ANTIMICROBIAL ACTIVITY OF *OCIMUM SUAVE* (WILLD*)* ESSENTIAL OILS AGAINST UROPATHOGENS ISOLATED FROM PATIENTS IN SELECTED HOSPITALS IN BUSHENYI DISTRICT, UGANDA

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MICROBIOLOGY OF KAMPALA INTERNATIONAL UNIVERSITY

2013

DEDICATION

I dedicate this thesis to the Almighty God, my dear parents Mr. Charles Zaribugire (RIP) and Mrs. Aidah Zaribugire, my brothers and sisters, for their care and support which has made me what I am today.

ACKNOWLEDGEMENT

I acknowledge my supervisors, Dr. Matilda A. Okech and Mr. Josephat Maniga Nyabayo for their enormous supervision and meticulous corrections while writing this disertation. My sincere gratitude goes to KIU for having granted me the study opportunity. I also extend my sincere appreciation to Mr. Pascal Mwebembezi, Department of Pharmacology, KIU, for having helped me in plant sample collection and essential oils extraction. I would like to thank Mr. Ambrose Shabohurira, Microbiology laboratory, KIU-TH for having provided me space and laboratory supplies. I also like to thank Dr. Jessica L. Nakavuma, Department of Biotechnical & Diagnostic Sciences, Makerere University, Uganda for her mentorship for the study. I also acknowledge Mr. Nathan L. Musisi, Department of Biotechnical & Diagnostic Sciences, Makerere University, Uganda for providing me with control organisms. I also wish to express my sincere gratitude to Mr. Hannington Baluku, Department of Medical Microbiology, College of Health Sciences, Makerere University, Uganda for having given me opportunity to use the laboratory facilities. Special thanks go to Mr. Josephat Maniga and all Microbiology and Immunology Department staff for their sincere support. I would like to thank Mzee Kaniora Zakaria from Bushenyi District, for providing me with the information on the traditional uses of *Ocimum suave* (Omujaja). Special thanks go to the laboratory staffs in the study hospitals in Bushenyi District for their support and collection of samples. This research received financial support from MESAU/MEPI Consortium, supported by Grant Number 5R24TW008886, OGAC, NIH and HRSA to academic staff of KIU.

ABSTRACT

Background: Microorganisms, which cause UTIs, exhibit drug resistance due to misuse and/or inadequate use of antimicrobial agents. Essential oils derived from aromatic medicinal plants have been reported to exhibit exceptionally good antimicrobial effects against bacteria, yeasts, filamentous fungi, and viruses.

Objectives: To determine the *in vitro* antibacterial activity, drug level interaction of *Ocimum suave* (Willd) essential oils and antibiotics against uropathogens.

Methods: A cross sectional and experimental study was carried out in six selected hospitals in Bushenyi District, Uganda. Midstream clean catch urine samples were collected and inoculated on CLED agar, using calibrated inoculation loop. The plates were incubated at 37ºC for 24 h to 48 h. The *Ocimum suave* (Willd) leaves were hydro distilled for 4 h using a Clevenger apparatus. The oil was collected and dried over anhydrous sodium sulphate (Na_2SO_4) and kept at 4°C till further use. The antimicrobial activity of *O. suave* (Willd) essential oils against uropathogen isolates was determined by agar well method. The MIC of reference antimicrobial drug and *Ocimum suave* (Willd) essential oil extract was carried out by micro-broth dilution method. The drug level interaction of the *Ocimum suave* (Willd) essential oil and ciprofloxacin in combination was obtained by calculating the fractional inhibitory concentration index (FICI).

Results: Out of 300 midstream clean catch urine samples, 67(22.33%) had significant bacterial growth and *E. coli* was the most frequent isolate 41(61.19%). The essential oil from *O. suave* (Willd) showed activity against uropathogens isolates of *E. coli, K. pneumoniae, S. aureus, E. feacalis, M. morganii, Citrobacter sp., Acinetobacter sp., Enterobacter sp.,* and *P. aeruginosa* with inhibition zone raging from (9-18mm). The essential oils enhanced the activity of ciprofloxacin when used in combination hence inhibiting the growth of uropathogens by two fold with inhibition zone ranging from 16-32mm. The essential oils had no inhibitory activity on *Acinetobacter* sp. The fractional inhibitory concentration indices (FICI) of *Ocimum suave* (Willd) essential oil and ciprofloxacin were calculated to be 0.35 for *E. coli,* 0.30 for *K. pneumoniae,* and 0.42 for *S. aureus.* The values of FICI for tested uropathogen isolates were found to be ≤ 0.5, which indicates synergism between *Ocimum suave* (Willd) essential oil and ciprofloxacin.

Conclusion: *E. coli* 41(61.19%) was the commonest organism detected. The study revealed synergism between ciprofloxacin and *Ocimum suave* (Willd) essential oil against uropathogens.

Recommendation: The *in vivo* studies are required to determine the efficacious dose of *Ocimum suave* (Willd) essential oil and assess the potential of the combination with commonly used antibiotics for therapeutic purposes.

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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
BA	Blood Agar
CFU ml^{-1}	Colony Forming Unit per milliliter
FICI	Fractional Inhibitory Concentration Index
HC IV	Health Center four
HIV	Human Immunodeficiency Virus
h	Hours
IREC	Institutional Research and Ethics Committee
KIU	Kampala International University
KIU-TH	Kampala International University-Teaching Hospital
MDR	Multidrug resistance
MEPI	Medical Education Partnership Initiative
MESAU	Medical Education for Equitable Services to All Ugandans
MIC	Minimum Inhibitory Concentration
MLC	Minimum Lethal Concentration
MoH	Ministry of Health
Na_2SO_4	Sodium sulphate
NCCLS	National Committee for Clinical Laboratories
UBoS	Uganda Bureau of Statistics
UK	United Kingdom
UNCST	Uganda National Council for Science and Technology
UTI	Urinary Tract Infection
WHO	World Health Organization

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CHAPTER ONE: INTRODUCTION

1.1 Background

The human urinary tract is a collecting and an emptying system, which comprises the kidneys, ureters, bladder and urethra. Urinary Tract Infections (UTIs) refers to colonization of the urinary tract as well as tissue invasion of organs of the urinary system by pathogenic microbes. UTIs are classified by the site of infection, bladder (cystitis), kidney (pyelonephritis), or urine (bacteriuria), which can be asymptomatic or symptomatic. The UTIs are usually characterized by a wide spectrum of symptoms, ranging from mild irritative voiding of urine, to bacteriamia, sepsis, or even death. UTIs that occur in a urinary tract, without prior instrumentation, are considered "uncomplicated" whereas "complicated" infections are diagnosed in urinary tracts that have structural or functional abnormalities, and are frequently asymptomatic (Stamm and Hooton, 1993; Gonzalez and Schaeffer, 1999; Betsy, 2002). Patients with an otherwise normal urinary tract but with symptomatic renal infection are diagnosed as having acute uncomplicated pyelonephritis.

Kidney and bladder urine are normally sterile, although the lower urethra in the female, and to a lesser extent in the male, may have detectable bacteria flora, which may include coliforms and *Staphylococci* species, with the number of microbes diminishing, upwards, as the bladder is approached (Getenet and Wondewosen, 2011). However, male infants are reported to have a higher rate of UTI than their female counterparts due to their being prone to congenital urinary disorders (Aaron, 2002). There are many different pathogenic microorganisms (bacteria, fungi, protozoa and viruses) which cause UTIs. Bacteria are usually more prevalent and invasive. *E. coli* and other Enterobacteriacae are the most common bacterial pathogens and accounts approximately 75% of the isolates (Getenet and Wondewosen, 2011). The relative frequencies of the pathogens vary with age, sex, catheterization, and hospitalization (Sefton, 2000; Getenet and Wondewosen, 2011).

The presence of over $10^5 CFUml^{-1}$ of uncentrifuged urine sample cultured is indicative of UTIs (Lucas and Cunningharm, 1993; Andabati and Byamugisha, 2010; Momoh *et al*., 2011). However, lesser counts may be strongly suggestive in some instances, especially, among pregnant women, where asymptomatic UTIs could predispose them to greater risk

of developing symptomatic UTIs and its attendant obstetric complications (Foxman and Fredrichs, 1985; Andabati and Byamugisha, 2010). The resulting infection may be symptomatic or asymptomatic; with the latter usually detected on routine examination (Manikandan *et al.,* 2010). UTIs are common problems in outpatient clinics daily particularly among patients in active reproductive ages (18-37 years), both young men and women (Momoh *et al*., 2011) About 150 million people worldwide are diagnosed with UTIs each year costing the global economy in excess of 6 billion US dollars (Gupta *et al*., 2001; Ava *et al.,* 2010; Manikandan *et al.,* 2010). UTIs are one of the most common bacterial infections encountered by both the general community and in hospitals.

Worldwide, *E. coli* causes 75-90% of acute uncomplicated cystitis while *S. saprophyticus* accounts for 5-15%, mainly in younger women (Gupta *et al.,* 2001; Ronald, 2002; Fihn, 2003; Mwaka *et al.,* 2011). *Enterococcus spp* and aerobic gram-negative rods, *K. pneumoniae* and *P. mirabilis*, were isolated from the cases of UTI (Finkelstein *et al.,* 1998; Allan, 2001, Fihn, 2003; Wanyama, 2003; Mwaka *et al.,* 2011). UTIs are commonly encountered diseases in developing countries, with an estimated annual global incidence of at least 250 million (Ronald *et al.,* 2001; Barisˇic´ *et al.,* 2003; Getenet and Wondewosen, 2011). The Uganda Bureau of Statistics (UBoS) 2009/2010, National Household Survey found the national prevalence of UTIs to be 0.2%. However, its impact and frequency vary in different populations. The prevalence of asymptomatic bacteriuria among the in-patients in medical wards in Uganda was found to be 8% in women and 6% in men (Tulloch *et al.,* 1963). More recent studies found the prevalence of UTIs to be 13.3%, and drug resistance to be 20-62% in Mulago Hospital, Uganda (Andabati and Byamugisha, 2010).

The search for antimicrobials of plant origin has been mainly stimulated by the fact that some of the major antibacterial agents have developed resistance. Traditional remedies utilizing plants products still occupy a central place among rural communities in developing countries for curing various diseases, in the absence of an efficient primary health care system (Ali *et al.,* 2001; Pandey, 2003; Pandey *et al.,* 2010). Although, the drug resistance development by microbes cannot be stopped, appropriate use of more

effective antibiotics including products of plant origin may reduce the mortality and health care costs (Ahmad and Beg, 2001; Pandey *et al.*, 2010).

Ocimum suave (Willd) belongs to the family *Lamiaceae*. It is found in the tropical Africa and warm temperature regions such as India (Sulistiarin, 1999). Some of the vernacular names include: Omujaja (Luganda), Kirumbasi (Kiswahili) Vambamanga (Giriama) Mukandu (Kamba) Mugio (Kikuyu) Olururuecha (Luo) Olemoran (Maa) (Hassanali, *et al*., 1990; Ssempereza, 2012). *O. suave* (Willd) has other synonyms like *O. viride* (Willd), and is also called *O. gratissimum* (Linn) (Sulistiarin, 1999). *O. suave* (Willd) is used in the treatment of different diseases such as respiratory tract infections, diarrhea, headache, conjunctivitis, skin diseases, teeth and gum disorders, fever and as a mosquito repellent (Onajobi, 1986; Ilori *et al.,* 1996; Obinna *et al.,* 2009). It is among the medicinal plants that have been reported to provide various culinary and medicinal properties. Its medicinal properties have bacteriostatic and bactericidal effects on some bacteria (Okigbo and Igwe, 2007; Obinna *et al.*, 2009).

Essential oils are fragrant substances contained in several plant organs (Cowan, 1999), and those derived from aromatic medicinal plants have been reported to exhibit exceptionally good antimicrobial effects against bacteria, yeasts, filamentous fungi, and viruses (Reichling *et al.,* 2009). The chemical composition of essential oils depends on a number of parameters such as environmental conditions, collection period, dehydration procedure, storage condition and isolation methods (Magiatis *et al.,* 2002; Pandey *et al.,* 2010). Essential oils have chemical compounds and active ingredients such as eugenol, linalool, methyl cinnamate, camphor, and thymol (Matasyoh *et al.,* 2007). Essential oils, either inhaled or applied to the skin, act by means of their lipophilic fraction reacting with the lipid parts of the cell membranes of microorganisms, and as a result, modifying the activity of the calcium ion channels (Buchbauer and Jirovetz, 1994; Svoboda and Hampson, 1999).

Essential oils have been the active principles of many important herbal remedies since ancient times (Guenther, 1948; Pandey *et al.,* 2010). The antimicrobial properties of essential oils are well recognized for many years and have been used as naturally

occurring antimicrobial agents in phytopathology, medical microbiology, food preservation, among other uses (Burt, 2004; Pandey *et al.,* 2010). Essential oils of many plants are known to have antimicrobial activity (Deans *et al.,* 1992; Piccaglia *et al.,* 1993). The inhibitory activity of essential oils and their components have been reported against bacteria, fungi, viruses and cancer by various researchers (Svoboda and Hampson, 1999; Jirovetz *et al*., 2006; Silva *et al*., 2008; Tripti and Singh, 2010). However, there are few reports on its activity against uropathogens (Pereira *et al*., 2004; Tripti and Singh, 2010). Therefore, this study was carried out to determine antibacterial activity of *O. suave* (Willd) essential oils against uropathogens.

1.2 Statement of the problem

UTIs in humans, especially in rural environment cause significant morbidity due to unsanitary conditions, poor personal hygiene, lack of patient's compliance to medication and economic burden. Many microorganisms, which cause UTIs, exhibit drug resistance due to misuse and/or inadequate use of antimicrobial agents. Despite the large number of antimicrobial agents available, these infections have remained a significant problem in medicine (Tripti and Singh, 2010). The indiscriminate use of antimicrobial drugs has led to resistance in uropathogens globally. Concurrent resistance to different antimicrobial agents has given rise to multi-drug resistance in uropathogens, which also complicates the therapeutic management of UTIs (Gupta *et al*., 2001; Akram *et al*., 2007; Tripti and Singh, 2010). In addition, antimicrobial agents are also associated with adverse effects on host, which include depletion of beneficial gut flora and mucosal microorganisms, immunosuppression, hypersensitivity and allergic reactions (Patel, 2007; Tripti and Singh, 2010).

According to WHO 2002 – 2005 Report, about 80% of the population of Uganda relies on traditional medicine, this is partly due to shortage of trained medical personnel and traditional healers are easily consulted since they live with the people in the same community. Furthermore, the country imports most of its drugs and often experiences shortages. This leads to the demand for traditional healers for medicinal plants and hence the majority of the people, rural and urban alike depend largely on herbal medicines for treating a variety of diseases. This reliance is mainly due to the fact that the community

regards herbal drugs to be safer than the conventional medicine as they are thought or reported to have less side effects (Armando and Yunus, 2009). The high cost of conventional medicine and inaccessibility of modern health care facilities in most areas complicates the situation further (WHO, 2002-2005; Armando and Yunus, 2009).

O. suave (Willd) has been used in the treatment of UTIs by traditional healers (personal communication), but no study has been carried out on its essential oils to determine its antibacterial properties against uropathogens in Uganda. It is upon this background that the study was carried out to determine the *in vitro* antibacterial properties of *O. suave* (Willd) essential oils against uropathogens.

1.3 Purpose of the Study

The aim of the study was to determine the antibacterial activity of *O. suave* (Willd) essential oils against uropathogens isolated from patients in selected Hospitals in Bushenyi District, Uganda.

1.4 Specific objectives

1. To isolate and identify the bacterial uropathogens.
2. To determine the antibacterial activity of essential oils from *O. suave* (Willd) against bacterial uropathogens.
3. To determine the drug level interaction of *O. suave* (Willd) essential oils and ciprofloxacin in combination.

1.5 Research questions

1. Which bacterial species are responsible for most of the UTIs?
2. Are essential oils from *O. suave* (Willd) having antimicrobial activity against bacterial uropathogens?
3. Do *O. suave* (Willd) essential oils have drug level interaction with ciprofloxacin in combination?

1.6 Significance of the study

The study findings will contribute to local and international knowledge on the types of uropathogens responsible for UTIs in the area of study. These research findings will

augmente information which will help Clinicians, Medical Officers, and Ministry of Health (MoH) in making decisions for empirical treatment of UTIs. At the community level, findings of the study will provide scientific backing to the claims by the traditional medicine practitioners, using extracts from *O. suave* (Willd) to treat UTIs. This could be a starting point for development of alternative treatment for UTIs in the local community.

1.7 Conceptual framework

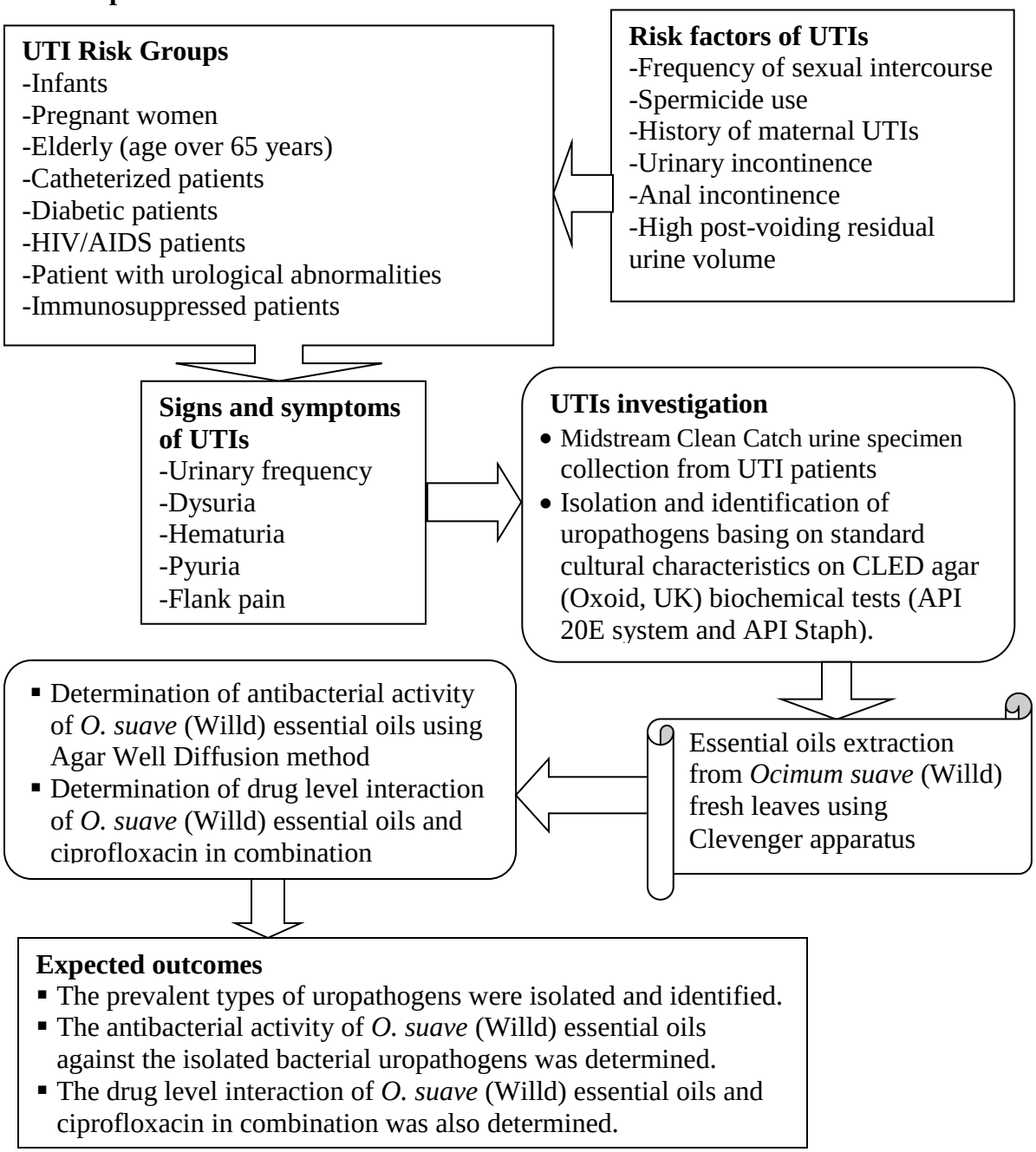

Fig 1.1: Conceptual framework

1.8 Scope

The study was conducted in selected hospitals in Bushenyi District, Uganda (Appendix II). The study samples were collected from UTI patients attending Kampala International University-Teaching Hospital (KIU-TH), Ishaka Adventist Hospital, Comboni Hospital, Bushenyi HC IV, Kyabugimbi HC IV, and Bushenyi Medical Center (BMC). Midstream clean catch urine samples were collected from in-and -out patients for isolation and identification of uropathogens, and determination of antibacterial activity of *O. suave* (Willd) essential oils. The leaves of *O. suave* (Willd) were collected from Ishaka, Bushenyi District, Uganda.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

Urinary tract infections (UTIs) refer to the presence of microbial pathogens within the urinary tract (Betsy, 2002). UTIs are some of the most common types of infections and nearly 10% of people will experience a UTI during their life time. They are serious ailments in humans due to the fact that their frequency, recurrence and difficulty in their eradication pose challenges to the medical professionals (Hoberman and Wald, 1997; Delanghe *et al*., 2000). Uncomplicated UTIs occur most often in young healthy adult women and are easy to treat. They are much more common in women than in men, due to anatomic and physiological reasons and up to 50% of women report having had at least one UTI in their lifetime (Barnett and Stephens, 1997; Fihn, 2003). Although it is not always possible to trace the mode of entry of bacteria into the urinary tract, many authors have suggested four possible routes of entry which include ascending infection, haematogenous spread and lymphogenous spread, and direct extension from another organ (Maripandi *et al.*, 2010).

The people at risk of UTIs include infants, pregnant women and the elderly aged over 65 years, as well as those with indwelling catheters, diabetes, underlying urologic abnormalities, and those under treatment with immunosuppressive drugs with immunocompromised status such as those with HIV infection and these normally have a complicated course that is more difficult to treat and recurrence is common (Johnson *et al.*, 1987; Hoepelman *et al.*, 1992; Foxman and Brown, 2003). It is also further reported that the incidence of UTIs in hospital environment is on the rise due to cross infection and lowered immune status of the patients (Maripandi *et al.*, 2010).

Different microorganisms like bacteria, viruses, fungi, and protozoa cause UTIs. Bacteria are the major causative organisms accounting for more than 95% of UTI cases (Bonadio *et al*., 2001). The common pathogens that have been implicated in UTIs are primarily gram-negative organisms with *E. coli* having a more prevalence than other gram-negative pathogens such as *K. pneumoniae*, *Enterobacter* spp., *P. aeruginosa*, *P. mirabilis* and *Citrobacter* spp., (McLaughlin and Carson, 2004; Llenerrozos, 2004; Mittal and Wing, 2005; Blair, 2007; Maripandi *et al.*, 2010). *E. coli* accounts for approximately 90% of

first UTI in young women (Jawetz, 2004; Momoh *et al.*, 2007; Momoh *et al*., 2011). Some enteric organisms such as *Pseudomonas* also adhere to the urinary catheters and form a biofilm on the surface, which then acts as a reservoir for growth (Shigemura *et al*., 2006). An accurate and prompt diagnosis of UTIs is important in shortening the disease course and for preventing the ascent of the infection to the upper urinary tract and renal failure.

The symptoms and signs of UTIs include frequent urination (polyuria), dysuria, hematuria and pyuria while flank pain is associated with upper tract infections. None of these symptoms or signs is specific for *E. coli* infection (Davidson, 2006). UTI can result in bacteriuria with clinical signs of sepsis (Eisenstein and Azalezink, 2000). Nephropathogenic *E. coli* typically produce a hemolysin. Most of the infections are caused by *E. coli* of a small number of somatic (O) antigen types. Its capsular (K) antigen appears to be important in the pathogensis of upper tract infection but pyelonephritis is associated with specific types of pilus which binds to the blood group substances (Bopp, 2003).

The spread of drug resistant uropathogens is one of the most serious threats to successful treatment of microbial diseases. Essential oils and other extracts of plants have evoked interest as sources of natural plant products. Some of these have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases (Tepe *et al.,* 2004). The World Health Organization (WHO) has also recognized the fact that the majority of the world's population depends on traditional medicine for primary healthcare. Among the sources of herbal remedies are medicinal aromatic plants which constitute a major source of natural organic compounds especially the essential oils (Seenivasan *et al*., 2006; Armando and Yunus, 2009).

2.2 Current situation of UTIs

About 150 million people worldwide are diagnosed with UTIs each year costing the global economy excess of 6 billion US dollars (Gupta *et al*., 2001; Ava *et al.,* 2010; Manikandan *et al.,* 2010), whereas in developing countries the annual estimate of at least 250 million (Ronald *et al.,* 2001; Barisˇic´ *et al.,* 2003; Getenet and Wondewosen, 2011).

In children approximately 5% of girls and 1% of boys have a UTI by 11 years of age (Jenson and Baltimore, 2006).

According to Uganda Bureau of Statistics (UBoS) 2009/2010, National Household Survey found the national prevalence of UTIs to be 0.2% and the detailed figures by region, age and residence are shown in tables 2.1 and 2.2 below. However, its impact and frequency vary in different populations.

Table 2.1: Prevalence of UTIs by region and age (%)

UBoS Uganda National Household Survey Report 2009/10	
Background Characteristics	**Urinary Tract Infection**
Region	
Kampala	0.2
Central	0.1
Eastern	0.3
Northern	0.2
Western	0.2
Age	
Under 5	0.2
5-17	0.1
18-30	0.3
31-59	0.2
60+	0.8
Uganda	**0.2**

Source: http://www.ubos.org

Table 2.2: Prevalence rates of UTIs by residence (%)

	2005/2006			**2009/2010**		
Type of illness	**Urban**	**Rural**	**Uganda**	**Urban**	**Rural**	**Uganda**
Urinary Tract Infection	0.1	0.3	0.3	0.1	0.2	0.2

Source: http://www.ubos.org

UTIs occur at the rate of 2 - 3% of hospital admission and account for 35 - 40% of all nosocomial infections (Nakhjavani *et al.,* 2007; Ava *et al.,* 2010). The urinary tract is the commonest source of nosocomial infection, especially when the bladder is catheterized (Ava *et al.,* 2010**)**. Most catheter-associated UTIs are derived from the patient's own normal flora and the catheter predisposes to UTIs in several ways. The most important

risk factor for the development of catheter-associated bacteriuria is the duration of catheterization (Tenke *et al.,* 2007; Ava *et al.,* 2010).

2.3 Epidemiology of UTIs

UTIs are more prevalent among premenopausal than postmenopausal women (Henn, 2010). In a study by Hooton *et al.,* (1996), the estimated incidence of cystitis in sexually active women in a university student population was found to be 0.5–0.7 episodes/person in a year. But a second infection was shown to occur within six months after a first UTI in 21% of young women (Foxman *et al.,* 2000). The incidence of culture confirmed acute cystitis in postmenopausal women was found to be 0.07 episodes/ person in a year (Jackson *et al.,* 2004). The peak incidence of infection occurs in young, sexually active women aged 18 to 24 years (Fihn, 2003). Bacteriuria is found in 2–3% of women aged 15–24 years, 20% of women 65–80 years and 25–50% of women older than 80 years (Rahn, 2008).

The natural history of most UTIs is acute, uncomplicated and it resolves spontaneously (clinical and microbiological) in about half of women within a few days or weeks. The antimicrobial treatment substantially shortens the duration of symptoms (Ferry *et al.,* 2004). UTIs are therefore mostly benign from the perspective of long-term outcomes, but each episode is associated with substantial disruption in a woman's life. Women report an average of 6.1 symptomatic days, 2 to 4 days restricted activity and 1.2 work days lost with each episode of cystitis. Also, 63% of women report that the infection had an impact on their usual activities, with a mean duration of 4.9 days (Nickel *et al.,* 2005).

Recurrent urinary tract infections (RUTIs) are also common among healthy women with structurally normal urinary tracts, with as many as 5% of women experiencing it at some stage during their life (Scholes *et al.,* 2000). According to Ikaheimo *et al.,* (1996), report in a primary health care setting, 44% of women presenting with an infection experienced a second infection within one year. Three aetiologies exist for RUTI: i) persistence of the original organism, ii) reinfection with the original organism, or iii) reinfection with a different strain of bacteria (Dwyer and O'Reilly, 2002). In women, the majority of RUTIs

are as a result of reinfection of the initial bacteria due to bacterial persistence in the faecal flora and subsequent recolonisation of the urethra (Russo *et al.*, 1995).

Patients with HIV/AIDS are predisposed to UTI by uncommon bacteria and pathogens (fungi, parasites and viruses). When the CD4 count declines to <200/mm^3 the risk of opportunistic infection increases dramatically. The reported incidence of bacterial UTI in patients with AIDS is 7–50%. They can experience the typical lower urinary tract symptoms (LUTS) such as dysuria and polyuria, although many patients are asymptomatic. Pyuria has been noted in up to 52% of patients, with associated UTI in only 20% (Steele and Carson, 1997; Hyun and Lowe, 2003). The most common bacterial pathogens in HIV-infected patients are *E. coli*, *Enterobacter*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Acinetobacter*, *S. aureus*, group D *Streptococcus*, *Serratia* and *Salmonella* spp (O'Regan *et al.*, 1990).

2.4 Uropathogens and drug resistance

The treatment of UTI cases is often started empirically and therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens (Wilson and Gaido, 2004). The antibiotics recommended for treatment of UTIs include; nitrofurantoin ampicillin, trimethoprim-sulfamethoxazole, and flouroquinolones. However, due to incessant abuse and misuse of these antibiotics, extensive resistance of microorganisms to them has developed and as a result drug resistance is now a huge problem in treating infectious diseases like UTIs. The improper and uncontrolled use of many antibiotics has resulted in the occurrence of antimicrobial resistance which has become a major health problem worldwide (Goldman and Huskins, 1997; Manikandan *et al.*, 2010).

There are many resistant strains which have been discovered e.g., Vancomycin Resistant Enterococci (VRE), Methicillin Resistant *S. aureus* (MRSA), Extended Spectrum Beta Lactamase (ESBL) resistant *Enterococci*, *S. marcescens*, and multidrug resistant *P. aeruginosa* (Gold, 2001; Wagenlehner and Naber, 2004; Bhattacharya, 2006; Kim *et al*., 2006; Linuma, 2007; Manikandan *et al.*, 2010). Drug resistance of pathogens causes serious medical problems because of fast development and spread of mutant strains that

are not susceptible to the common drugs. Microorganisms use varied mechanisms to acquire drug resistance e.g., horizontal gene transfer (plasmids, transposons and bacteriophages), recombination of foreign DNA in bacterial chromosome and mutations in different chromosomal locus (Klemm *et al*., 2006; Manikandan *et al.,* 2010).

Furthermore, there have been a lot of reports in the scientific literature on the inappropriate use of antimicrobial agents and the spread of bacterial resistance among microorganisms causing UTIs (Tenover and McGowan, 1996; Hryniewicz *et al*., 2001; Kurutepe *et al*., 2005; Manikandan *et al.,* 2010). The changing patterns in the uropathogens and their sensitivities to commonly prescribed antibiotics are reported (Jacoby and Archer, 1991; Hryniewicz *et al*., 2001; Kurutepe *et al*., 2005; Mordi and Erah, 2006; Manikandan *et al.,* 2010). The emergence of antibiotic resistance in the management of UTIs is a serious public health issue, particularly in the developing world.

2.5 Management of UTIs using Antimicrobial agents

Low dose antimicrobial therapy remains an effective intervention to manage recurrent, acute uncomplicated UTIs (Henn, 2010). Women receiving long term prophylactic therapy have four times less episodes of UTI compared to those without (Albert *et al.*, 2004). The antimicrobial agent may be given as continuous daily or every-other-day therapy, usually at bedtime, or as postcoital prophylaxis. First line treatments are nitrofurantoin, trimethoprim and sulphamoxazole, or fosfomycin. Fluoroquinolone antimicrobials are effective, but should be reserved for women who are unable to tolerate first line agents or who experience recurrent infection with resistant organisms while receiving first line regimens (Henn, 2010). The initial suggested duration of prophylaxis is six months; however, 50% of women will experience recurrence by three months after discontinuation of the prophylactic antimicrobial. When this occurs, prophylaxis may be reinstituted for as long as one or two years and remain effective (Schooff and Hill, 2005).

2.6 Non-antimicrobial management of UTIs

Daily cranberry products (juice or tablets) or lingonberry juice decreases the frequency of RUTI by about 30–35% at 12 months compared to placebo (Stothers, 2002; Jepson and

Craig, 2008). The exact mechanism of action is not clear, but the belief is that they prevent bacteria (particularly *E. coli*) from adhering to uroepithelial cells that line the wall of the bladder and that without adhesion; *E. coli* cannot infect the mucosal surface of the urinary tract (Henn, 2010). Topical vaginal oestrogen is also a potential intervention to decrease recurrent episodes for postmenopausal women. Vaginal oestrogens compared to placebo reduced the number of UTIs in postmenopausal women with RUTI (Perrotta *et al.,* 2008). The recommended treatment is to use a vaginal cream for a minimum period of six months. Herbal products have also been proposed as a means of preventing RUTI and have shown promise, but larger sample sizes and confirmatory studies are needed (Albrecht *et al.,* 2007).

2.7 *Ocimum suave* (Willd)

The genus *Ocimum* belongs to the family Lamiaceae and is comprised of more than 50 species of herbs and shrubs distributed in tropical and subtropical regions of Asia, Africa and America. Several species and varieties of this family such as *Hyptis*, *Thymus*, *Origanum*, *Salvia* and *Mentha* species are considered economically useful because of their essential oils of diverse nature. Studies by Wossa *et al.,* (2008), have reported composition of essential oils to be eugenol, linalool, methyl cinnamate, camphor, and thymol. Various species of *Ocimum* have been reported for their numerous medical uses attributed to their essential oils primarily composed of monoterpenes and sesquiterpenes hence the subject of extensive studies due to their economic importance (Lawrence, 1993; Wossa *et al.,* 2008).

Table 2.3: General description of *Ocimum suave* (Willd)

Family: LAMIACEAE (LABIATAE)
Scientific name: *Ocimum suave (*Willd)
Synonyms: *Ocimum gratissimum* Linn and *Ocimum viride* Willd
Source: http://www.horizonherbs.com/group

Description	Aromatic branched perennial erect shrub, 1-3m tall.
Habitat	In its native area occurs from sea-level up to 1500m altitude in coastal scrub, along lake shores, in savanna vegetation, in submontane forest, and disturbed land. Its greatest variability occurs in tropical Africa (from where it possibly originates) and India.
Uses	Naturally used in the treatment of respiratory tract infections, diarrhea, headache, conjunctivitis, skin diseases, tooth and gum disorders, fever and as mosquito repellants.
Constituents	Include eugenol, mono- and sesquiterpenoids.

2.8 Nature and chemical composition of essential oils from *O. suave* (Willd)

Essential oils are aromatic oily liquids obtained from plant materials including flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots. They are complex mixtures comprising of many single compounds. Chemically, they are derived from terpenes and their oxygenated compounds. They can be obtained by expression, fermentation or

extraction but the methods of hydrodistillation and steam distillation are the most commonly used for commercial production (Wossa *et al.*, 2008).

In essential oils, four main chemotypes and numerous other sub-chemotypes were established on the basis of the structural features of the main constituents as belonging to either the phenylpropanoid group (methyl chavicol, eugenol, methyleugenol and methyl cinnamate) or the terpenic group (linalool and geraniol), which are derived from the shikimic acid and the mevalonic acid biosynthetic pathways respectively. Other latter studies on the essential oils from other geographical regions have added new chemotypes to the list based on the established classification scheme (Lawrence, 1992; Grayer, 1996; Wossa *et al.*, 2008). Some of chemotypic entries include terpenen-4-ol type from *O. canum;* thymol type from *O. gratissimum;* geranyl acetate type from *O. minimum;* citral and camphor types from *O. americanum;* and p-cymene type from *O. suave* (Sanda *et al*. 1998; Yusuf *et al*. 1998; Keita *et al*. 2000; Mondello *et al*. 2002; Ozcan and Chalchat, 2002; Wossa *et al.*, 2008).

The chemical compositions in the essential oils are mainly of monoterpenes or sesquiterpenes with predominant features representing the terpenic chemotype group such as linalool and geraniol or the phenylpropenic chemotype groups, while the observed biological activities are attributable to either the individual components within the matrix of the oil or due to a synergistic effect of the components (Lachowicz *et al*. 1998; Sinha and Gulani, 1990; Holm, 1999; Vasudaran *et al*. 1999; Carleton *et al*. 1992; Svoboda *et al*. 2003; Wossa *et al.*, 2008). The prospect of further developing and using essential oils exhibiting broad spectrum biological activities holds promise in medicine and agriculture, owing to its low mammalian toxicity, biodegradability, non-persistence in the environment and affordability (Wossa *et al.*, 2008).

2.9 Mode of action of essential oils

According to Buchbauer and Jirovetz (1994), essential oils, either inhaled or applied to the skin, act by means of their lipophilic fraction reacting with the lipid parts of the cell membranes of microorganisms, and as a result, modifying the activity of the calcium ion channels. At certain levels of dosage, the essential oils saturate the membranes and show

effects similar to those of local anaesthetics. They can interact with the cell membranes by means of their physicochemical properties and molecular shapes, and can influence their enzymes, carriers, ion channels and receptors. The physiological effects on humans include; brain stimulation, anxiety-relieving, sedation and antidepressant activities, as well as increasing the cerebral blood flow (Svoboda and Hampson, 1999).

Studies by Svoboda and Hampson, (1999), describe the effects of odours on cognition, memory, and mood. The fragrant compounds are absorbed by inhalation and are able to cross the blood-brain barrier and interact with receptors in the central nervous system. Bioassays used for the description and explanation of essential oil action, are usually carried out on mice, rats and toads screening for analgesic properties (Fogaca *et al.,* 1997; Svoboda and Hampson, 1999). Increasing numbers of aromatherapists and physiotherapists are using essential oils both in private practice and within hospitals and hospices, and their reports in all the main aromatherapy journals stress the positive effects of oils (Svoboda and Hampson, 1999).

2.10 Applications of essential oils

Essential oils are a rich source of biologically active compounds. About 3000 essential oils are known, of which 300 are commercially important in the fragrance market (Van de Braak and Leijten, 1999). Essential oils such as aniseed, calamus, camphor, cedarwood, cinnamon, citronella, clove, eucalyptus, geranium, lavender, lemon, lemongrass, lime, mint, nutmeg, orange, palmarosa, rosemary, basil, vetiver and wintergreen have been traditionally used by people for various purposes in different parts of the world (Seenivasan *et al.,* 2006). Some other oils have been used in food preservation, aromatherapy and fragrance industries (Van de Braak and Leijten, 1999). Anti-inflammatory activity has been found in essential oils (Singh and Majumdar, 1999). Lime oil has shown immunomodulatory effect in humans (Arias and Ramon-Laca, 2004).

Antiviral activity of the essential oil from *Houttuynia cordata* was tested against herpes simplex virus, influenza and HIV-1 (Hayashi *et al.,* 1995). It was suggested that the antiviral activity of the oil may be due to interference with the virus envelope. In another experiment, essential oil from several species of the genus *Heracleum* showed promising activity against influenza virus (Tkachenko *et al.,* 1995; Svoboda and Hampson, 1999).

Further studies are required to substantiate claims of antiviral activities and to elucidate the mode of action. The brine shrimp (Artemia salina) bioassay was used to test the toxicity of essential oils, (i.e. terpinen-4-ol, carvone, camphor, limonene, menthone and citral) and showed relatively low toxicity if used between 500-1800ppm (Svoboda, and Hampson, 1999). Further tests are required to assess specific activities of the oils and their individual components.

Plant essential oils as antioxidants were researched in detail and were found positive for highly unsaturated lipids in animal tissues (Deans *et al.,* 1993; Svoboda and Hampson, 1999). Lemon and rosemary oils possess antioxidant properties (Aruoma *et al.,* 1996; Calabrese *et al.,* 1999). However, it is very important to realize that in certain cases, antioxidants can be pro-oxidant and can stimulate free radical reactions (Svoboda and Hampson, 1999). Essential oils from *Ocimum basilicum* L have been reported to be cytotoxic to human cancer cells (Manosroi *et al*., 2006; Gutierrez *et al*., 2008; Hussain *et al*., 2008; 2006; Hanan *et al.,* 2010). Peppermint and orange oils have shown anticancer activity (Kumar *et al.,* 2004; Arias and Ramon-Laca, 2004). Also, Lawrence (1993) reported that Holy basil (*Ocimum sanctum*) and Sweet basil (*Ocimum basilicum*) possess antitumor activity in mice. There has been an increased interest in looking for antimicrobial properties of extracts from aromatic plants particularly essential oils (Milhau *et al.,* 1997). Essential oils of many plants are known to have antimicrobial activity (Deans *et al.,* 1992; Piccaglia *et al.,* 1993; Svoboda and Hampson, 1999). This activity could act as chemical defense against plant pathogenic diseases. It is also suggested that complex oil presents a greater barrier to pathogen adaptation than would a more simple mixture of monoterpenes (Carlton *et al.,* 1992; Svoboda and Hampson, 1999).

Essential oils derived from aromatic medicinal plants have been reported to exhibit exceptionally good antimicrobial effects against bacteria, yeasts, filamentous fungi, and viruses (Burt, 2004; Kordali *et al.,* 2005; Reichling *et al.,* 2009). Cinnamon, clove and rosemary oils had shown antibacterial and antifungal activity; cinnamon oil also possesses antidiabetic property (Ouattara *et al.,* 1997). Citronella oil has shown inhibitory effect on biodegrading and storage-contaminating fungi (De Billerbeck *et al.,* 2001). Lavender oil has shown antibacterial and antifungal activity (Cavanagh and Wilkinson,

2002; Matashoy *et al.,* 2011). The *Ocimum* oil has been described to be active against several species of bacteria and fungi. These include *Listeria monocytogenes, Shigella, Salmonella* and *Proteus*, for fungi *Trichophyton rubrum, Trichophyton mentagrophytes, Cryptococcus neoformans, Penicillum islandicum*, and *Candida albicans* (Begum *et al.*, 1993; Nwosu and Okafor, 1995; Akinyemi *et al.*, 2004; Janine de Aquino *et al.*, 2005; Lopez *et al.,* 2005).

Deans and Ritchie (1987) examined 50 plant essential oils for their antibacterial properties against 25 genera of bacteria, using an agar diffusion technique. Essential oils exhibited various reductions in growth of microorganisms, depending on the oil concentration and chemical composition. The inhibitory activity of essential oils and their components have been reported against bacteria, fungi, viruses and cancer by various researchers (Svoboda and Hampson, 1999; Jirovetz *et al*., 2006; Silva *et al*., 2008; Tripti and Singh, 2010). However, there are few reports of their activity against uropathogens (Pereira *et al*., 2004; Tripti and Singh, 2010).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study design

A cross sectional and experimental study.

3.2 Area of Study and study population

According to Ministry of Local Government (http://www.molg.go.ug), Bushenyi District has a population of 117,000 and 124,000 male and female, respectively, totalling to 241,500 people. The population distribution in rural and urban areas was projected to stand at 89 per cent rural and 11 per cent urban. The population density of 282 people per square km with a household size of 6 (5.4) was estimated. The study samples were obtained from UTI patients attending the selected hospitals namely; Kampala International University-Teaching Hospital (KIU-TH), Ishaka Adventist Hospital, Comboni Hospital, Bushenyi HC IV, Kyabugimbi HC IV, and Bushenyi Medical Center (BMC).

3.3 Sampling Procedure

A total of three hundred (300) midstream clean catch urine samples were collected from in-and-out patients with the help of trained nursing staff. The urine samples were collected using random sampling method. A total of 50 samples were collected from each of the study areas. The samples were then transported to the laboratory on ice for standard microbiological analysis within 30 minutes of collection. Baseline data such as patients' age, sex, and clinical history was recorded at the time of sampling.

3.4 Inclusion and Exclusion criteria

The study included patients attending out-and-in patient clinics that had UTI signs and symptoms at the selected hospitals, patients aged 18 to 51 years, patients with no history of antimicrobial drug administration in the last two weeks and patients who consented to participate.

The study excluded women who were in their menstruation period, patients aged below 18 to 51 years, patients with history of antimicrobial drug administration in the last two weeks and patients who had not consented to participate.

3.5 Sample size

A total of 300 patients were sampled for uropathogens isolation for the study. The sample size (n) was calculated using the standard formula (Martin *et al*., 1987).

$$n = \frac{Z^2QP}{I^2} \qquad n = \frac{Z^2(100-P)}{I^2} \qquad n = \frac{(1.96)^2(100-79.1)}{5^2} = 279.063664$$

n = 300 Urine samples

Where n = Sample size, Q = 100-P

Z=Level of significance (1.96) for confidence interval of 95%.

P =Prevalence of UTIs in Uganda 76.1 % (Kees and Serigne, 2010)

I = margin of error of setting a significance level of 0.05 (i.e. 5%).

3.6 Isolation and Identification of isolates

Midstream clean catch urine samples were inoculated on CLED agar (Oxoid, UK) plates using calibrated loop delivering 0.001ml of urine. Inoculated plates were incubated at 37ºC for 24 h to 48h (Pezzlo and York, 2004). The samples were considered positive for UTI if pure culture of 10^5CFU/ml were obtained from uncentrifuged urine sample and ≥5 pus cells observed from urine sample per field under microscope (Lucas and Cunningharm, 1993; Andabati and Byamugisha, 2010; Momoh *et al*., 2011).

The presumptive identification of the isolates was based on the cultural characteristics on CLED agar (Oxoid, UK) plates, and identification confirmed by standard identification protocol namely; Gram staining, motility test, and biochemical tests using API 20E (bioMérieux S.A), and coagulase test) (Collee and Marr, 1996; Foxman *et al*., 2000; Foxman and Brown, 2003; Sohely *et al.*, 2010).

3.8 Plant collection and identification

The leaves of *O. suave* (Willd) were collected from Bushenyi District, Uganda and the collected specimen shoot with leaves and flowers was used for identification at the Department of Botany, Makerere University. Voucher specimen (JT 001) was deposited at the Makerere University Herbarium.

3.8.1 Extraction of Essential Oils

Fresh mature leaves of *O. suave* (Willd) were collected and thoroughly washed with distilled water twice. The excess water was drained off on paper towel. The leaves were cut into small pieces and hydro distilled for 4h using a Clevenger apparatus (Clevenger, 1928; Loghmani *et al.,* 2007). The oil was collected and dried over anhydrous sodium sulphate (Na_2SO_4). The extracted oil was stored in glass bottle at 4°C wrapped with aluminium foil.

3.9 Screening for Antibacterial Activity of Essential Oils

The antimicrobial activity of *O. suave* (Willd) essential oils was screened against uropathogen isolates by Agar well method described by Kirimuhuzya *et al.,* (2009). *E. coli* ATCC 25922, *S. aureus* ATCC 12692 were used as reference strains (obtained from Department of medical microbiology, Makerere University). Ciprofloxacin was used as positive control in the assay.

Three well-isolated colonies were selected from the pure culture and transferred into a tube containing 4–5ml of normal saline. The turbidity of the mixture was adjusted to match 0.5 McFarland standard. The mixture was diluted so that the final inoculum concentration in each well is 5 X 10^5CFU/ml. The inoculator delivers 0.01ml (1:10 dilution) into each well.

The Muller Hinton agar plates (Oxoid, UK) were inoculated using the surface spraeding method so that a uniform surface distribution of inoculum was obtained. Wells of 6mm diameter were punched into the previously inoculated medium using sterile cork borer. To 100µl of essential oil was diluted with dimethyl sulfoxide (DMSO) at working concentrations of 25-50µg/ml. The working concentrations of essential oils were sterilized using 0.2µm single use filters (Sterile Acrodisc®).

The well in the first quadrant was filled with 20µl of ciprofloxacin, while 20µl of the essential oil was dispensed into the well of the second quadrant. The well in the third quadrant was left as a control, while fourth quadrant was filled with DMSO as a control. The Petri dishes were left to settle for 2-5 minutes to allow diffusion of the essential oils

and ciprofloxacin. The plates were then incubated at 37°C for 18-24h. The activity of essential oil extract and Ciprofloxacin against test organisms was determined by measuring the zone of inhibition using a transparent millimetre scale. The actual diameter of zone of inhibition was obtained by subtracting the diameter of the well. The results were compared with the standards and reported as sensitive (S), intermediate (I) or resistant (R) recommended by (CLSI, 2006).

3.10 Minimum Inhibitory Concentration (MIC) of essential oils

The MIC of reference antimicrobial drug and *O. suave* (Willd) essential oils extract was carried out by micro-broth dilution method in broth media Mueller-Hinton (Oxoid, UK) (Hammer *et al*., 1999; Chander, 2002; Tripti *et al.,* 2011). *E. coli* ATCC 25922, *S. aureus* ATCC 12692 were used as reference strains (obtained from Department of Medical Microbiology, Makerere University).

Three well-isolated colonies were selected from the pure culture and transferred into a tube containing 4–5ml of normal saline. The turbidity of the mixture was adjusted to match 0.5 McFarland standard. The mixture was diluted so that the final inoculum concentration in each well is 5 X 10^5CFU/ml. The micropipette delivering 0.01ml (1:10 dilution) of the standard inoculum into each well was used. The 100µl of essential oil was diluted with dimethyl sulfoxide (DMSO) at working concentrations of 25-50µg/ml.

The test was carried out in 96-well microtitre plates, each well was dispensed with 95µl of Muller Hinton broth; 5µl essential oil was serially diluted in the wells and 5µl of inoculum added to each well. The plates were then incubated at 37°C for 18-24h. The lowest concentration showing no visible growth was considered as the MIC. The bactericidal or bacteriostatic activity was determined by culturing a tenfold dilution of all the wells that had no apparent growth. The lowest concentration showing absence of growth was considered as Minimum Bactericidal Concentration (MBC).

3.11 Drug level interaction of *O. suave* (Willd) essential oil & ciprofloxacin in combination

The drug level interaction of *O. suave* (Willd) essential oil and ciprofloxacin in combination was obtained by calculating the fractional inhibitory concentration index (FICI) using the following formulae below:

$$FIC_{O.suave\ \text{Essential oils}} = \frac{\text{MIC of Essential oils in combination}}{\text{MIC of } O.suave \text{ Essential oils alone}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (i)$$

$$FIC_{\text{Resistant antibiotic}} = \frac{\text{MIC of Resistant antibiotic in combination}}{\text{MIC of Resistant antibiotic alone}} \ldots\ldots\ldots\ldots\ldots (ii)$$

$$\text{FICindex (FICI)} = FIC_{O.suave\ \text{Essential oils}} + FIC_{\text{Resistant antibiotic}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (iii)$$

The FIC index was interpreted as: (i) synergistic effect when ≤0.5, (ii) additive or indifferent effect when >0.5 and <1 and (iii) antagonistic effect when >1 (Rosato *et al.*, 2007; Tripti *et al.*, 2011).

3.12 Quality Control

There was monitoring of the quality and quantity of specimens, sample preparation and testing, decontamination, reagents and equipment, and reviewing test results and controls using established laboratory operating procedures as reported by (Cheesbrough, 2006).

3.13 Data analysis

The data was entered in EpiData version 3.1.2701.2008, and statistical analysis was done by descriptive statistics using SPSS version 11.5. The antibacterial activity was reported in terms of diameters of the zones of inhibition (mm). The results were expressed as mean and presented as tables.

3.14 Ethical considerations

The research ethical approval was obtained from Mbarara University of Science and Technology (MUST), Institutional Research and Ethics Committee on Human Research (IREC) on behalf of Uganda National Council for Science and Technology (UNCST).

The procedures followed were in accordance with the ethical standards of the committees on human experimentation, and with the Helsinki Declaration of 1975 as revised in 2000. Oral and written consents were obtained from participants before starting the study and participation was voluntary for all those who agreed and signed the consent form. The identity of all participants was protected and the information given would not be traced to their names. The responses and concerns of participants were only used for research; names were not used in analysis and the only link with the results was via identity numbers (ID No).

The traditional healer who provided the information on the use of the plant was consulted and his consent obtained. He was requested to sign the consent form authorizing the use of his information. He was acknowledged for his contribution to the study.

The safety of the investigators was ensured by carrying out the work in collaboration with, and under the guidance of the Laboratory staff that had the necessary knowledge for handling infectious samples. Protective wears including laboratory coats and gloves were used, to minimize the risk of exposure to infectious organisms.

CHAPTER FOUR: RESULTS

Three hundred (300) midstream clean catch urine samples were collected from patients attending the selected hospitals. Sixty seven samples 67(22.33%) had significant bacteriuria. A total of 104 male urine samples, 22(21.15%) had positive cultures, female 45(22.96%) out of 196 female samples had positive cultures. The prevalence of UTIs was found to be high (46.27%), in the age group 18-28 years. The results of the relationship of UTIs with the sex and age of the patients are presented in (Table 4.1).

Table 4.1: Prevalence of UTIs in different groups

Age Groups (Yrs)	Male		Female		Total samples (n=300)	Total positive samples (n=67)	% UTIs Prevalence
	Samples (n=104)	Positive samples	Samples (n=196)	Positive samples			
18-28	48	7	86	24	134	31	46.27
29-39	12	6	55	11	67	17	25.37
40-50	19	3	32	6	51	9	13.43
Above 51	25	6	23	4	48	10	14.93

Nine bacterial uropathogens were isolated from 67 midstream clean catch urine samples of which *E. coli* was the most frequent isolate 41(61.19%), followed by *Staphylococcus sp*., 10(14.93%), *K. pneumoniae*, *E. feacalis* 4(5.97%), *M. morganii* 3(4.89%), and *Citrobacter sp.* 2(2.99%). The least isolated were *Acinetobacter sp., Enterobacter sp*., and *P. aeruginosa* 1(1.49%). The distribution of uropathogens in patients of different sex and age groups was significant. The results of bacterial uropathogens with the age groups of the patients are presented in (Table 4.2).

Table 4.2: Uropathogens isolated from patients of different groups

Isolates	Age Groups (Yrs)				Total Isolates (n = 67)	% Isolates
	18-28	29-39	40-50	Above 51		
E. coli	22	0	3	6	41	61.19
Staphylococcus sp.	5	3	1	1	10	14.93
K. pneumoniae	3	1	0	0	4	5.97
E. feacalis	1	3	0	0	4	5.97
M. morganii	1	0	2	0	3	4.48
Citrobacter sp.	0	0	1	1	2	2.99
Acinetobacter sp.	0	0	1	0	1	1.49
Enterobacter sp	0	0	0	1	1	1.49
P. aeruginosa	0	0	1	0	1	1.49

Fresh leaves of *O. suave* (Willd) yielded 0.20% of essential oil. The antimicrobial activity of *O. suave* (Willd) essential oil tested against uropathogens isolates is shown in (Table 4.3).

Table 4.3: Antimicrobial activity of *O. suave* (Willd) essential oils

Isolates	**Zones of inhibition (mm) of *O. suave* (Willd) essential oils**													**Control**
	EO						**EO+ CIP**							
	F (300µg)			**CIP (5µg)**										
	12	**16**	**18**	**14**	**18**	**22**	**0**	**9**	**14**	**18**	**0**	**16**	**23**	**32**	
E. coli (n=41)		5	36	1	7	33		8	14	19		21	3	17	0
S. aureus (n=10)		1	9	1	1	8		1	6	3		4	6		0
K. pneumoniae (n=4)	1	1	2	2		2		4				1	3		0
E. feacalis (n=4)		2	2			4			2	2		1	3		0
M. morganii (n=3)		2	1	1		2		3				2	1		0
Citrobacter sp. (n=2)		1	1			2				2			1	1	0
Acinetobacter sp. (n=1)	1			1			1				-				0
Enterobacter sp. (n=1)			1			1				2			1		0
P. aeruginosa (n=1)			1			1		1				1			0
E. coli ATCC 25922	1			1				1				1			0
S. aureus ATCC 12692	1			1					1				1		0

F – Nitrofurantoin, CIP – Ciprofloxacin, EO – Essential oil

The fractional inhibitory concentration indices (FICI) of *O. suave* (Willd) essential oil and ciprofloxacin were determined to be 0.35 for *E. coli*, 0.30 for *K. pneumoniae* and 0.42 for *S. aureus* (Table 4.4). The values of FICI for tested uropathogens isolates were found to be ≤0.5, which indicates synergism between *O. suave* (Willd) essential oil and ciprofloxacin.

Table 4.4: Fractional inhibitory concentration (FIC) and fractional inhibitory concentration indices (FICI) of *O. suave* (Willd) essential oil and ciprofloxacin

Isolates	**MIC$_{O.\ suave}$ essential oils (µg/ml)**	**FIC$_{O.\ suave}$ essential oils (µg/ml)**	**MIC$_{Ciprofloxacin}$ (µg/ml)**	**FIC$_{Ciprofloxacin}$ (µg/ml)**	**MIC (EO+CIP) (µg/ml)**	**FICI**
E. coli	13	1.35	4.50	3.88	9	0.35
K. pneumoniae	16	1.30	4.75	4.37	10	0.30
S. aureus	10	1.42	4.20	3.38	8	0.42
E. coli ATCC 25922	12	1.38	4.53	3.65	7	0.38
S. aureus ATCC 12692	11	1.39	4.25	3.59	6	0.39

EO – Essential oil, CIP – Ciprofloxacin

CHAPTER FIVE: DISCUSSION

Urinary tract infections (UTIs) are the most common infections which affects all age groups, men, women and children worldwide (McLaughlin and Carson, 2004; Llenerrozos, 2004; Blair, 2007; Maripandi *et al.,* 2010). In this study, 300 patients were assessed for UTIs and sixty seven 67(22.33%) of sampled urine had significant bacterial growth. The results obtained could be due to the fact UTI signs and symptoms are not reliable indicators of the infection. Early diagnosis, timely and appropriate antimicrobial treatment are considered key factors for elimination of the uropathogens, prevent urosepsis and reduce the risk of renal scarring (Maripandi *et al.,* 2010).

Urinary tract infections (UTIs) are caused by bacteria and the findings in this study shows that *E. coli* 41(61.19%) was the most common uropathogens followed by *Staphylococcus spp.* 10(14.93%), *K. pneumoniae* and, *E. feacalis* 4(5.97%), *M. morganii* 3(4.48%), *Citrobacter spp.* 2(2.99%), *Acinetobacter spp., Enterobacter spp.,* and *P. aeruginosa* 1(1.49%). These findings are in agreement with most previous studies on UTIs (Allan, 2001; Wanyama, 2003; Cheesbrough, 2006; and Mwaka *et al..,* 2011). UTIs due to *E. coli* are common because of its inherent virulence for urinary colonization particularly its adhesive abilities and the association with microorganisms ascending from the periurethral areas contaminated by fecal flora due to the close proximity to the anus and warm moist environment in women (Andabati and Byamugisha, 2010).

Similar results were observed by Taneja *et al*., (2010), in which a total of 1974 clean catch midstream urine samples were investigated and significant bacteriuria was found in 558 samples (28.3%). Common uropathogens isolated were *E. coli* (47.1%), *Klebsiella* spp. (15.6%), *E. fecalis* (8.7%), members of tribe *Proteae* (5.9%), *P. aeruginosa* (5.9%) and *Candida* spp., (5.5%). Tambekar *et al*., (2009), investigated a total of 174 urine samples from which, 68 were found to be significant bacteriuria with *E. coli* (59%), followed by *P.* a*eruginosa* (15%), *K. pneumoniae* (10%), *P. mirabilis* (9%), *S. aureus* (6%) and *C. freundii* (1%). The UTIs were found to be most frequent in female (63%) than male (37%).

According to Amin *et al*., (2009), report 68% females' and 32% males' urine cultures were positive for bacteria. The predominant isolate was *E. coli* with frequency rate of 59%. The other isolates were *Klebsiella* spp. (11.6%), *Enterobacter* sp. (9.8%), *Pseudomonas* spp. (7.2%), *Proteus* spp. (2.9%), *Acinetobacter* sp. (2.7%), coagulase positive *Staphylococci* (2.2%), coagulase negative *Staphylococci* (2.3%), *Citrobacter* spp. (1.3%) and *Streptococci* α hemolytic (1.1%). Other studies have also reported higher incidence of *E. coli* (47.30%) in urine samples (Wazait *et al*., 2003). The presence and distribution of other pathogens namely *S. aureus* correlate with earlier reports (Ronald, 2002). It is interesting to note that only few have reported the presence of *Citrobacter* sp., in UTIs (Chawla *et al*., 1998; Kim *et al*., 2003).

The essential oil from *O. suave* (Willd) showed activity against uropathogens isolates of *E. coli, K. pneumoniae, S. aureus, E. feacalis, M. morganii, Citrobacter sp., Acinetobacter sp., Enterobacter sp.,* and *P. aeruginosa* with inhibition zone raging from (9-18mm). The essential oils enhanced the activity of ciprofloxacin when used in combination hence inhibiting the growth of uropathogens by two fold with inhibition zone ranging from 16-32mm. The fractional inhibitory concentration indices (FICI) of *O. suave* (Willd) essential oil and ciprofloxacin were calculated to be 0.35 for *E. coli*, 0.30 for *K. pneumoniae,* and 0.42 for *S. aureus.* The values of FICI for tested uropathogens isolates were found to be ≤0.5, which indicates synergism between *O. suave* (Willd) essential oil and ciprofloxacin.

These findings are comparable with the similar study in which synergism was reported between *Pelargonium graveolens* essential oils and ciprofloxacin (Tripti *et al.,* 2011). The values of FICI were found to be 0.375 for both *K. pneumoniae* KT2 and *S. aureus* ST 2, while the value of FICI for *P. mirabilis* PRT3 was found to be 0.5. These results are also comparable with the similar study in which synergism was reported between *P. graveolens* essential oil and norfloxacin (Rosato *et al.,* 2007). The values of FICI were found to be 0.51, 0.50, 0.37, 0.38 and 0.57 for *B. subtilis* ATCC 6633, *B. cereus* ATCC 11778, *S. aureus* ATCC 6538, *S. aureus* ATCC 29213 and *E. coli* ATCC 35218, respectively. Partial and complete synergy has been proved by combinations of methyl

gallate + nalidixic acid and carvacrol + methyl gallate against nalidixic acid resistant bacteria (Choi *et al.,* 2009).

According to Lopez *et al.,* (2005), *Ocimum* oil has been described to be active against several species of bacteria and fungi. The chemical compositions in the essential oils are mainly of monoterpenes or sesquiterpenes with predominant features representing the terpenic chemotype group such as linalool and geraniol or the phenylpropenic chemotype groups, while the observed biological activities are attributable to either the individual components within the matrix of the oil or due to a synergistic effect of the components (Lachowicz *et al*. 1998; Sinha and Gulani, 1990; Holm, 1999; Vasudaran *et al*. 1999; Carleton *et al*. 1992; Svoboda *et al*. 2003; Wossa *et al.,* 2008). The prospect of further developing and using essential oils exhibiting broad spectrum biological activities holds promise in medicine and agriculture, owing to its low mammalian toxicity, biodegradability, non-persistence in the environment and affordability (Wossa *et al.,* 2008).

The synergistic effect of *O. suave* (Willd) essential oils that was demonstrated in this study depicts reduction in the potential side effects of ciprofloxacin when used in combination. The combination therapy also overcomes the problem of multidrug resistance. When ciprofloxacin is applied alone, bacterial efflux pumps are responsible for resistance in pathogenic bacteria (Mahamoud *et al.,* 2007; Tripti *et al.,* 2011). Plant derived products have augmented the activity of antibiotics by inhibiting MDR efflux systems in bacteria (Tegos *et al.,* 2002; Tripti *et al.,* 2011). Since the combination affects several targets at a time in an agonistic-synergistic manner, this multitarget approach is advantageous over the conventional single target approach (Hemaiswarya *et al.,* 2008; Tripti *et al.,* 2011).

CHAPTER SIX

6.0 Conclusion

Urinary tract infections (UTIs) are the most common infectious diseases which affect all age groups, men, women and children worldwide. *E. coli* 41(61.19%) was the commonest organism detected in this study. The study revealed synergism between ciprofloxacin and *O. suave* (Willd) essential oil against uropathogens. Therefore, this effective combination may be suitably applied for the treatment of UTIs and thus minimize the side effects of ciprofloxacin.

6.1 Recommendation

The *in vivo* studies are required to determine the efficacious dose of *O. suave* (Willd) essential oil and assess the potential of the combination with commonly used antibiotics for therapeutic purposes.

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APPENDICES

Appendix I: Clearance from Ethics Committee

MBARARA UNIVERSITY OF SCIENCE AND TECHNOLOGY
INSTITUTIONAL REVIEW COMMITTEE
P.O. Box 1410, Mbarara, Uganda
Tel. 256-4854-33795 Fax: 256 4854 20782
Email: irc@must.ac.ug Web site : www.must.ac.ug

Our Ref: MUIRC 1/7

Date: June 21, 2012

Mr. Julius Tibyangye
KIU.
Western Campus
Ishaka

Re: Submitted Protocol on: "Anti microbial activity of ocimum suave (willd) essential oils against uropathegens isolated from patients from selected Hospitals in Bushenyi District, Uganda" No.13/05-12

Reference is made to the above study protocol which was resubmitted to the Institutional Review Committee for reconsideration and approval.

It's noted that you have addressed all the concerns raised by the Committee at its sitting of 31st May 2012.

I am glad to inform you that your study has been approved for a period of one year up to June 21, 2013.

You are required to register the study with Uganda National Council for Science and Technology, and submit progress and end of study reports to MUST IRC.

You can now proceed with the rest of the research activities as per your work plan.

I wish you all the best.

Simon K. Anguma
CHAIRMAN- MUST IRC

cc Secretary –IRC

Appendix II: UNCST Approval Letter

Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

Our Ref: HS 1211

11th September 2012

Dr. Julius Tibyangye
Kampala International University
Western Campus
Bushenyi

Dear Dr. Tibyangye,

RE: RESEARCH PROJECT, "ANTIMICROBIAL ACTIVITY OF OCIMUM SUAVE (WILD) ESSENTIAL OILS AGAINST UROPATHOGENES ISOLATED FROM PATIENTS IN SELECTED HOSPITALS IN BUSHENYI DISTRICT, UGANDA"

This is to inform you that the Uganda National Council for Science and Technology (UNCST) approved the above research proposal on **25th July 2012.** The approval will expire on **25th July 2013.** If it is necessary to continue with the research beyond the expiry date, a request for continuation should be made in writing to the Executive Secretary, UNCST.

Any problems of a serious nature related to the execution of your research project should be brought to the attention of the UNCST, and any changes to the research protocol should not be implemented without UNCST's approval except when necessary to eliminate apparent immediate hazards to the research participant(s).

This letter also serves as proof of UNCST approval and as a reminder for you to submit to UNCST timely progress reports and a final report on completion of the research project.

Yours sincerely,

Leah Nawegulo
for: Executive Secretary
UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Appendix III: Office of the President Research Secretariat Clearance Letter

OFFICE OF THE PRESIDENT

PARLIAMENT BUILDING P.O. BOX 7168 KAMPALA, TELEPHONE: 254881/6, 343934, 343926, 343943, 233717, 344026, 230048, FAX: 235459/256143
Email: secretary@op.go.ug www.uganda2012.ug www.officeofthepresident.go.ug

ADM 154/212/01

September 5, 2012

The Resident District Commissioner
Bushenyi District

This is to introduce to you **Dr. Tibyangye Julius** a Researcher who will be carrying out a research entitled **"Anti Microbial activity of ocimum suave essential oils against uropathogens isolated from patients from selected hospitals in Bushenyi District, Uganda"** for a period of **01 (one) year** in your district.

He has undergone the necessary clearance to carry out the said project.

Please render him the necessary assistance.

By copy of this letter **Dr. Tibyangye Julius** is requested to report to the Resident District Commissioner of the above district before proceeding with the Research.

Alenga Rose
FOR: SECRETARY, OFFICE OF THE PRESIDENT

Copy to: Dr. Tibyangye Julius

MBARARA UNIVERSITY OF SCIENCE AND TECHNOLOGY
INSTITUTIONAL REVIEW COMMITTEE (MUST-IRC)

Appendix IV: Informed Consent Format

Study Title:

Antimicrobial Activity of *Ocimum suave* (Willd) Essential Oils against Uropathogens Isolated from Patients in Selected Hospitals in Bushenyi District, Uganda

Principal Investigator(s):

Julius Tibyangye

INTRODUCTION

What you should know about this research study:

- You are being asked to join this research study.
- This consent form explains the research study and your role in the study
- Please read it carefully and take your time to decide
- You are a volunteer. You can choose not to take part and if you join, you may quit at any time. There will be no penalty if you decide to quit the study

Purpose of this research

To determine the antibacterial properties of the essential oils from *Ocimum suave* (Willd) against uropathogens isolated from patients presenting at selected Hospitals in Bushenyi District, Uganda.

Why you are being asked to participate

You are being asked to participate because you reside and attend the in-and-out patient clinics in the selected hospitals (study areas) in Bushenyi District, Uganda.

Procedures

The target population is UTI patients attending the selected hospitals namely; Kampala International University-Teaching Hospital (KIU-TH), Ishaka Adventist Hospital, Comboni Hospital, Bushenyi HC IV, Kyabugimbi HC IV, and Bushenyi Medical Center (BMC).

Patient's assessment for signs and symptoms of UTIs, midstream clean catch urine specimens will be collected from patients using the systematic random sampling method, where every third patient assessed for signs and symptoms of UTIs by the attending Clinicians or Medical Officers and suspected to have UTIs will be referred for Midstream Clean Catch urine specimens collection, getting a total of 50 samples from each of the six study areas totaling to 300 samples.

Sample analysis, Isolation and identification of uropathogens, using calibrated loop technique, Antibiotic sensitivity testing by Kirby-Bauer disk diffusion technique, Extraction of Essential Oils-Hydrodistillation using a Clevenger apparatus, Screening for

Antibacterial Activity of Essential Oils using Agar well method, Potentiation effect will be obtained by calculating the fractional inhibitory concentration index (FICI) using formulae by (Tripti *et al.,* 2011).

Risks / discomforts

There are no risks involved in the study.

Benefits

The study will increase local and international knowledge on the types of uropathogens responsible for the UTIs in the area of study. It will also increase information to Clinicians, Medical Officers, and policy makers which assist them in making decisions for empirical treatment of UTIs and interpreting the trends and variations in new, emerging or developing levels of drugs resistance.

At the community level, the results of this study will provide scientific backing to the claims by the traditional medicine practitioners, who are using extracts from *O. suave* (Willd) to treat symptoms of UTIs, and if positive results are obtained, this could provide the starting point for development of an alternative treatment for UTIs in the local community.

Incentives / rewards for participating

There will be no rewards for participating but your participation will be highly appreciated.

Protecting data confidentiality

No names will be involved in the analysis of results. The only link between participants and the results will be via patient's identification numbers (ID No.).

Protecting subject privacy during data collection

Confidentiality will be observed at all levels of data collection and any questions raised will be answered accordingly. No names will be involved in the analysis of results. The only link between any participant and the results will be via patient's identification numbers (ID No.).

Right to decline / withdraw

Participants will be voluntarily recruited after consenting and they will be able to withdraw from the study at any time even after agreeing.

What happens if you leave the study?

Withdrawal from the study will not deny you of any benefits from the hospital.

Who do I contact if I have questions or a problem?

- **Contact for principal investigator**

  Julius Tibyangye
  Email: tibya2005@yahoo.com
  tibyangye@hotmail.co.uk
  Mobile: +256-782-683182,
  +256-703-798795

- **Contact for IRC office**

  Mr. Simon Anguma
  Chairman, MUST- IRC
  P.O. Box 1410, Mbarara
  Tel: 0485433795

What does your signature (or thumbprint/mark) on this consent form mean?

Your signature on this form means

- You have been informed about this study's purpose, procedures, possible benefits and risks
- You have been given the chance to ask questions and response given before you sign
- You have not waivered any of your human rights
- You have voluntarily made an informed decision to participate in this study

Print Name of adult participant	Signature of adult participant	Date
Print Name of person obtaining consent	Signature	Date
Thumbprint/mark		
Signature of witness	Date	

KAMPALA
INTERNATIONAL
UNIVERSITY
WESTERN CAMPUS

P.O.BOX 71 Bushenyi, Uganda
Tel: +256-485-443629
E-mail: admin@kiu.ac.ug
Website: www.kiu.ac.ug

FACULTY OF BIOMEDICAL SCIENCES
Department of Microbiology and Immunology

Appendix V: Okukushaba Kwetaba Omu Kucondooza Kwangye (Runyakitara)

Sebo/Nyabo

Nyowe Julius Tibyangye, Owa Kampala International University, Western Campus, ndiyo ninkora okucondoza aharurengo rwokujanjaara kw'obukooko burikukwata abarwire omubicweka byekyama. Omushaho waawe otwikiriize kukushaba kwenyigira omukucondoza oku obwo orikunyikiriza nkakwihaho enkari omumuringo gwobujunanisibwa reero tukabikyebera kureba okutwakuha ahabujanjabi obworikutunga. Okwenyigyira omukucondoza oku nokwabusha, kandi noikirizibwa kushazamu okwikiriza kwawe ohorahurire wayendera. Ebirarugye omukukyebera ebiturakwiheho nibiza kuhebwa omushaho waawe kugira ngu kimuyambe okukujanjaba.
Kworabe waikiriza kwenyigyira omukucondoza oku, noshabwa kuteeka omukono gwawe neinga ekinkumu aheifo.

Okwikiriiza
Nyowe nayetegyereza okushoborora nebigyendererwa byokucondoza okwagambwaho omururiimi orundikumanya nebirungi ebukwakubaasa kureeta ohantura yabantu bomukikweeka nyeitu kandi
Naikiriza kwenyigyira omukucondoza oku.

Ninye

_____	_____
Signature of Participant/ Ekinkumu kyomurweire	Date/ Ebiro
_____	_____
Owabariho (Witness)	Date/ Ebiro
_____	_____
Signature of Researcher/ Omucondozi	Date/ Ebiro

Appendix VI: Urinary Tract Infection Assessment Form

Name: _____ Date of Birth: _____
OPD/IPD No._____ Date: _____
Age: _____ Sex: _____

1. Please circle the UTI symptoms you're experiencing.
   A. Frequency: How many times an hour do you urinate? _____
   B. Dysuria: (Burning or pain on urination)
   C. Hematuria: (Blood in urine)
   D. Urgency: (sudden need to urinate)
   E. Nocturia: (awakening during sleep to urinate)
   How many times during your sleep? _____
   F. Incontinence: (loss of control)
   G. Back pain: If yes, right side, left side or both? _____
   H. Fever: If yes, highest temp_____ for how many days? _____
2. How long (days) have you had these symptoms? _____
3. Have you had a previous urinary tract infection (UTI)? Yes No
   If yes, more than 2 per year? Yes No
   Please list medication taken for past UTI: _____
   _____
4. Have you ever had an infection of the kidney? Yes No
5. Have you taken any medication for current symptoms? Yes No
   List all prescription, over the counter medication, or herbs that you have taken in the last 2 days:
   _____
   _____

6. Females only: when did your last menstrual cycle begin? _____
7. Are you sexually active? Yes No
   If yes, when did you last have sex? _____

Tests ordered

Urinalysis results: Color: _____ Turbidity: _____ pH: _____ Sp. Gr.:_____
Uristrips Results: _____
Microscopy Results: _____
C&S Results:
_____
_____

Treatment Plan

_____
_____
Physician/Nurse Practitioner Signature

Appendix VII: Map of Bushenyi District showing the study area

Source:http://www.bushenyi.go.ug/index.php?option=com_content&view=article&id=88&Itemid=109

Appendix VIII: Antimicrobial sensitivity test zone diameter interpretive standards

Zone diameter interpretive standards for selected antimicrobial agents used against *Enterobacteriaceae* and other gram negative bacteria (CLSI, 2007)

Test condition Medium: Mueller – Hinton agar Inoculum: Equivalent to a 0.5McFarland standard. Incubation: 35 - 37^{o}C; Ambient air; 16 – 18 hour					Minimum QC recommendations *Escherichia coli* ATCC^{R}25922
Antimicrobial agent	**Disk Content**	**Zone diameter, nearest whole mm**			**Comment**
		R	I	S	
Ampicillin	10ug	≤ 13	14 - 16	≥ 17	*Class representative for Ampicillin and Amoxicillin*
Ciprofloxacin	5ug	≤ 15	16 - 20	≥ 21	
Nitrofurantoin (urine)	300ug	≤ 14	15 - 16	≥ 17	
Trimethoprim-sulfamethoxazole	25ug	≤ 10	11 – 15	≥16	

Zone diameter interpretive standards for selected antimicrobial agents used against *Staphylococcus* spp. (CLSI, 2007)

Test condition Medium: Mueller – Hinton agar Inoculum: Equivalent to a 0.5McFarland standard. Incubation: 35 - 37^{o}C; Ambient air; 16 – 18 hour, 24hrs for Oxacillin, methicillin, Nafcillin and Vancomycin.	Minimum QC recommendations *Staphylococcus aureus* ATCC^{R}25923

Antimicrobial agent	**Disk Content**	**Zone diameter, nearest whole mm**			**Comment**
		R	I	S	
Ampicillin	10ug	≤ 13	14 - 16	≥ 17	*Class representative for Ampicillin and Amoxicillin.*
Ciprofloxacin	5ug	≤ 15	16 - 20	≥ 21	
Nitrofurantoin (urine)	300ug	≤ 14	15 - 16	≥ 17	
Ofloxacin	5ug	≤ 12	13 - 15	≥ 16	
Trimethoprim-sulfamethoxazole	25ug	≤ 10	11 – 15	≥16	