Introduction
1.0. INTRODUCTION

1.1. Descriptions about UTI

Infectious disease is the number one cause of death accounting for approximately one-half of all deaths in tropical countries. Perhaps it is not surprising to see these statistics in developing nations, but what may be remarkable is that infectious disease mortality rates are actually increasing in developing and developed countries. Death from infectious disease, ranked 5th in 1981, had become 3rd in 1992, with an increase of 58% (Pinner et al., 1996). It is estimated that, 8% of the death in USA is caused by infectious disease (Pinner et al., 1996). This is alarming number was once believed that would be eliminated by the end of the millenium. The increases are attributed to increases in respiratory tract infections and HIV/AIDS. Other contributing factors are an increase in antibiotic resistance in nosocomial, urinary tract infections and community acquired infections. Furthermore, the most dramatic increases are occurring in the 25-44 year old age group (Pinner et al., 1996). These negative health trends called for a renewed interest in infectious disease in the medical and public health communities and strategic plans must be advocated on the treatment and control of the disease. Proposed solutions are multi-pronged approach that includes prevention, (such as vaccination, improved monitoringsystems and
development of newer drugs for effective treatment. This development of newer antimicrobial drugs should be given high importance to tackle present serious situation (Fauci, 1998).

The urinary system consists of the kidneys, ureters, bladder, and urethra. The key elements in the system are the kidneys, a pair of purplish-brown organs located below the ribs toward the middle of the back. The kidneys remove excess liquid and wastes from the blood in the form of urine, keep a
keep a stable balance of salts and other substances in the blood, and produce a hormone that aids the formation of red blood cells. Narrow tubes called ureters carry urine from the kidneys to the bladder, a sack-like organ in the lower abdomen. Urine is stored in the bladder and emptied through the urethra. The average adult passes about a quart and a half of urine each day. The amount of urine varies, depending on the fluids and foods a person consumes. The volume formed at night is about half that formed in the daytime (Guyton, 1991; Ganong, 1999).

1.2 What are the causes of UTI?

Normally, urine is sterile. It is usually free of bacteria, viruses, and fungi but does contain fluids, salts, and waste products. An infection occurs when tiny organisms, usually bacteria from the digestive tract, cling to the opening of the urethra and begin to multiply. The urethra is the tube that carries urine from the bladder to outside the body. Most infections arise from one type of bacteria, *Escherichia coli* (*E. coli*), which normally lives in the colon. In many cases, bacteria first travel to the urethra. When bacteria multiply, an infection can occur. An infection limited to the urethra is called urethritis. If bacteria move to the bladder and multiply, a bladder infection, called cystitis, results. If the infection is not treated promptly, bacteria may then travel further up the ureters to multiply and infect the kidneys. A kidney infection is called pyelonephritis. Microorganisms called *Chlamydia*
and *Mycoplasma* may also cause UTIs in both men and women, but these infections tend to remain limited to the urethra and reproductive system. Unlike *E. coli*, *Chlamydia* and *Mycoplasma* may be sexually transmitted, and infections require treatment of both partners. The urinary system is structured in a way that helps ward off infection. The ureters and bladder normally prevent urine from backing up toward the kidneys, and the flow of urine from the bladder helps wash bacteria out of the body. In men, the prostate gland produces secretions that slow bacterial growth. In both sexes, immune defenses also prevent infection. But despite these safeguards, infections still occur. Some people are more prone to getting a UTI than others. Any abnormality of the urinary tract that obstructs the flow of urine (a kidney stone, for example) sets the stage for an infection. An enlarged prostate gland also can slow the flow of urine, thus raising the risk of infection. A common source of infection is catheters, or tubes, placed in the urethra and bladder. A person who cannot void or who is unconscious or critically ill often needs a catheter that stays in place for a long time. Some people, especially the elderly or those with nervous system disorders who lose bladder control, may need a catheter for life. Bacteria on the catheter can infect the bladder, so hospital staff should take special care to keep the catheter clean and remove it as soon as possible. People with diabetes have a higher risk of a UTI because of changes in the immune system. Any other
disorder that suppresses the immune system raises the risk of a urinary infection.

UTIs may occur in infants, both boys and girls, who are born with abnormalities of the urinary tract, which sometimes need to be corrected with surgery. UTIs are rare in boys and young men. In adult women, though, the rate of UTIs gradually increases with age. Scientists are not sure why women have more urinary infections than men. One factor may be that a woman’s urethra is short, allowing bacteria quick access to the bladder. Also, a woman's urethral opening is near sources of bacteria from the anus and vagina. For many women, sexual intercourse seems to trigger an infection, although the reasons for this linkage are unclear. According to several studies, women who use a diaphragm are more likely to develop a UTI than women who use other forms of birth control. Recently, researchers found that women whose partners use a condom with spermicidal foam also tend to have growth of \textit{E. coli} bacteria in the vagina (Harry \textit{et al.}, 1996; West, 2006).

\subsection*{1.2.1 Recurrent infections}

Many women suffer from frequent UTIs. Nearly 20 percent of women who have UTI will have another infection, and 30 percent of those will have yet another. Of the last group, 80 percent will have recurrences.
1.2.2 Other contributing factors of UTI

Normally, human system has its own defense mechanisms against UTI. For instance, Urine has specific types of proteins that prevent attachment of bacteria such as *E. coli* to the urinary tract. As age advances urine excretes lesser quantity of this proteins and therefore increase the risk of UTI. Some medicines and diseases alter the chemical composition of the urine for example increased glucose in the urine due to diabetes increase the growth of the bacteria in the urinary tract. However, risk factors vary with age, sex and presence of some diseases. In infants and children, who have not had circumcision are at a higher risk of developing UTI because intestinal bacteria can easily grow under the prepuce of the penis. Women who clean their external genitalia from behind increase the risk of the intestinal bacteria to enter the urethra. In adult men, *E. coli* infections are more common among homosexuals than heterosexuals.

The increased risk of UTI among elderly people is due to (a) Obstruction to the passage of urine due to prostrate enlargement and increased insertion of instrument in the urethra. (b) Decreased secretion of the prostrate gland. These secretions prevent growth of the bacteria. (c) Decreased protein in urine which prevents attachment of the bacteria to the lining of the urinary tract system. (d) Increased soiling around the anus in
women. Moreover, People with blood group ‘A’ and some with “AB” have a higher tendency for bacterial attachment in their urinary tract. Urinary tract stones increase the risk of infections because of Obstruction to the flow of urine increased opportunity for the bacteria to grow, irritation of the lining of the urinary tract, which allows attachment of the bacteria to the lining and persistent focus of the bacteria. Other causes are injury to the urethra or bladder due to sexual intercourse infection of the vulva and vagina and inflammation of the urethra and bladder. Men who have acute bacterial infections of the prostrate gland are likely to have symptoms of urinary tract infection.

1.3 What are the Symptoms of UTI?

Not everyone with a UTI has symptoms, but most people get at least some symptoms. These may include a frequent urge to urinate and a painful, burning feeling in the area of the bladder or urethra during urination. It is not unusual to feel bad all over—tired, shaky, washed out—and to feel pain even when not urinating. Often women feel an uncomfortable pressure above the pubic bone, and some men experience fullness in the rectum. It is common for a person with a urinary infection to complain that, despite the urge to urinate; only a small amount of urine is passed. The urine itself may look milky or cloudy, even reddish if blood is present. Normally, a UTI does not cause fever if it is in the bladder or urethra. A fever may mean that the
infection has reached the kidneys. Other symptoms of a kidney infection include pain in the back or side below the ribs, nausea, or vomiting.

In children, symptoms of a urinary infection may be overlooked or attributed to another disorder. A UTI should be considered when a child or infant seems irritable, is not eating normally, has an unexplained fever that does not go away, has incontinence or loose bowels, or is not thriving. Unlike adults, children are more likely to have fever and no other symptoms. This can happen to both boys and girls. The child should be seen by a doctor if there are any questions about these symptoms, especially a change in the child's urinary pattern. In addition to the symptoms of lower and upper UTI causes high fever, chills, rigour and pain in the loin. Vomiting may also be present. UTI may be present without any symptoms and are detected accidentally when the urine is tested. When symptoms are absent, the infection is called asymptomatic. This is commonly seen in individuals above age of 65 years and 5-10% pregnant woman.

1.4 How is UTI Diagnosed?

To find out whether you have a UTI, your doctor will test a sample of urine for pus and bacteria. You will be asked to give a "clean catch" urine sample by washing the genital area and collecting a "midstream" sample of urine in a sterile container. This method of collecting urine helps prevent
bacteria around the genital area from getting into the sample and confusing
the test results. Usually, the sample is sent to a laboratory, although some
doctors' offices are equipped to do the testing. In the urinalysis test, the urine
is examined for white and red blood cells and bacteria. Then the bacteria are
grown in a culture and tested against different antibiotics to see which drug
best destroys the bacteria. This last step is called a sensitivity test. Some
microbes, like Chlamydia and Mycoplasma, can be detected only with special
bacterial cultures. One could suspect this type of infection when a person has
symptoms of a UTI and pus in the urine, but a standard culture fails to grow
any bacteria. When an infection does not clear up with treatment and is
traced to the same strain of bacteria, it is necessary to carry out some more
tests to determine whether patient's system is normal or not. One of these
tests is an intravenous pyelogram, which gives X-ray images of the bladder,
kidneys, and ureters. An opaque dye visible on X-ray film is injected into a
vein, and a series of X-rays is taken. The film shows an outline of the urinary
tract, revealing even small changes in the structure of the tract. In case of
recurrent infections, it is normally advised to perform an ultrasound
examination, which gives pictures from the echo patterns of sound waves
bounced back from internal organs. Another useful test is cystoscopy. A
cystoscope is an instrument made of a hollow tube with several lenses and a
light source, which allows the doctor to see inside the bladder from the.
1.5 How is UTI Treated?

UTIs are treated with antibacterial drugs. The choice of drug and length of treatment depend on the patient's history and the urine tests that identify the offending bacteria. The sensitivity test is especially useful in helping the doctor select the most effective drug. The drugs most often used to treat routine, uncomplicated UTIs are trimethoprim (Trimpex), trimethoprim/sulfamethoxazole (Bactrim, Septra, Cotrim), amoxicillin (Amoxil, Trimox, Wymox), nitrofurantoin (Macroductin, Furadantin), and ampicillin (Omnipen, Polycillin, Principen, Totacillin). A class of drugs called quinolones includes four drugs approved in recent years for treating UTI. These drugs include ofloxacin (Floxin), norfloxacin (Noroxin), ciprofloxacin (Cipro), and trovafloxin (Trovan).

1.6 History of herbs used in Medicine

Historically, plants have provided a source of inspiration for novel drug compounds since plant derived medicines have made major contributions to human health and well-being from ancient days. There are numerous examples of plant derived drugs. The isoquinoline is an alkaloid emetine obtained from the underground part of *Cephaelis ipecacuanh*. Related species of this plant has been used as amoebicidal drug as well as for the treatment of abscesses due to the spread of *Entamoeba histolytica* infections for
many years. Another important drug of plant origin with a long history of use is quinine. This alkaloid occurs naturally in the bark of Cinchona tree. Apart from its continued usefulness in the treatment of malaria, it can be also used to relieve nocturnal leg cramps. Currently, the widely prescribed drugs are analogs of quinine such as chloroquine. Some strains of malarial parasites have become resistant to the quinines; therefore anti-malarial drugs with novel mode of action are required.

Similarly, some other plants have made important contributions beyond anti-infective nature, such as cancer therapies. Early examples include the antileukaemic alkaloids vinblastine and vincristine. Both alkaloids are obtained from the Madagascan periwinkle (Catharanthus roseus syn. Vinca roseus) (Nelson, 1982). Other cancer therapeutic agents of plant origin include taxol, homoharringtonine and several derivatives of camptothein. A well-known benzylisoquinoline alkalioid, papaverine, has been shown to have a potent inhibitory effect on the replication of several viruses including cytomegalovirus, measles and HIV (Turano et al., 1989). Most recently, three new atropisomeric naphthylisoquinoline alkaloid dimers, michellamines A, B, and C were isolated from a newly described species tropical liana Ancistrocladus korupensis from the rainforest of Cameroon. The three compounds showed potential anti-HIV among which michellamine B showed most potent effect against HIV. These compounds
are capable of inhibiting the cytopathic effects of HIV-1 and HIV-2 on human lymphoblastoid target cell *in vitro* (Boyd *et al.*, 1994).

First generation of plant drugs were usually employed in more or less in their crude form. Several effective medicines used in their natural state such as cinchona, opium, belladonna and aloe were selected as therapeutics agents based on empirical evidence of their clinical application by traditional societies from different parts of the world. Following the industrial revolution, a second generation of plant based drugs emerged based on scientific processing of the plant extracts to isolate “their active principles.”

The second-generation phytopharmaceutical agents were pure molecules and some of the compounds were even more pharmacologically active than their synthetic counterparts. Notable examples were quinine from *Cinchona*, reserpine from *Rauvolfia*, and more recently taxol from *Taxus* species. These compounds differed from the synthetic therapeutic agents only in their origin. They followed the same method of development and evaluation as other pharmaceutical agents. The sequence of development of pharmaceuticals usually begin with the identification of active constituent, detailed biological assays, and formulation of dosage followed by several phases of clinical studies designed to establish safety, efficacy and
pharmacokinetic profiles of the new drug. Possible interaction with food and other medications may be investigated from the clinical trials.

In the development of “Third Generation” phytotherapeutic agents a top to bottom approach is usually adopted. This consists of conducting a clinical evaluation of the treatment modalities first and therapy as administered by traditional doctors or as used by the community as folk medicine. This evaluation is then followed by acute and chronic toxicity in animals including cytotoxicity studies whereever applicable.

Formulation and trial production of the dosage forms are structured to mimic the traditional use of the herb. Careful attention being paid on the stability of the finished product and formulation of the final dosage form. This is a unique blend of the empiricism of the earlier first generation botanicals with the experimental research used to prove the efficacy and safety of second generation isolated pure compounds. Several pharmaceuticals companies are engaged in the development of natural product drugs through the isolation of the so-called active molecules from plant extracts.

To day it is estimated that plant materials have provided the sources of 50% Western drugs (Robbers, 1996). Many commercially proven modern drugs were initially used in crude form in traditional or folk healing
practices, or for other purposes that suggested potentially useful biological activity. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and at more affordable costs.

Much of the exploration and utilization of natural products such as antimicrobials arise from microbial sources. It was the discovery of penicillin that led to later discoveries of antibiotics such as streptomycin, aureomycin and chloromycetin (Trease and Evans, 1972). Though most of the clinically used antibiotics are produced by soil microorganisms or fungi, higher plants have also been a source of antibiotics (Trease and Evans, 1972). Some of the typical examples of these are the bacteriostatic and antifungicidal properties of Lichens, the antibiotic action of allinone in Allium sativum (garlic), or the antimicrobial action berberines in goldenseal (Hydrastis canadensis) (Trease and Evans, 1972) etc. Plant based antimicrobials represent a vast untapped source for medicines. Continued effort on the further exploration of plant antimicrobials would be of high value to meet the challenges of the future. In this direction plants based antimicrobials have enormous therapeutic value. They are very effective in the treatment of many infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. They are effective and harmless in most instances. Many active ingredients of plant materials have tropisms to
specific organs or systems in the body. Some phytomedicines usually have multiple effects on the body. Their actions often associated beyond the symptomatic treatment of disease. A typical example of this type of action is reported in *Hydrastis Canadensis*. This plant not only possesses antimicrobial activity, but also increases blood supply to the spleen promoting its ability to release mediating compounds (Murray, 1995).

There has been a renewed interest in natural products worldwide. This interest created the consumer’s belief that natural products are superior to conventional medicines. The phenomenal changes in many aspects newer drug discovery, its result oriented efficacy, more liberal advertising, laws permitting to sell these products, and national concern for health care cost provided impetus to the industry of herbal medicine worldwide. Sale herbal products in the market have increased considerably in the last decade. Sales of these products have reached have reached $3.1 billion of the $10.4 billion dollar dietary supplement industry 1996 in the United States (Iwu *et al.*, 1999). The industry anticipates a growth of 15-20% in the years to come (Johnston, 1997). This growth rate will be maintained in this industry that is still considered as its infancy. Many plants that were previously considered wild crafted will be grown domestically to meet the consumer demand. This makes many opportunities open for the cultivation of crops for this industry, modern drug development and latest treatment for many incurable diseases.
The need for plant based antimicrobials is well documented in market of herbal products. In a review undertaken clearly delineates that the plants are used as anti-infectives and the primary plant used as an antimicrobial is *Hydrastis*. These anti-infective agents make up 24% of the pharmaceutical market (Gruenwald, 1997). *Hypericum* is used as an antiviral but primarily used for its anti-depressant activity. Until 1995 it was not among the top selling herbs, but it had become an overnight success in 1997 with sale increasing over 20,000% in the mass market. The meteoric increase in the sales of *Hypericum* is multi-factorial, but the primary factor for its popularity was the existence of an unexploited market opportunity. Approximately 21% of drugs sold in 1994 were drugs affecting the central nervous system mostly prescribed for depression. During this period of time, none of top selling herbs had primary indication for depression. This market hole, coupled with the media exposure produced a market success. There are many market holes exist. When a similar analogy applied for antimicrobial agents, there are very prospects for anti-infectives in the same light as *Hypericum* analogy.

Again *Hydrastis*, one of the top selling antimicrobials in the US herbal market, represents an example of a herb that has undergone domestication. Originally this plant, native to eastern North America, was wild crafted. *Hydrastis*, has been used by Native Americans for many conditions, including as an antimicrobial for infections. Efforts to cultivate this plant
were undertaken in order to supply the demands of the herbal products market and to battle its threatened extinction.

It is vital to be in the position to capitalize on the phytomedicine market, providing environmentally responsible solutions to public health concerns presented by new trends in infectious disease. In order to be prepared, the industry must be able to sustainably harvest and supply the herbal market. That means we must be able to anticipate the market needs and develop products to satisfy this market.

1.7. Descriptions about mangroves

Mangroves are defined as an assemblage of tropical trees and shrubs that inhabit the coastal intertidal zone. A mangrove community is composed of plant species whose special adaptations allow them to survive the variable flooding and salinity stress conditions imposed by the coastal environment. Therefore, mangroves are defined by their ecology rather than their taxonomy. From a total of approximately 20 plant families containing mangrove species world wide, only two, Pellicieraceae and Avicenniaceae are composed exclusively of mangrove. In the family Rhizophoraceae, for example only four of its sixteen genera live in mangrove ecosystems (Duke et al., 1998)
Mangroves worldwide cover an approximate area of 240000 km² of sheltered coastlines (Lugo and Senedecker, 1974). They are distributed within the tropics and subtropics, reaching their maximum development between 25° N and 25°S. Their latitudinal distribution is mainly restricted by temperature since perennial mangrove species generally cannot withstand freezing conditions. As a result, mangroves and grass dominated marshes in middle and high latitudes fill a similar ecological niche. The global distribution of mangroves is divided into two hemispheres, Atlantic East Pacific and the Indo West Pacific. The Atlantic East Pacific has 12 species compared to 58 in Indo West Pacific hemispheres. Species composition is also very different between the two hemispheres.

Mangroves are salt tolerant forest eco-system found mainly in tropical and sup-tropical regions of the world. They are trees or shrubs that have the common trait of growing in shallow and muddy salt water or brackish water especially along quiet shorelines and salt - water tolerance. The mangrove plants somewhat prefer a daily tidal wash while others find their optimum conditions in shallow areas subject to occasional high tides. This process governs their distribution. Mangroves do not appear on sandy beaches and rocky shores. A muddy substratum of varying depth and consistency is necessary for their growth.
Mangrove eco-system provides unique and valuable range of resources. The collective noun of mangrove designates a tidal wetland eco-system formed by a very special association of plants and animals that live in the inter-tidal areas of low lying tropical latitudes. It also used to designate halophytic marine tidal ground forms and grasses. They are one of the easiest tropical forest types to generate because of their reproductive biology and adaptations to inter-tidal conditions. Mangrove plants are the source of organic nutrients for propagation of planktonic organism. It is the feeding and breeding ground for crusteacean, Molluscs, Shrimps and other marine organisms. It is also called “Cradle of Prawn”. It protects the seashore from tidal erosion (Lugo and Senedecker, 1974).

Mangrove can be classified into three categories, firstly true mangrove are mainly restricted to inter tidal areas between the high water levels of neap and spring tides. Plant species from true mangrove belong to at least different families. About 20 species make a significant contribution to the structure of mangrove forest. The species composition and structure of mangrove forest varies as a function of geophysical, geographical, hydrographic, biogeographically, climatic and edaphic factors and the environmental conditions. *Rhizophora* species occur in all three regions. Secondly minor species of mangroves are distinguished by their inability to form conspicuous elements of the vegetation and they rarely form pure communities. The third category, the mangal associates are not found
exclusively in the proximity of mangroves and may occur only in transitional vegetation, landwards, and seawards. However, they do interact with true mangroves. These are salinity tolerant plant species such as Terminalia, Hibiscus, Thespesia, Ficus, Chlophyllum, Casuarina. Some legumes and milk weeds (Asclepiadaceae and Apocyanaceae). It is a common feature of tropical estuarine brackish water bordered by mangroves, that the standing stock of phytoplankton is dense in the lower reaches where it is dominated by diatoms, especially those of the genera Coscinodiscus, Pleurosigma and Biddulphia. The leaf fall from the mangrove trees also contributes substantially to formation of detritus, which support coastal fisheries.

Mangrove plants are rich sources of saponins, alkaloids, and flavonoids. Mangrove forests have great potential for medicinal uses. Materials from different species can be used to treat many diseases especially urinary tract infection, sore throat, constipation, fungal infections, fever, kidney stones, rheumatism and dysentery. Some of the bioactive substances obtained from these plants also possess antifungal, antibacterial, and pesticidal properties (Bandaranayaka, 1998). However, no systemic study have been carried out to find out the bioactive principle available in mangrove plants and its effectiveness in the treatment of urinary tract infection. Hence, a systemic approach was made to extract bioactive compounds from selected mangrove plants and their antibacterial activity on antibiotic resistant bacterial species isolated from urinary tract infections.