaeffer, A. and Hooton, T. M. (2005). Infectious Diseases Society of America; American Society of Nephrology; American Geriatric Society.Infectious Diseases Society of America guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults. Clinical Infectious Diseases.40:643-654.

[37] Nicolle, L.E. (2008). "Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis". Urological Clinic North American 35 (1): 1-12.

[38] Obiogbolu, C.H. (2004). Incidence of Urinary Tract Infection among pregnant women within Awka Metropolis. A B.Sc project in the department of Applied Microbiology and Brewing, NnamdiAzikweUnversity, Awka, Anambra State, Nigeria p.55.

[39] Odugbemi TO, Akinsulire OR, Aibinu IE, Fabeku PO (2007). Medicinal plants useful for malaria therapy in Okeigbo, Ondo State, Southwest Nigeria. African Journal of Traditional, Complementary and Alternative Medicines. 4(2):191–198.

[40] Okigbo RN, Eme UE, Ogbogu S (2008). Biodiversity and conservation of medicinal and aromatic plants in Africa. Biotechnology of Molecular Biology. 3(6): 127-134.

[41] Okonko, I. O., Ijandipe, L. A., Ilusanya, O. A., Donbraye-Emmanuel, O. B., Ejembi J., Udeze A. O., Egun O. C., Fowotade A. and Nkang A. O (2009).Incidence of urinary tract infection (UTI) among pregnant women in Ibadan, South-Western Nigeria. African Journal of Biotechnology. 8 (23), pp. 6649-6657.

[42] Omonigbo, S.E., Obasi, E.E and Akukalia, R.N (2001). In vitro Resistance of urinary isolates of Escherichia coli and Klebsiella species to Nalidixic Acid. Nigerian Journal of Microbiology. 15(1):25-29.

[43] Onifade, A.K., Omoya, F.O and Adegunloye, D.V. (2005). Incidence and control of urinary tract infections among pregnant women attending antennal clinics in government hospitals in Ondo State, Nigeria. Journal of .Food, Agricultural and Environment. 3 (1): 37-38.

[44] Opoku F, Akoto O (2015) Antimicrobial and Phytochemical Properties of AlstoniaBoonei Extracts. Organic Chemistry Current Research 1:137.

[45] Osuntokun OT, Olajubu FA (2014). Comparative study of phytochemical and proximate analysis of seven Nigerian medicinal plants. Applied Science Research Journal of Applied Science Research. J Pon Publishers. Ekpoma Edo state Nigeria. 2(1):10-26.

[46] Owoabi J., Omogbai E.K.I. and Obasuyi O (2007). Antifungal and antibacterial activities of the ethanolic and aqueous extract of Kigellaafricana (Bignoniaceae) stem bark. Africal Journal of Biotechnology. 6: 882-885.

[47] Prescott, M., Harley, P. and Klein, A. (2008). Microbiology 7th edition. McGraw – Hill , New York. pp. 124-126

[48] Ramzan M, Bakhsh S, Salam A. (2004). Risk factors in urinary tract infection. Gomal Journal of Medical Science. 2: 50-53.

[49] Rizvi M, Khan F, Shukla, (2011). Rising prevalence of antimicrobial resistance in urinary tract infections during pregnancy: necessity for exploring newer treatment options. Journal Laboratory Physicians. 3:98–103.

[50] Salvatore, S., Cattoni, E., Siesto, G., Serati, M., Sorice, P. and Torella, M. (2011). "Urinary tract infections in women". European journal of obstetrics, gynecology, and reproductive biology 156(2):131-6.
[51] Schwenger EM, Tejani AM, andLoewen PS (2015). Probiotics for preventing urinary tract infections in adults and children. Cochrane Database Syst Rev. 23;12.
[52] Solberg OO, Ajiboye R, Riley LW (2006). Origin of class 1 and 2 integron and gene cassettes in a population-based sample of uropathogenic Escherichia coli. Journal Clinical Microbiology. 44(4): 1347-1351.
[53] Talha H., Imam, MD, Kaiser (2016). Introduction to Urinary tract Infections.
[54] Tankeshwar, A., (2015). Biochemical test in microbiology, Bacteriology.
[55] Thomson, R. E. (Jr.), Miller, J. M., Murray, P. R., Baron, E.J., Jorgensen, J. H., Pfaller, M. A. and Yolken, R. H. (2003).Specimen collection,transport, and processing: bacteriology and manual of ClinicalMicrobiology, American Society for Microbiology. 320-322.
[56] Zaffanello, M., Malerba, G. and Cataldi, L. (2010). Genetic risk for recurrent urinary tract infections in humans: a systematic review. Journal of Biomedical Biotechnology. 10:321082.