1.1. Introduction

Urinary tract infections (U.T.Is) represent one of the most frequently registered cases of malady daily in hospitals of Diwaniya and the remaining provinces of Iraq. It is as such in other countries all over the world. These infections are caused by different pathogens like viruses, chlamydia and bacteria. *Proteus* species stands as the important bacteria that causes (U.T.I).*Proteus* spp. is one of the components of the normal flora within the intestine as saprophytes, and so it dose not carry any risk to the health of human as long as it is in the intestine. But it can be one of the opportunistic pathogens when it is carried to the urinary tract to cause U.T.I. which is considered one of the leading causes for renal stone formations [1, 2].

Proteus spp. becomes resistant to many antibiotics that were commonly used for the treatment of U.T.I [3], such as the resistance to beta-lactam aminoglycoside and other types of antibiotics. The abuse of antibiotics is the main cause for the appearance of resistant generation of *Proteus* spp. which leads to non effective treatment for U.T.I [4]. This resistance is probably gained by possessing the ability to change the permeability of their membrane or by producing enzymes like beta-lactamase which destroys the beta-lactam antibiotics. Many studies reveal that the probable cause for the increase in the prevalence of resistance to beta-lactam antibiotics are the hereditary factors by genes that are transmitted for bacterial generation to succeeding generations [5].

It is also noted that there is an increase in the resistance toward the new antibiotics like cephalosporin due to the production of beta-lactamase with a broad spectrum activities [6].

Researchers working in the field of medicine pay more attention to the presence of multi-drug resistant *Proteus* spp., since it leads to serious clinical and public health problem of U.T.I. that could lead to serious complications as renal failure and death. The ability of *Proteus* spp. to induce U.T.I. could be due to many factors as an example; *Proteus mirabilis* produces urease, hemolysin, and proteins of the outer membrane, proteolytic enzymes that have the ability of fimbria and invasion of the cells lining of the urinary tract [7].

Urease is one of the main factors of virulence since it's production by *Proteus mirabilis* can lead to the formation of urinary calculi in patients with U.T.I. also it can lead to damage and necrosis of renal tissues[8].

Many measures were taken to remove urinary stones: either by surgical operations or by destruction of these stones by ultrasounds waves or laser or by chemicals. To each of these measures there are pitfalls and side effects in addition to the probability of the recurrence of stones and infections. The empirical choice of an effective treatment is becoming more difficult as urinary pathogens are increasingly resistant to commonly used antibiotics [9].

Consequently it is necessary to prepare a series of a novel (urea, thiourea and Schiff bases) derivatives compounds, most of these new derivatives have antimicrobial activity against gram-negative bacteria especially against *Proteus* spp., because they act as urease inhibitors in order to compact U.T.I. and urolithiasis.

In order to understand the occurrence and development of U.T.I. many experiments were employed on laboratory animals by researchers to induce U.T.I. in such animals through mechanical manipulation inside the urinary tract such as introducing foreign bodies inside the urinary bladder [10], or by surgical operation legating the ureter [11].

On the other hand, few studies on the urease of *Proteus* spp. in U.T.I. and urolithiasis .In a study of extraction and characterization of urease of *Proteus* spp. isolates from patients with U.T.I. [12], showed that 1 out of 67 isolates of *Proteus* spp. was multi-resistant to the commonly used antibiotics. While in study [13] the synthesis of urea analogues and application of these compounds as urease inhibitor which is an *in vitro* study, reveal of the presence of antimicrobial activity of these compounds on laboratory measures. But no study was done to explore the effect of a new heterocyclic compounds on the stone formation and U.T.I. due to infection of *Proteus* spp.

The aim of this study is to study the effect of new heterocyclic compounds (urea or thiourea and Schiff bases) which are considered urease inhibitors with antimicrobial activity to treat urolithiasis in U.T.I. caused by *Proteus* spp., and the relation of this infection with some biochemical parameters (Microbial-Chemistry study).

The steps of the study are as follows:-

1. Isolation and identification of *Proteus* spp. from patients with U.T.I. and urinary stones, using routine cultural techniques.

2. Preparation and studying the physical properties of new compounds (urea, thiourea derivatives, and Schiff bases). T.L.C. technique was used to examine the formation and purity of these compounds that act by competitive inhabitance on urease.

3. Studying the sensitivity of the isolated *Proteus* spp. toward commonly used antibiotics and the prepared compounds *in vitro*.

4. Conducting a biochemical investigation on patients with U.T.I. and urinary calculi with a control group for statistical comparison. Those investigations include (Blood Urea, Uric Acid, Random Blood Sugar, Serum Creatinine and Serum Calcium).

1.2. Review of Literature

1.2.1. Description of Genus *Proteus*

1.2.1.1. Historical prospective

Genus *Proteus* has been discovered for the first time by Hauser in 1885, and has isolated from stool, waste water and decompose organic matters, and he has called it *Proteus* because this kind of bacteria has a pleomorphism property [14, 15].

Genus *Proteus* belongs to group five according to Bergy's classification in 1994 [16]; this group includes a set of bacteria which is called Gram- negative bacteria (Entrobacteriaceae) and known as proteeae [17]. According to modern classification depending upon the biochemical reaction, the genus of *Proteus* includes five species: *P.mirabilis, P.vulgaris, P.penneri, P.myxofaciens and P.hauseri* [14].

Proteus myxofaciens have isolated from Gypsy moth larval (porthertria dispar), it is not known yet whether there is a relation between it and the infection to human [14], while other kinds could be isolated from clinical specimens.[18,19].

Proteus bacteria have a characteristic which can not form spores and do not contain any capsule, but it has the ability to produce urease, and phenylalanine deaminase [18] as well. It is also considered a producer for hydrogen sulfide gas (H_2S) when it grows on triple sugar iron agar, which is negative oxidase test. While it is positive to catalase test and the methyl red test, All types of *Proteus* give a negative result to indole test, except *P.vulgaris*. [18]

Proteus bacteria colonies appear to be in a pale yellow color in a media of MacConkey agar, because it does not fermentate lactose sugar. But it fermentates galactose, sucrose and glucose [16, 20], and it does not produce beta-galactosides enzyme in ONPG test [18, 21].

Proteus bacteria has peritrichous flagella which enables it to move so fast and this so clear on the culture media as blood agar and in a form just like circle of the same center and like waves, this is known as a swarming phenomenon [22-24]. It represents a unique characteristic for only *Proteus* spp. in Enterobacteriaceae, but in using MacConkey agar which prevents the *Proteus* characteristic swarming motion because MacConkey agar contains bile salts [25].

The motion of *Proteus* has a major role in Urinary Tract Infection, so *Proteus* bacteria invades urinary tract via the fast motion of their flagella which increases the pathogenic of *Proteus* and enables it to ascend to the top of urinary system and consequently builds a colonies [23, 24, 26].The optimum temperature for *Proteus* bacterial growth is 37°C, and best pH is 7.4 [27].

1.2.1.2. Clinical significance of *Proteus*

Proteus is considered an opportunistic microorganism that belongs to Entrobacteriaceae for its effect on health of human and animals [28, 29]. It causes gastrointestinal infection and genitourinary infection and its clinical significance comes after *E.coli*, *Klebsiella pneumoni* as they cause U.T.I. [30, 31]. It has been isolated from urinary tract, wounds, skin, eyes, ears, nose, and larynx and from alimentary tract [32].

Proteus spp. causes U.T.I. in elderly patients [30], as it constitutes 15 % from bacterial infection of the urinary system [31, 33, 34], in addition to other surgical operations [35].

Proteus spp. also causes nosocomial infections [36], it was clear 50 years ago that ways of prevention of such infections inside hospital was considered a great difficulty because of emerging resistant strains of bacteria [37]. Spread of infection is either endogenous that is present on the skin, intestine and pulmonary systems from

the patients in hospital or exogenous by medical staff, tools used in instrumentations like catheterization [38].

Chane found that 70.4 % of the infection sources are tools and contaminated instruments with *Proteus* [39]. *Proteus mirabilis* constitutes about 70-90 % of the infection by other class of *Proteus* spp.[40].While *Proteus vulgaris* causes about 25 % , then other strains constitute (5-8) % and the urinary system is considered the most common site for infection with this bacteria and especially in women as it causes (10-20) % of infection [41].

Ankler [42], shows that urinary system infections caused by *Proteus* occur by the movement of these bacteria upward through its ability to move fast by flagella and this is called (ascending infection), and the infection may be complicated to be pyelonephritis , and in some cases these bacteria may spread in the blood stream causing septicemia[43].

This bacteria has many virulence- factors that increase the percentage of infection, as its presence in urine, rapid growth rate, and fast movement in addition to many factors that increase the health problems especially in elderly and causes infections, renal stones, bladder stones, renal failure and urinary tract obstruction [44]. It is thought that *Proteus* has an important role in rheumatoid arthritis as antibodies for the bacteria found in those patients [45- 47].

1.2.1.3. Virulence factors

Proteus have many virulence factors that help in destruction of host's defense mechanism due to the ability of secreting many enzymes and toxins in addition to have certain surface structures increasing their virulence [43]:

1.2.1.3.1. Urease production

The *Proteus* bacteria have the ability to increase the pH of urine by secreting urease that splits urea to ammonia (NH_3) and carbon dioxide (CO_2) that precipitate magnesium ion (Mg^{+2}) and calcium ion (Ca^{+2}) which are present in urine [20]. Struvite [$MgNH_4$ $PO_4.6H_2O$], is considered the main constituent of the urinary stones [48], in addition to the stones that are rarely found of apatite carbonate [Ca_{10} $(PO_4)_6.CO_3$], [49].

The increase of the pH of the urine 7 to 9 increases the average of stone formation [50, 51], and the absorption of the evolved ammonia from the decomposition of urea by urease may lead to increase the ammonia concentration in blood (hyperammonemia), and may cause death to the patient [52].

1.2.1.3.2. Hemolysin production

Proteus bacteria can secrete hemolysin that destructs RBC and is one of factors that help in invasion [53]. Hemolysin performs many functions, one of them making pores in both RBC membranes (erythrocytes) and epithelial cells, and due to its cytotoxicity, it may lead to tissue damage [26].

Hemolysin is produced from many gram-negative bacteria, such as *E.coli, Proteus,* and gram-positive bacteria as well, such as *Staphylococcus* [54].

Senior and Hughs have indicated that there is a relationship between the virulence of *Proteus mirabilis* that causes U.T.I. and hemolysin production [54].

1.2.1.3.3. Flagella and swarming motility

A *Proteus mirabilis* posse more than 100 peritrichous flagella [55]. These flagella are responsible for the movement of bacteria and facilitate its adherence to the cells forming the outer surface of different tissues. Then after penetration of these tissues and it initiates its pathogenicity [15, 26]. These flagella also give the *Proteus* spp. one of its features which are the swarming phenomena [24].

1.2.1.3.4. Fimbrial and adherence ability

These bacteria can adhere to uroepithelial cells and the presence of fimbria in large numbers on bacterial surface cause severs infection leading to pyelonephritis (inflammation of its pelvis and kidney parenchyma) due to bacteruria [56-58].

1.2.1.3.5. Lipopolysaccharide-O-antigen endotoxin

This is one of gram negative virulence factor that help in activities of bacterial antibodies present in serum like phagocytes [59]. LPS consist of O-specific sequence, core zone and lipid A (lipophilic zone) that act as fixation of LPS on outer membrane of *Proteus* spp.[49].

1.2.1.3.6. Natural resistance to polymyxin

Proteus bacteria resist cyclic antibiotics of (PMX) kind because of connection of positive charge present in their structure with negative charge of lipid A region [60, 61].

1.2.1.3.7. Invasiveness

Infection occurs after *Proteus* penetration of the host that causes secretion of a protein which helps them to change their shape to small rods with fast movements and multiplication inside the host [62, 63].

1.2.1.3.8. Capsule polysaccharide (CPS)

The capsule of *Proteus* bacteria has relation with their pathogenicity because of its ability to collect metallic magnesium ions as the result of presence of some acids like uric acid, pyruvic acid or phosphate groups that result in urolithiasis [46, 64].

1.2.1.3.9. Protease production

Proteus can resist the immune system by producing of protease enzyme that splits peptide chains present in immunological activities that permit the establishment of infection [48].

The urinary tract infection is common in community and hospitals [65].In most cases infection is chronic and recurrent and 20 % in chronic cases infection stops after therapy [66].

Pathogenic bacteria transport to the urinary tract via ascending route from the colon region via urethra and the surrounding tissue to the bladder; its presence in the kidney depends on the virulence factors of the bacteria and host factors. Bacteria might spread via hematogenous route from the blood as in causes of bacteremia, or lymphatogenous route from lymphatic [67], urinary system is infected with many infections leading to disturbances of renal infections some of which lead to end stage renal disease [67-69]. Most diseases that affect the urinary tract are caused by pathogens, fungi, viruses like pyelonephritis and tuberculosis (T.B.). The kidneys might be affected by metabolic disorders and hereditary

nephritis, nephritis syndrome, lipoid nephrosis, and congenital malformation and anomalies [70], or the disease occurs as mutation in alleles like renal coloboma syndrome while bladder is affected by benign or malignant lesion [70].

1.2.2. Urease (urea amido hydrolase)

Urease is one of the enzymes belongs to the group (hydrolases) which has classification number (E.C. 3.5.1.5) [71], and this enzyme contains nickel ion (metalo-protein) to induce urea hydrolysis that evolves ammonia and carbomate molecule and carbonic acid according to the equation [72], as in Figure (1-1):

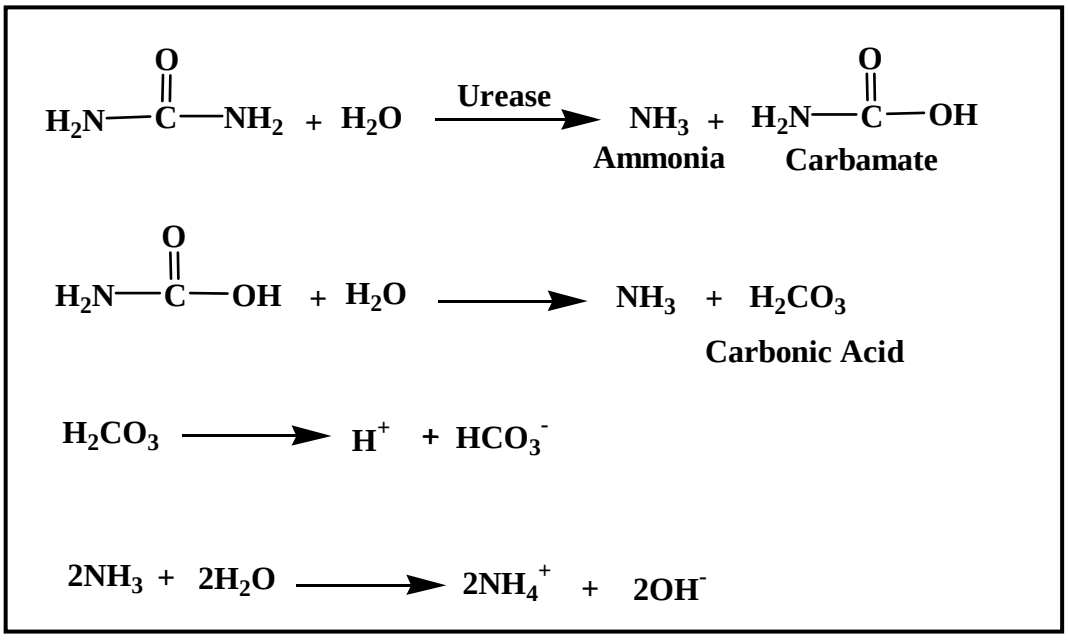


Figure (1-1): The equations of urea hydrolysis to evolve ammonia carbomate molecule and carbonic acid [72].

After equilibrium between ammonia and water the ammonium hydroxide occurs that rapidly increases pH [72]. Musculus (1876) is considered to be the first that study the bacterial urease after that Miquel

(1890) diagnosed the production of urease in many microorganisms including bacteria [73-76].

Urease is produced by pathogenic or nonpathogenic bacteria [77,78], that helps causing disease and considered a virulence factor in many bacteria. It has been shown the production of such enzyme in a bout 100 types of bacteria [72].

The infection with the *Proteus*, *Pseudomonas*, *Staphylococcus*, *Corynbacterium*, *Morganella*, *Micrococcus*, *Klibsiella*, cause U.T.I. and urolithiasis [79].

All these bacteria produce urease that has a major role in urolithiasis by increasing the pH from 5 to 9 causing the mineral salts to precipitate in mucous material [79], which is produced by the bacteria and entered in its cellular structure and acts as naives around which salts are precipitated to form stones. It also has been found that bacterial cells inside renal stones in proteins are treated with antibiotics [20].

1.2.3. Cellular localization of urease

Urease is an extracellular enzyme [80, 81], and most studies show that urease localized in yeasts and cytoplasm parts of most bacterial strains [20, 82], as enzyme is detected in the cytoplasm of 22 bacteria [83]. Electronic microscope shows the conjugation of the inner membrane of *Staphylococcus*, while in the periplasm and outer membrane of *Proteus mirabilis* [20].

While *Helicobacter pylori* is the only bacteria that has enzyme in cytoplasm and on cell membrane, because of the ability of the bacteria outer membrane to absorb the enzyme that is released from adjacent cell autolysis [84].

Mobley *et.al.*[15] have indicated the relationship between the production of urease and differential stages during the growth of bacterial

cells. It was noticed that there was a difference in the urease activity in swarming property of the *Proteus* spp. from the non-swarming bacteria cells, since the activity of urease in swarming cells was greater than the non-swarming bacteria cells.

1.2.4. Urease mechanisms

It is possible to demonstrate urease mechanism as assumed by Zerner and called Zerner model in the active site [85], and the presumed mechanism for the action of urease [86], as in Figure (1-2):

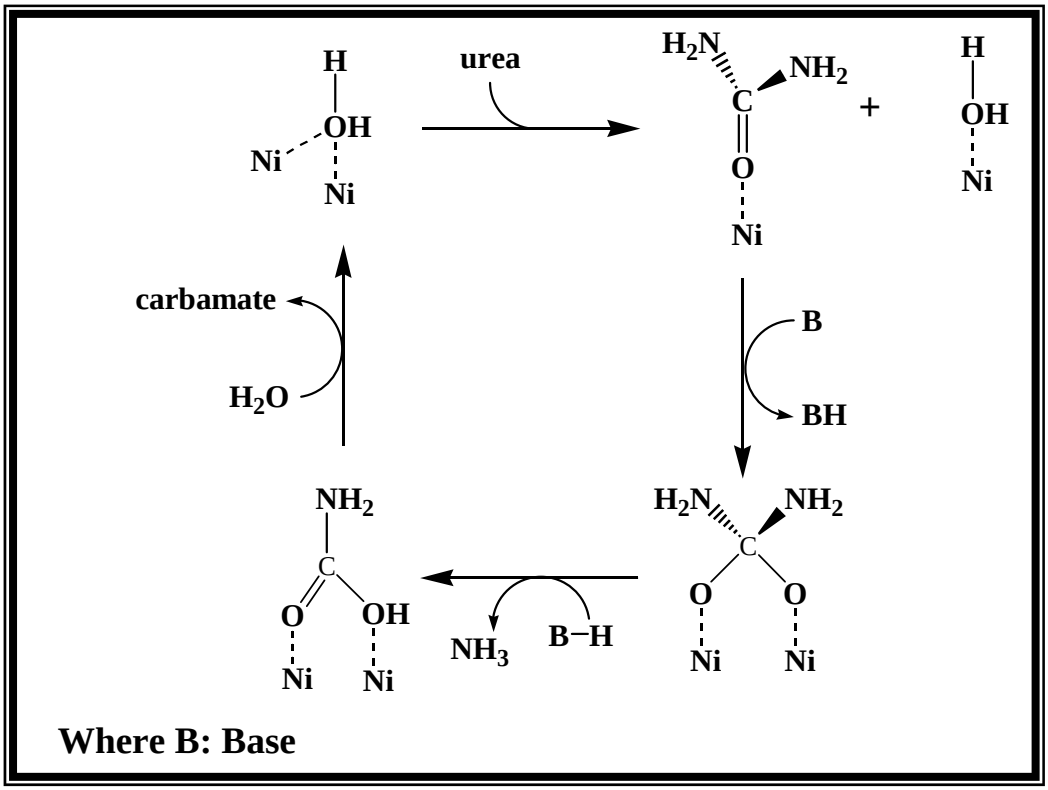


Figure (1-2): Presumed mechanism for the action of urease [86].

It is thought to be an association between nickel ions with water molecule or nickel ions with hydroxide. The mechanism of urease action depends on different nickel ions [87].

A study was done on the crystal form of urease structure and it was found that urease binds covalently with first nickel ion substitute water ion through covalent bond of oxygen atom of urea to first nickel ion with the assistance of histidine [88, 89].

1.2.5. Importance of urease for different organisms

1.2.5.1. Animals

For ruminants, they can utilize urea presence in blood from to portions in nitrogen cycle that involves urea transport from blood to stomach then hydrated to ammonia and carbon dioxide by bacterial urease presence in stomach. Ammonia is used as source of nitrogen by microbes to form proteins, then digested and absorbed microbial proteins in alimentary canal [90] .

1.2.5.2. Microorganisms

Urease is considered a virulence factor for bacteria (*Proteus mirabilis*) that cause urinary tract infection [73, 91]. Many studies show that urease plays a role in alimentary canal and pathogenesis causing dangerous disease like stomach cancer [92, 93].

1.2.6. Urinary tract infection

U.T.I. is the microbial invasion of any tissue of the urinary tract, extending from the renal cortex to the urethral meatus [94]. 95 % of the U.T.I. occurs by microorganisms include *E.coli* and other Entrobacteriaceae such as *Proteus* spp., *Staphylococcus sapraphyticus, Enterococci and Candida*. 5 % of the infection occurs via the hematogenous route, and may occur after bacteremia [94].

1.2.7. Classification of urinary tract infection

The use of U.T.I. is considered a general term and the U.T.I. could be classified as follows:

1.2.7.1. Location of infection

1.2.7.1.1. Upper U.T.I.

It includes severe and chronic kidney and pyelonephritis infection, and it is one of the most common diseases that affect the kidney [95].

1.2.7.1.1.1. Severe kidney and pyelonephritis infection

Severe kidney and pyelonephritis infection is usually accompanied with the obstruction of urinary tracts; in general the (stones, cervical prolapsed, foreign bodies, and tumors) are considered one of the main causes of the common obstruction [96, 97].

Liaw *et. al.*[23] have indicated that *Proteus mirabilis* infect upper urinary tracts and causes pyelonephritis infection, kidney stones and bladder stones.

1.2.7.1.1.2. Chronic kidney and pyelonephritis infection

Edwards and Macleod [98] have defined the chronic kidney and pyelonephritis infection as the infection that occurs due to the continuous attack and continuous infection and healing from the incomplete treatment for these cases of severe kidney and pyelonephritis infection which may sequentially lead to a chronic state with a slow rate and with no clear symptoms being observed, so it is so difficult to diagnose this infection accurately [99].

The chronic urinary infection occurs due to the severe urinary infection, so the bacteria have a higher pathogenicity and resistance

toward anti-microbial agents. Chronic bladder infection is accompanied with urethra obstruction, which leads to urea stasis in bladder and it commonly infects males only [99].

1.2.7.1.2. Lower U.T.I.

It includes both cystitis and urethritis: As for cystitis it is a superficial infection of the mucus layer [100], and it commonly infects young and married females, especially pregnant ones [101], while it occurs in elder ages of both sexes, but in a small rate for males. It could be attributed to the wide spread of this infection in female to their short and wide urethra and the absence of prostate which has an anti-bacterial feature [102].

During pregnancy, the baby is pressing on the bladder and the effects from progesterone dilate the upper collecting system, making it easier for microorganisms to reach the kidney [103], and it also adheres to the mucus layer of bladder [104].

1.2.7.2. Pathogenesis

Urinary tract infection could be classified according to their pathogenicity into two categories: complicated U.T.I. and uncomplicated U.T.I.:

1.2.7.2.1. Complicated U.T.I.

It has noticed that *Proteus mirabilis* infections to urinary tracts usually accompanied with complicated U.T.I., accompanied also with the catheterization patients, structural and congenital malformations and so obstructions in the urinary tract [105-107].

This category of infection is so difficult to be treated by using ordinary antibiotics [108] because of the presence of the bacteria inside the stone matrix [49, 106].

1.2.7.2.2. Uncomplicated U.T.I.

In this category the urinary tract is natural from the anatomic and physiologic aspects, and the renal functions are regular with or without any symptoms [109]. Further more, a study in Japan has indicated that most of infections by this category were found in females [110].

1.2.8. The risk-factors for U.T.I.

The most important factors on U.T.I., in terms of the location of infection and pathogenicity are (stones, diabetes mellitus, sex, age, pregnancy, catheterization process, sircumsition, renal failure, and congenital malformations of urinary tract [49, 95, 96, 98, 111- 113]. These factors may facilitate the infection process by *Proteus* bacteria, and consequently U.T.I [114]. While congenital malformations are considered to be the main causes of urinary tract observation which leads to urinary stasis, and then increases the probability of U.T.I. [105, 115].The formation of stones inside the bladder and kidney is one of the characteristic features of *Proteus* spp. [105] which needs a surgical operation or other medical interventions [116].

1.2.9. Urinary tract stones

A kidney stone is a solid mass that consists of a collection of tiny crystals. There can be one or more stones present at the same time in the kidney or in the ureter [117].

Kidney stone may form when the urine becomes too concentrated (supersaturated urinary environment) with certain substance. These substances may create crystals that become stones [117] as illustrated in the following Figure (1-3):

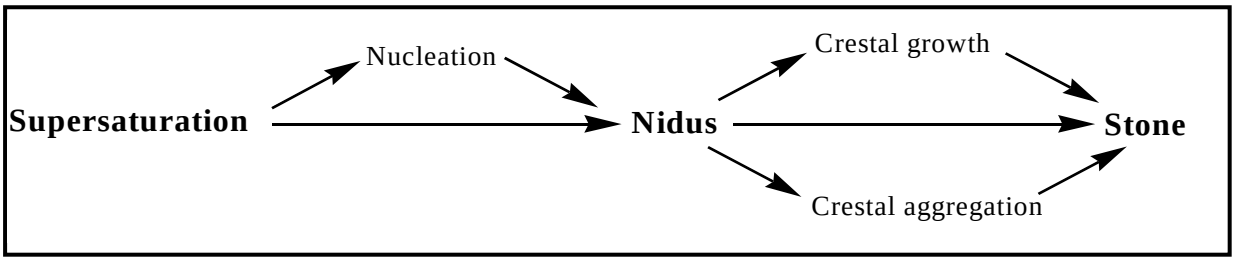


Figure (1-3): Schematic reaction to stones formation [117].

1.2.9.1. Types of stones

The urinary tract stones could be classified in two kinds:

1.2.9.1.1. Metabolism of stones

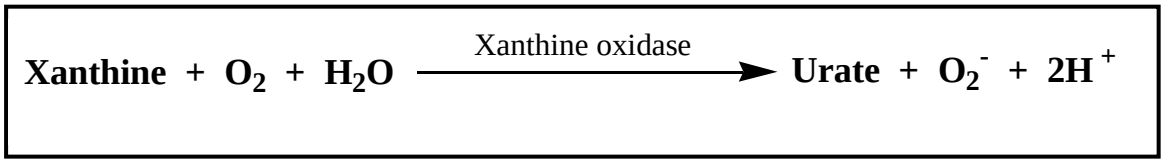
A stone that may be formed in the pure urine and could be divided into six kinds [118]:

1.2.9.1.1.1. Calcium stones

These kinds are most common in about (65 – 80) % from other types of stones. The calcium may combine other substance such as oxalate (the most common substance), phosphate, or carbonate to form stone (sparingly soluble salts) [119]. Oxalate is present in foods. Diseases of the small intestine increase the tendency to form calcium oxalate stones [120].

1.2.9.1.1.2. Xanthene's stones

They rarely infect urinary tract, and it is found in (4:10000) patients, and the cause of this stone is the increase of xanthine formation in urine due to the genetic deficiency in xanthine oxidase [121, 122].



1.2.9.1.1.3. Uric acid stones

They represent about 10 % of the total stones, and they are largely formed in bladder more than in kidney and commonly infect males [117]. And these kinds of stones are formed due to a high consumption of animal protein [123].

1.2.9.1.1.4. Drugs stones

There are many drugs that facilitate the stone formation like analgesics, and anti-virus drugs (e.g. indivar) which is used for treatment HIV [122].

1.2.9.1.1.5. Silica stones

These kinds of stones are rarely found in human, but they are so common in animals. They may be formed in patients that take anti-acid drugs which contain magnesium trisilicate. When they react with any acid. They may yield silica crystals (stones) [122].

1.2.9.1.1.6. Cystine stones

They represent about 1 % of the total stones and they are formed due to the presence of cystinurea [119].

1.2.9.1.2. U.T.I. stones

Bacteria have a major role in formation of such type of stones and they represent 7- 31 % of the total stones [119, 124]. It is produced from ammonium magnesium phosphate ($MgNH_4PO_4.6H_2O$) and called struvite [49, 105, 109].

These stones are considered a major threat more than other stones. This is so because they are continuous and bigger in size therefore it's dangerous for the kidney damage [105].

1.2.10. Chemotherapy of infections

Infection is a major category of human disease and skilled management of antimicrobial drugs is of first importance. According to the formulation originally made by Paul Ehrlich (1906), what is wanted is "specific chemotherapy", "selective toxicity rather than absolute". We are indebted to the microorganisms themselves for producing many chemotherapeutic agents, so the antibacterial or the antibiotics (chemical substances) [125, 126].

The chemotherapy is used for the drug treatment of parasitic infections in which parasites (bacteria, viruses, fungi, protozoa, and worms) are destroyed or removed without damaging host cells and tissues [127]. They are also called (antibacterial, antiviral, antifungal, antiprotozoal, and antihelminthic) drugs [128].

Antibiotics are used for treatment of U.T.I. and the properties of antibiotics are wide spectrum and secreted in an optimum concentration in urine, have a slight effect on intestine flora or on other places and not lead to a resistance between creatures [129], safe and not affected by pH of the urine [130].

1.2.10.1. Mechanism of antimicrobial action

It should always be remembered that drugs are seldom the sole instruments of cure but act together with the natural defenses of the body [131].

The mechanism of action of most antimicrobial drugs is not completely understood. However, these mechanisms of action can be placed under four headings [127, 132]:

1.2.10.1.1. Inhibition of cell wall synthesis

Bacteria possess a rigid outer layer of the cell wall .They maintain the shape of the microorganism and provide protection against much lower osmotic pressure of the environment (the internal pressure is three to five times greater in gram-positive than in gram-negative bacteria) [133].

Bacterial multiplication involves a breakdown and an extension of the wall, interference with these processes prevents the organism from resisting osmotic pressures, so that it bursts. As these cells of higher organisms, e.g. human, these organisms do not possess this type of wall. Drugs which act here, may be especially selective, obviously the drugs are effective only against growing cells. They include bacitracin, cephalosporins, cycloserine, penicillins, and vancomycin [142,135,136].

1.2.10.1.2. Inhibition of cell membrane function

The cytoplasm of all living cells is bonded by the cytoplasmic membrane, which serves as a selective permeability barrier, which carries out active transport functions, and thus controls the internal composition of the cell. If the functional integrity of the cytoplasmic membrane is disrupted macromolecules and ions escape from the cell, and cell damage

or death ensues [133]. Drugs that interfer with its functions includes; polyenes (nitration, amphotericin B), azoles (fluconazole, itraconazole, minconazole), polymixins (colistin, poly-myxin B) [137,138].

1.2.10.1.3. Inhibition of proteins synthesis

It is established that (Chloramphenicol, Tetracycline, Erythromycin, lincomycin and aminoglycoside) drugs can inhibit proteins synthesis in bacteria. The precise mechanism of action is not fully establishing for theses drugs [139]. Bacteria have 70S ribosmes.The subunit of each type of ribosome is different in chemical composition, and their functional specification are sufficiently different, therefore antimicrobial drugs can inhibit proteins synthesis in bacterial ribosome (inhibition of translation and transcription of genetic material) without having a major effect on mammalian ribosome [133, 140].

Aminoglycosides include (kanamycin, Neomycin, Gentamycin, Tobramycin, Amikacin, Streptomycin, Sisomycin and Netilmycin) and these drugs probably all act similarly [141]. The first step is the attachments of the aminoglycoside to a specific receptor protein as on the 30S subunit of the microbial ribosome. Second, the aminoglycoside blocks the normal activity of the "initiation complex" of peptide formation (mRNA + formylmethionih + tRNA).

Third, the mRNA message is misread on the recognition region of the ribosome; consequently, the wrong amino acid is inserted into the peptide, resulting in a nonfunctional protein. Fourth, aminoglycoside attachments result in the breakup of polysomes and their separation into monosomes incapable of proteins synthesis [133, 142, 143].

1.2.10.1.4. Inhibition of Nucleic Acid Synthesis

Drugs may interfere directly with microbial DNA or its replication or repair as include e.g. (all quinolones and fluroquinolones) by blocking DNA gyrase [144], or with RNA synthesis include e.g. (rifampin , sulfonamides , trimethoprim , and trimetrexate) [145].

1.2.10.2. Use of antimicrobial drugs

The general rule is that selection of antimicrobials should be based on the diagnosis (identification of the microbe), and sensitivity test. All appropriate specimens (blood, urine, pus, spinal fluid) must therefore be taken for examination before administering any antimicrobial [146].

This process inevitably takes time and therapy at least more serious infections must usually start on the basis of the "best guess" with the world wide rise in prevalence of multiply in resistant bacteria, in the last decade. Knowledge of local antimicrobial resistance rate is an essential prerequisite choice as empirical antimicrobial therapy. Knowledge of the likely pathogens (and their current local susceptibility rates to antimicrobials) in the clinical situation [133]. Thus aminoglycosides (Tobramycin) is used as first choice for treatment the urinary tract infection that get from (*Proteus* or *Pseudomonas aeruginosa*) bacteria , and benzyl-penicillin for meningitis in the adult (*Meningococcal* or *Pneumococcal*) [129, 146]. Another use as rapid diagnostic tests and these tests undergo a revolution with the widespread introduction of affordable, sensitive and specific nucleic acid detection assays (especially those based on the polymerase chain reaction – P.C.R.) [131]. The route of administration parental therapy (which may be IM. or IV.) is preferred to treatment of serious infections because high therapeutic concentration

is achieved reliably and rapidly. Initial parental therapy should be switched to the oral route whenever possible [128,147].

1.2.10.3. Resistance to antimicrobial drugs

Bacterial resistance for the chemical compounds may occur through many mechanisms, as: First , microorganisms produce enzymes that destroy the active drug , example : *Staphylococci* resistant to penicillin G by produce a ß-Lactamase that destroys the drug as e.g. Penicillinase destroys the ß-Lactam of penicillin and converted to inactive drugs (penicilloic acid)as more stable compound [148], as following reaction:

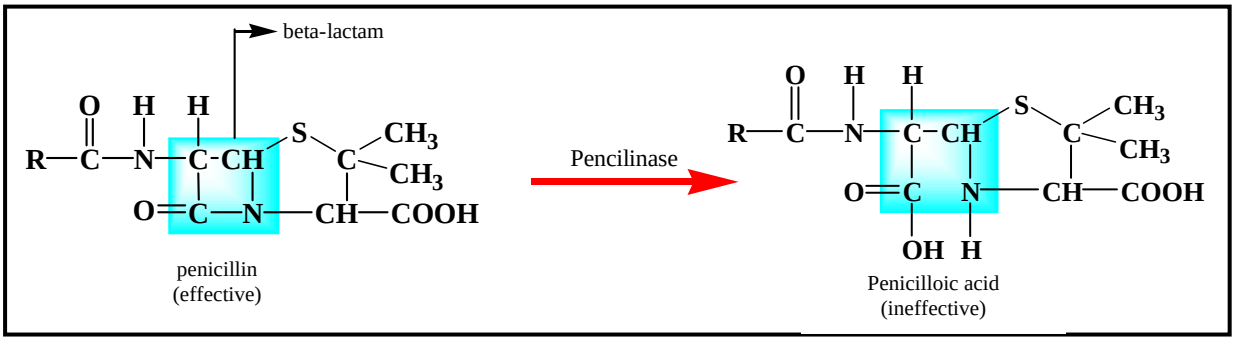


Figure (1-4): The mechanism of enzyme action (Penicillinase) [148].

Gram- negative bacteria may be resistant to Chloramphincol if they produce a Chloramphincol acetyltransferase [133]. Second, microorganisms change their permeability to the drug, example: tetracycline accumulates in susceptible bacteria but not in resistant bacterial , apparently due to an outer membrane change that impairs active transport into the cell or due to thick cell wall as it contains high lipid in the wall. Third, genetic mutations, example: chromosomal mutation allows bacteria to aminoglycosides by alteration of a specific protein in the 30S subunit of the bacteria ribosome.

Fourth, microorganisms develop an altered metabolic pathway that bypasses the reaction inhibited by the drug, example: sulfonamide

resistant bacteria, do not require extracellular PABA, but like mammalian cell, can utilize preformed folic acid [133, 149].

U.T.I. is considered one of the infections that are caused by bacteria with an increased ability for multi-drug resistance [112].In many studies it is noticed that researchers could isolate many spp. of bacteria in one Genus some of which are sensitivities and others are resistant to antibiotic [150]. It is also noticed that an increase in the occurrence of resistance to new broad spectrum antibiotics [151].

Multi-drug resistance is registered all over the world with special consideration to hospitalized patients .This fact ushers a big problem [142], that it is one of the most important health and economic world wide problem carries a great challenge to researchers who lead them to search for new antibiotics in order to concur those emergent strains of resistant pathogens and so to lower ever increasing rate of mortalities and morbidities due to infections with these agents [140,153], therefore this is one of the aims of this work by preparation of novel heterocyclic compounds.

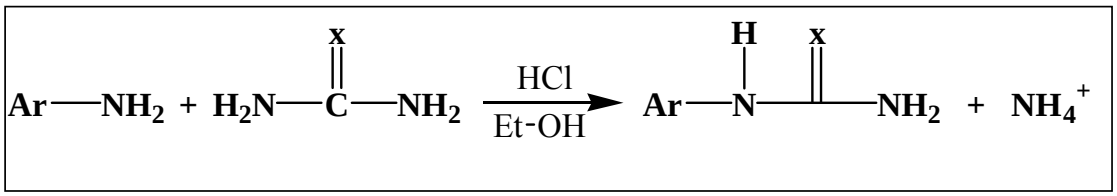
Heterocyclic are compounds ring containing, in addition to carbon other kinds of atoms most commonly nitrogen, oxygen, and sulfur for example pyrrole, furan, and thiophen respectively. These compounds classes to either five-membered systems or six-membered systems, and both systems consist of one hetero-atom, more than one hetero-atom, and benzoderivatives [154].

3. Results & Discussion

3.1. The chemical compounds

3.1.1. Urea and thiourea derivatives compounds

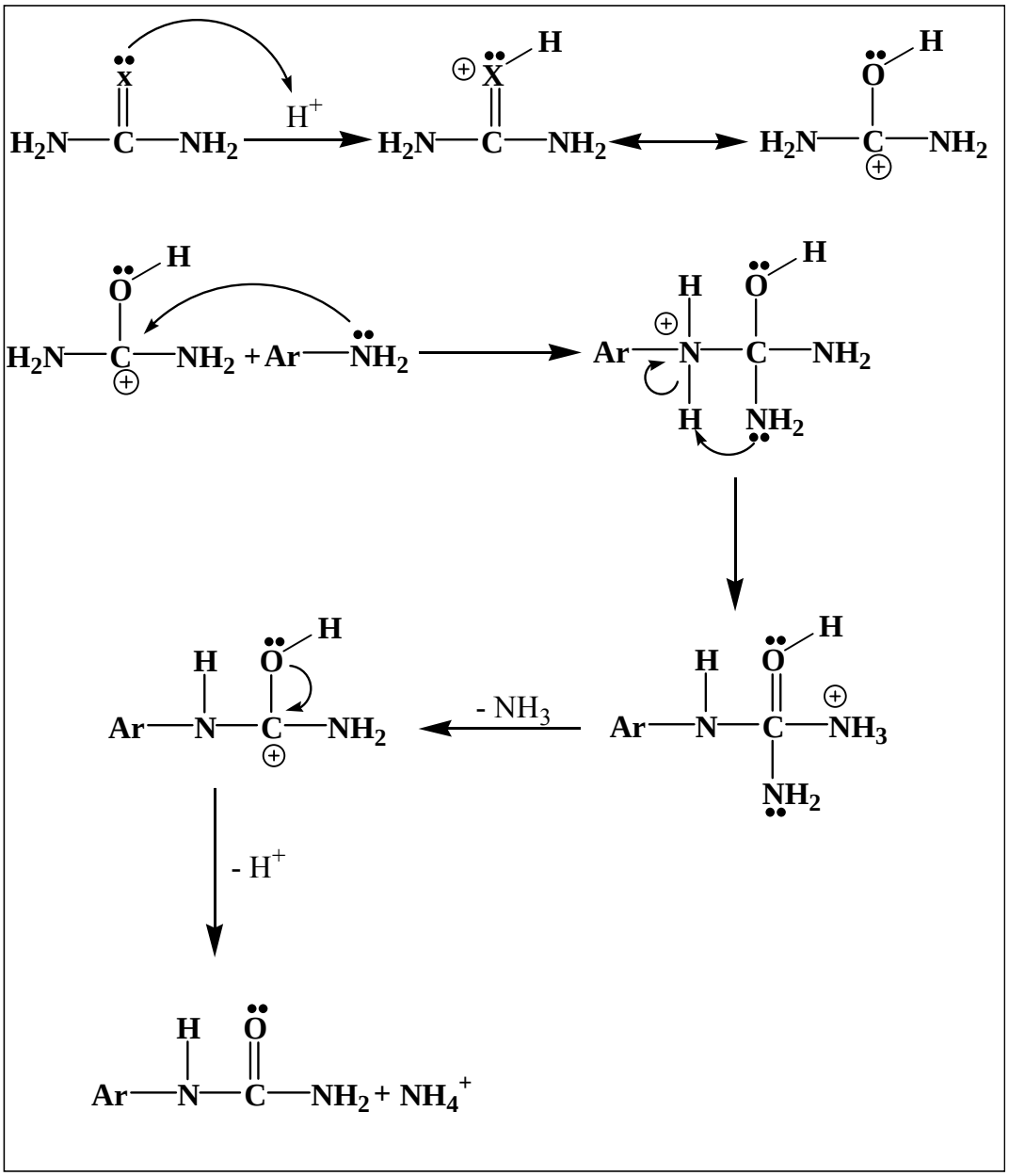
Compounds in (2.2.2.1 - 2.2.2.14) these are urea and thiourea derivatives. They were prepared from the reaction of urea or thiourea with aromatic amines in absolute ethanol as solvent in presence of concentrated HCl as catalyst according to the following general equation:



Where X= O or S

This type of reaction is considered nucleophilic-electrophilic one [176]. Where amines groups represent the nucleophil and carbon atom of carbonyl group in urea or thiourea represents the electrophile.

The concentrated HCl act as catalyst to increase the rate of reaction in r. d. s., through urea or thiourea salts formation which is considered as strong electrophile (increase the electrophilicity of carbon in carbonyl group), then strong nucleophil amines attack urea or thiourea salts to form urea derivatives by release NH_4^+ as demonstrated through the following general suggested mechanism:



For all prepared compounds T.L.C. technique was used to examine the formation and purity of these compounds by using a suitable mixture as eluent. Also F.T.I.R. was used for characterization the functional groups these prepared compounds. Physical properties of 14 prepared compounds were measured and the results showed in Table (3-1):

Table (3-1): The physical properties of prepared (urea and thiourea) compounds.

No.	Name of compounds	Chemical formula	M.wt.	Melting point(ºC)	Color
1	N-4-aminobenzylurea	$C_8H_{11}N_3O$	165	169-171	White
2	N-4-aminobenzylthiourea	$C_8H_{11}N_3S$	181	177-179	White
3	N-pyrimidine-6-ylurea	$C_6H_8N_4O$	152	287-289	Brown
4	N-pyrimidine-6-ylthiolurea	$C_6H_8N_4S$	168	295-297	Brown
5	N-pyridine-4-ylurea	$C_7H_9N_3O$	151	232-234	Brown
6	N-pyridine-4-ylthiourea	$C_7H_9N_3S$	167	238-240	Brown
7	N-benzothiazole-2-ylurea	$C_8H_7N_3OS$	193	>350	Orange
8	N-benzothiazole-2-ylthiourea	$C_8H_7N_3S_2$	209	>350	Orange
9	[N-(5-phenyl-1,3,4-oxadiazole)-2-yl]urea	$C_9H_8N_4O_2$	204	216-218	Dark brown
10	[N-(5-phenyl-1,3,4-oxadiazole)-2-yl]thiourea	$C_9H_8N_4OS$	220	224-226	Dark brown
11	[N-(5-phenyl-1,3,4-thiadiazole)-2-yl]urea	$C_9H_8N_4OS$	220	255-257	Grey
12	[N-(5-phenyl-1,3,4-thiadiazole)-2-yl]thiourea	$C_9H_8N_4S_2$	236	263-265	Grey
13	[N-(5-phenyl-1,3,4-triazole)-2-yl]urea	$C_9H_9N_5O$	203	350	Deep-violet
14	[N-(5-phenyl-1,3,4-triazole)-2-yl]thiourea	$C_9H_9N_5S$	219	350	Deep-violet

3.1.2. Infrared spectra for prepared (urea and thiourea) derivative compounds [177-180]:

The F.T.I.R. spectra for compounds (1-14) as shown in Table (3-2), the compound no.1, Figure (1) showed absorption band of ($-NH_2$) group at (3350-3310 cm^{-1}) could be a attributed to (N-H) stretching mode, and band of ($-CH_2$) aliphatic at (2937-2858 cm^{-1})could be attributed to (C-H) aromatic stretching mode in (3010 cm^{-1}), band of amide group at (1610 cm^{-1}) could be attributed to (C=O) stretching mode, (C=C) aromatic group of benzene showed absorption band at (1548 cm^{-1}), para-substitution in ring absorption band at (810 cm^{-1}) could be attributed to *p*-substitution bending mode. In F.T.I.R. spectrum for the compound no.2, Figure (2) showed absorption band of thioamide group at (1076.2cm^{-1}) attributed to (C=S) stretching mode.

The F.T.I.R. spectrum for compound no.3, Figure (3), showed absorption band of (N-H) stretching group at (3359.62 cm^{-1}) attributed to (N-H) stretching mode. The absorption band of amide group at (1610.45 cm^{-1}) attributed to (C=O) stretching mode. Absorption band of aromatic (C-N) group at (1369.37 cm^{-1}) attributed to (C-N) stretching mode. Ortho-substitution showed absorption band at (752.19 cm^{-1}). In the compound no.4, Figure (4) showed the absorption band of thioamide group at (1116.71 cm^{-1}) attributed to (C=S) stretching mode. The F.T.I.R. spectrum for compound no.5, Figure (5) showed the absorption band of amide group at (1623.24 cm^{-1}). Absorption band at (1565.8 cm^{-1}) attributed to (C=C) aromatic stretching mode. The absorption band of (N-H) group at (3371.05 cm^{-1}) attributed to (N-H) stretching mode.

In F.T.I.R. spectrum for the compound no.6, Figure (6) showed the absorption band of thioamide group at (1135.99 cm^{-1}).The F.T.I.R. spectrum for the compound no.7, Figure (7) showed absorption band of of (N-H) group at (3340.20 cm^{-1}) attributed to (N-H) stretching mode,

(C-H) aromatic group at (3060.55 cm^{-1}), the carbonyl of amide group absorbed at (1577.66 cm^{-1}), and the (C-S) absorption band at (1377.08 cm^{-1}),ortho-substitution absorption band at (744.47 cm^{-1}).In the compound no.8, Figure (8) showed the absorption band of (C=S) at (1137.92 cm^{-1}).

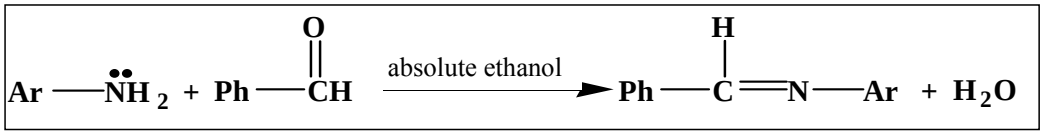
The F.T.I.R. spectrum for the compound no.9, Figure (9) showed the absorption band of (N-H) group at (3313.5 cm^{-1}) attributed to (N-H) stretching mode, and absorption band of (C-H) aromatic group at (3051.0 cm^{-1}), the carbonyl of amide group at (1643.2 cm^{-1}) attributed to ($CO-NH_2$) streching mode. The (C=C) aromatic gave band at (1554.5 cm^{-1}). The absorption band of cyclic (C-O-C) group at (983.6 cm^{-1}). In the F.T.I.R. spectrum of compound no.10, Figure (10) showed the absorption band of thioamide group at (1210 cm^{-1}).

The F.T.I.R. spectrum for the compound no.11, Figure (11) showed the absorption band of ($-NH_2$) at (3438.29 – 3330 cm^{-1}) attributed to (N-H) streching mode, and (C-H) aromatic group at (1616.24 cm^{-1}).In the compound no.12, Figure (12) showed the absorption band of thioamide group at (1095.49 cm^{-1}).

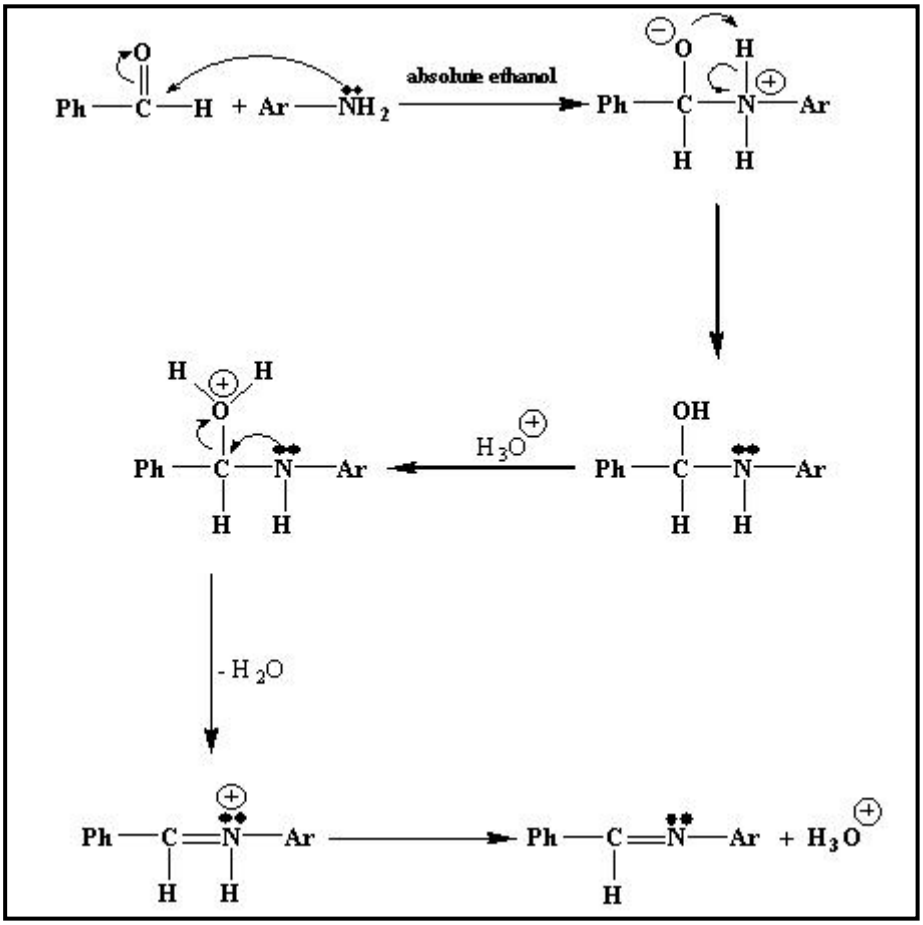
The F.T.I.R. spectrum for the compound no.13, Figure (13) showed the absorption band of (N-H) group at (3305.06 cm^{-1}), (C-H) aromatic group at (3076.25 cm^{-1}). The absorption band of ($-CO-NH_2$) group at (1610.45 cm^{-1}), (C=C) aromatic group absorbed band at (1566.09 cm^{-1}). In the compound no.14, Figure (14) showed the absorption band of thioamide group at (1079.95 cm^{-1}) streching mode.

3.1.3. Schiff bases compounds

Other six compounds of Schiff bases (2.2.2.15.1- 2.2.2.15.6) were also prepared in addition to the previous compounds (urea and thiourea derivatives) in this study, due to the biological importance of these compounds, and also to compare the biological (inhibitory) activity with commercially antibiotics were also done in this study. The reaction occurs between the aromatic amine with aromatic aldehyde (benzyldehyde) by using absolute ethanol or glacial acetic acid as catalyst, as in the following equation:



In this reaction, amine acts as nucleophil and attacks the carbon of the carbonyl group of aldehyde as electrophile to form N-substituted hemiaminals (unstable product) and that losses water molecule to yield stable compound as shown in this mechanism [181]:



The Table (3-3) shows the physical properties of schiff bases compounds as follow:

Table (3-3): The physical properties of prepared schiff bases

No.	Name of compounds	Chemical formula	M.wt.	Melting point(ºC)	Color
15.1	[benzylidene-6-aminopyrimidine]carboamide	$C_{13}H_{12}N_4O$	240	277-279	Yellow
15.2	[benzylidene-6-aminopyrimidine]carbothioamide	$C_{13}H_{12}N_4S$	256	285-287	Yellow
15.3	[benzylidene-4-aminopyridine]carboamide	$C_{14}H_{12}N_3O$	239	295-297	Brown
15.4	[benzylidene-4-aminopyridine]carbothioamide	$C_{14}H_{12}N_3O$	255	298-300	Brown
15.5	[benzylidene-2-aminobenzothiazol]carboamide	$C_{15}H_{11}N_3OS$	281	>350	Orange
15.6	[benzylidene-2-aminobenzothiazol]carbothioamide	$C_{15}H_{11}N_3S_2$	297	>350	Pale orange

3.1.4. Infrared spectra of the prepared Schiff bases [182, 183]

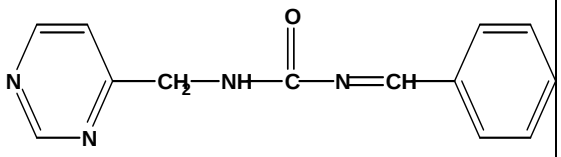
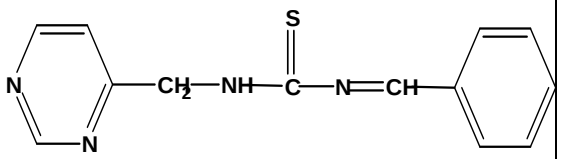
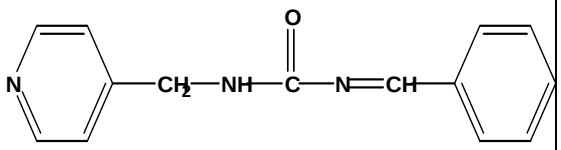
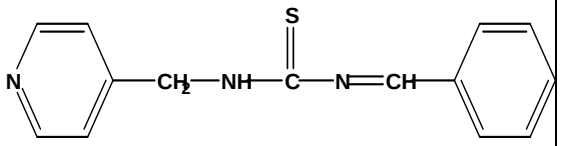
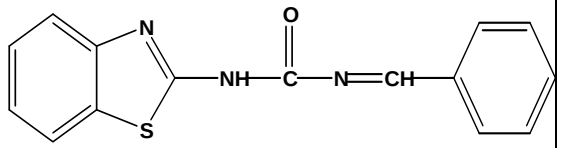
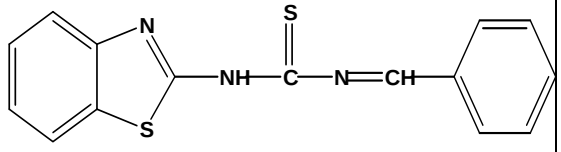
The F.T.I.R. spectra for the compounds (15.1-15.6) as shown in Table (3-4), and for the compounds no.15.1, Figure (15) showed absorption band at (1603.43 cm^{-1}) which could be attributed to (C=N). This imine group is characteristic to Schiff bases streching vibration mode, absorption band of (C-H) aromatic group at (3058.10 cm^{-1}) could be attributed to (C-H) streching mode, also absorption band of ($-CH_2$) aliphatic group at (2934.09 – 2851.52 cm^{-1}), (C=C) aromatic ring at (1571.88 cm^{-1}),

and (C-N) bond shows absorption band at (1097 cm^{-1}) attributed to (C-N) aromatic stretching mode. In the compound no.15.2, Figure (16) showed the absorption band of (C=S) at (1140 cm^{-1}) streching mode.

The F.T.I.R. spectrum for the compound no.15.3, Figure (17) showed the absorption band of (N-H) group at (3320.69 cm^{-1}) , (C-H) aromatic group at (3033.62 cm^{-1}), also absorption band of (C=N) group at (1612.38 cm^{-1}) attributed to (C=N) aromatic stretching mode, (C-N) bond shows absorption band at (1022.20 cm^{-1}), and para- substitution could be showed absorption band at (883.34 cm^{-1}). In the compound no.15.4, Figure (18) showed the absorption band of (C=S) group at (1180.4 cm^{-1}) stretching mode.

The F.T.I.R. spectrum for the compound no.15.5, Figure (19) showed the absorption band of (N-H) group at (3380.05 cm^{-1}), absorption band of (C-H) aromatic group at (3109.04 cm^{-1}) , also absorption band of (C=N) group at (1612.38 cm^{-1}), (C=C) aromatic ring shows absorption band at (1571.88 cm^{-1}) could be attributed to (C=C) streching mode, the (C-N) and (C-S) showed absorption band at (1020.27 cm^{-1}) and (919.76 cm^{-1}) respectively. In the compound no.15.6, Figure (20) showed the absorption band of (C=S) group at (1135.85 cm^{-1}) stretching mode.

Table (3-4): Characteristic infrared absorption bands of the prepared Schiff bases compounds

No.	Structures	υ (C =N) cm^{-1}	υ (C=C) Arom. cm^{-1}	υ (C-N) cm^{-1}	υ (C=S) cm^{-1}	Others band cm^{-1}
15.1		1603.43	1571.88	1118.64	–	(C-H) Aliph. 2934.09
15.2		1600	–	–	1097	(C-H) Arom. 3058.10
15.3		1612.38	1554.52	1022.20	–	(N-H) 3320.69
15.4		1604.7	–	–	1180.4	P-sub. 883.34
15.5		1683.74	1504.34	1020.27	–	(N-H) 3380.05
15.6		1616.24	–	–	1135.8	(C-H) Arom. 3109.04

3.2. The microbiologic study

3.2.1. Isolation and identification of causative agents of U.T.Is.

A. Isolation

From each of 200 patients with urinary tract infection and/or urolithiasis, a urine specimen was taken and cultured on MacConkey's agar and Blood agar. 138 out of the 200 patients gave positive results for bacterial growth as shown by colonies on culture media i.e. 69 %. This result is in agreement with results of the studies by Kumamoto *et.al.*[92] and Keizu *et.al.*[184] who found that the bacterial growth was positive in 70.7 % and 67 % respectively. Beside the shape of the colonies the Api 20 E system used showed that 23 isolates were identified as *Proteus* spp. i.e. 16.66 %.These results are in agreement with the results of these studies done by Sharmn *et.al.*[185], Orrette [186] and [12], who they found that percentages of isolating for *Proteus* spp. were 19.62 %, 17.41 % and 15.47 % respectively. But it was not disagree with results of studies done by Mohr *et.al.*[187], Warren *et.al.*[188] and Khurana *et.al.*[189] who found that 5 %, 28 % and 33 % respectively.

Other positive bacterial growth represent only 115 out of the 138 was neglected for the purpose of this study. On the other hand, *Proteus* spp. with regard to gender, *Proteus* spp. were found in 17 females out of the 23 i.e. 73.91 % with 26.09 % in males as shown in Figure (3-1).

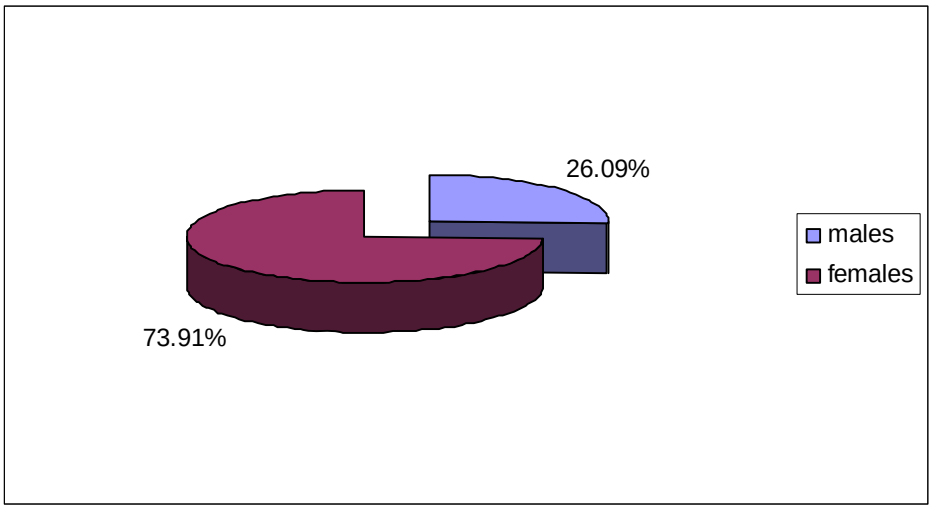


Figure (3-1): Percentage for urinary tract infection by *Proteus* spp. between females and males.

U.T.I. is caused by *Proteus* spp. occurs in both sexes and in all ages [43]. Some researches found that females were infected more than males by this type of infection as for 74.6 % females, there was 25.4 % males [125].This result is in agreement with the results of the present study. Mean while, other researchers showed the reverse, as for 68.66 % males, there was 31.34 % females [190,186], which are not in agreement with the present study.

It is known that U.T.I. occurs more frequently in females than in males which could be explained by the fact the urethra of females is wider and shorter than that of males and also by the fact that the prostates fluid in males posses antibacterial activity [102].

B. Identification

Depending on the morphology of the colonies of the bacterial growth on MacConkey's agar and Blood agar, 23 primarily were presumed to belong to the Genus of *Proteus*.

This is showed by the characteristic swarming phenomenon and the presence of the odor of fish on blood agar, also by the pale color of colonies on MacConkey's agar which indicates the absence of the ability to ferment lactose by the *Proteus* spp. [21].

Depending on microscopically examination of smears taken from the colonies of the bacterial growth after staining by Gram stain, this results of which is showed in Figure (3-2) are identified as Gram-negative bacilli by the shape of the bacterial, its size, arrangement and its reaction to Gram stain.

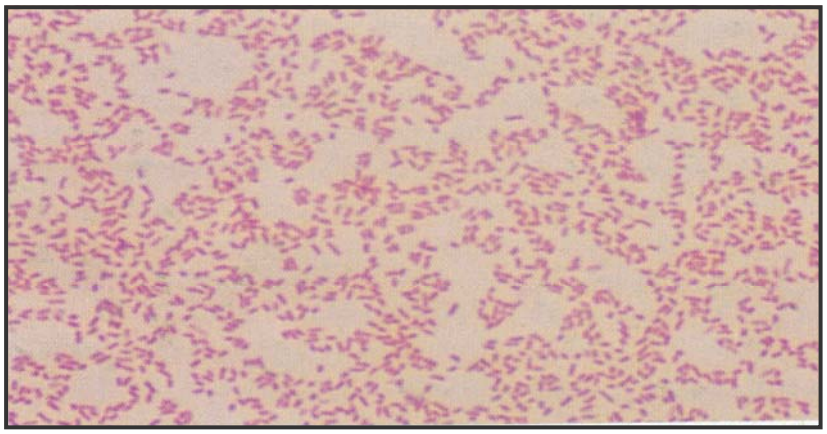


Figure (3-2): Gram stains of *Proteus* spp. under microscopically examination showed Gram-negative bacilli (100 X).

Proteus spp. was also identified by its ability to change the color of urea broth base in tubes from the yellow-orange to the pinkish-red color. This is because of the ability by *Proteus* to produce urease which elevate the degree of the pH of the broth base in turn effects phenol-red reagent [26,162].

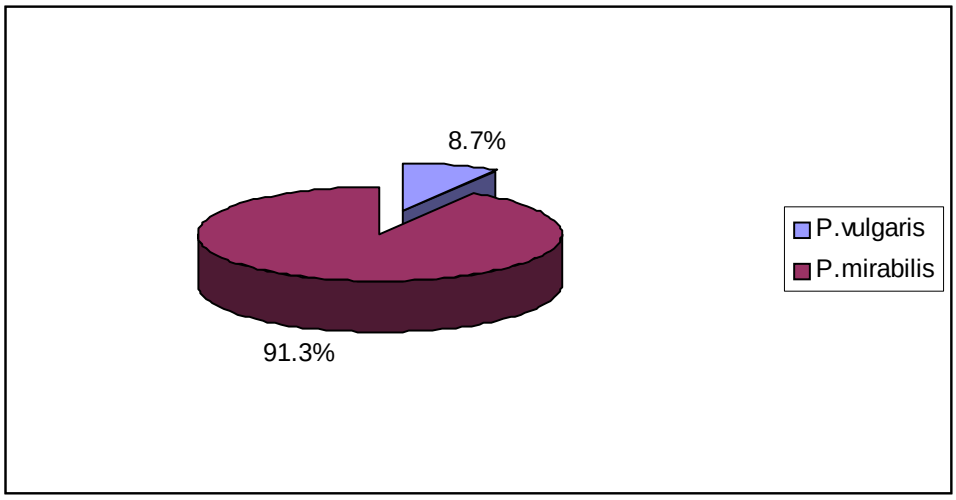
Also the results of modern method Api 20E were identical to that of *Proteus* spp.. The results were observed from the colors change in 20 microtubes according to the company supplied, as shown in Figure (3-3):

Figure (3-3): Api 20E results identical that of *Proteus* spp.

A- *Proteus vulgaris*. B- *Proteus mirabilis*.

From this result also distinguish between *Proteus* species, by indole test as in Table (2-7) was negative test for *Proteus mirabilis* and positive test for *Proteus vulgaris* [162].

The isolation *Proteus mirabilis* in this study was 21 isolates out of 23 isolated *Proteus* bacteria with percent 91.3 %. While 2 of *Proteus vulgaris* was found with 8.7 %, as shown in Figure (3-4)



Figure(3-4): The percentage between the *Proteus mirabilis* and *Proteus vulgaris* from isolated *Proteus* species.

This result is in an agreement with the Orrette [186] whose percentage of the isolates of *Proteus mirabilis* was 87.8 %, but the isolates of *Proteus vulgaris* was 12.2 %.

From all these characteristics weight also to be seen with other bacteria, but with the presence of the swarming phenomenon these characteristics are confirmative for the identification of *Proteus* spp. [160] as shown in the following Figure (3-5).

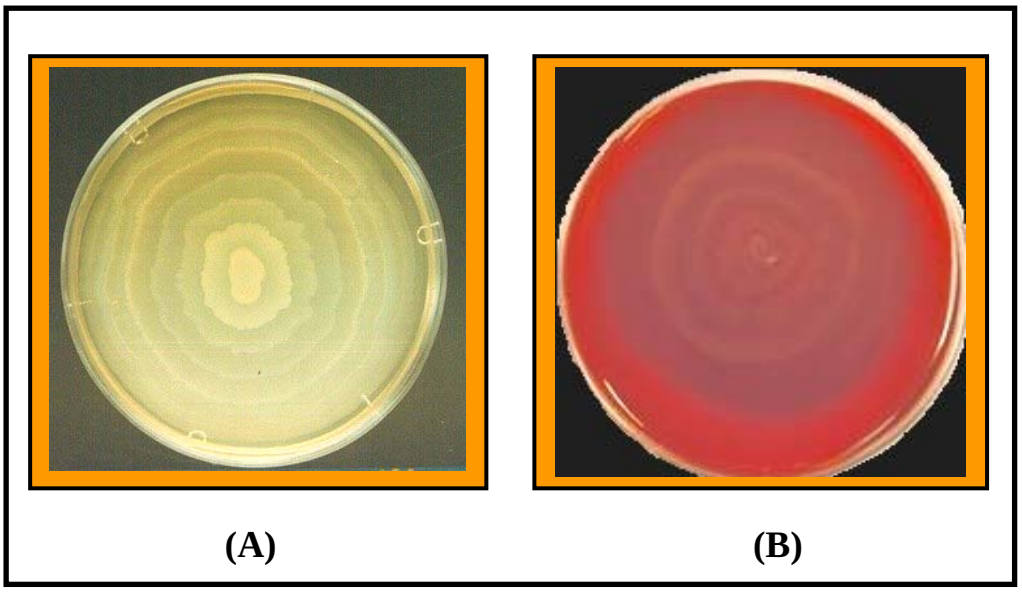


Figure (3-5): Swarming phenomenon of *Proteus* spp. A-on nutrient agar. B- on blood agar.

3.2.2. Bacterial sensitivity against commercially used antibiotics

A disc diffusion method was used to determine the inhibiting power of the 9 types of common used antibiotics as in Table (2-4) against the 23 isolates of *Proteus* spp.. This method involves the exposure of the zone of inhibition toward the diffusion of microorganisms on agar plates.

This inhibition growth is shown by the appearance of a clear zone around each disc to which the bacteria is sensitive. Hence no growth of *Proteus* spp., the power of inhibition is indicated by wider zone. As shown by Table (2-4), in which the measurement is made by a ruler in

mm. for the clear zone around each disc and compared by the standard Tables supplied by the manufacturing company, the Table (3-5) showed results of sensitivity test for isolates of *Proteus* bacteria towards the antibiotics used in this study.

Table (3-5): The results of sensitivity test for isolates of *Proteus* spp. towards the commercially antibiotics used in this study, with the no. and percentage of resistance.

Antibiotics / ***Proteus* spp.**	A	Cb	Ce	C	Cf	E	G	L	T
1	R	R	S	S	S	R	S	R	R
2	I	R	S	R	S	R	S	I	S
3	R	R	R	R	S	I	S	R	I
4	S	I	I	R	R	R	I	R	R
5	I	R	S	S	S	R	S	R	R
6	S	S	R	R	S	R	S	R	R
7	R	S	S	R	S	R	S	S	R
8	R	I	S	R	S	R	S	R	R
9	I	R	S	R	I	R	I	R	S
10	R	S	S	R	S	R	S	I	R
11	I	R	S	R	I	R	I	R	R
12	R	I	S	R	S	S	S	S	R
13	R	S	S	R	S	R	S	R	R
14	R	R	R	R	R	R	R	R	R
15	S	R	S	I	I	R	S	I	S

---Continued

Antibiotics / Isolates	A	Cb	Ce	C	Cf	E	G	L	T
16	R	R	S	R	I	R	I	R	R
17	R	I	S	R	I	R	I	I	R
18	R	I	R	R	S	R	S	R	R
19	I	R	S	R	S	R	S	R	S
20	I	R	S	R	S	R	S	R	R
21	R	S	I	R	S	R	S	R	R
22	S	S	S	S	S	S	S	S	S
23	R	R	S	R	S	R	S	R	R
No. of isolates that resistance to antibiotics	13	12	4	19	2	20	1	16	17
Resistant percentage %	56.5	52.2	17.4	82.6	8.6	86.9	4.3	69.5	73.9

Where:
R = Resistance.
S = Sensitive.
I = Intermediate.

Figure (3-6) shows the percentage of resistance of the isolates of bacteria under this study against 9 listed antibiotics.

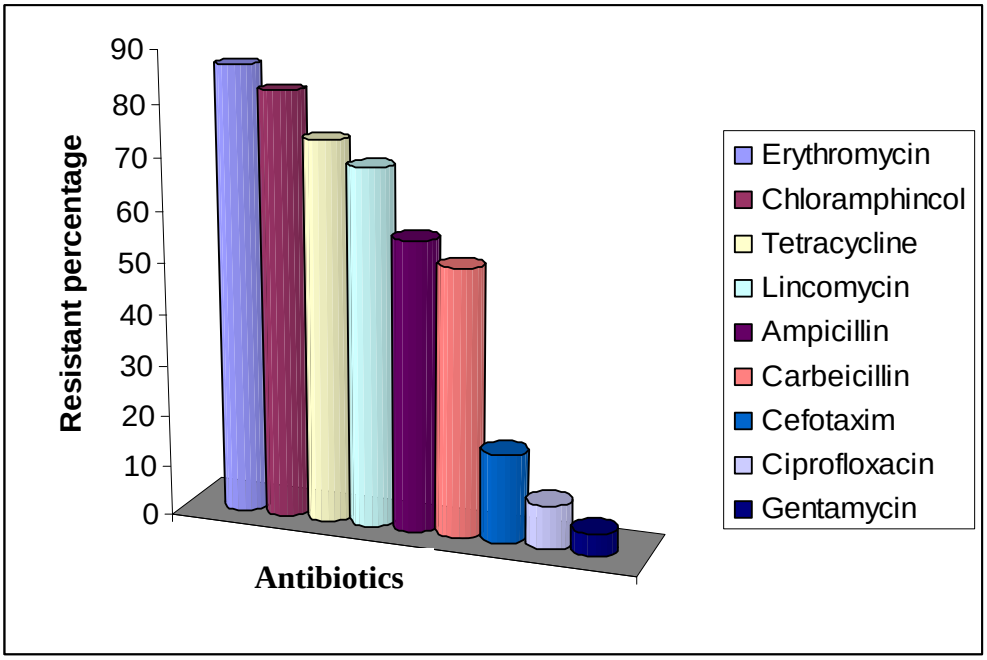


Figure (3-6): Resistant percentage of antibiotics listed against *Proteus* spp.

The following Figure (3-7) showed the some of tested antibiotics against *Proteus* spp. in this study

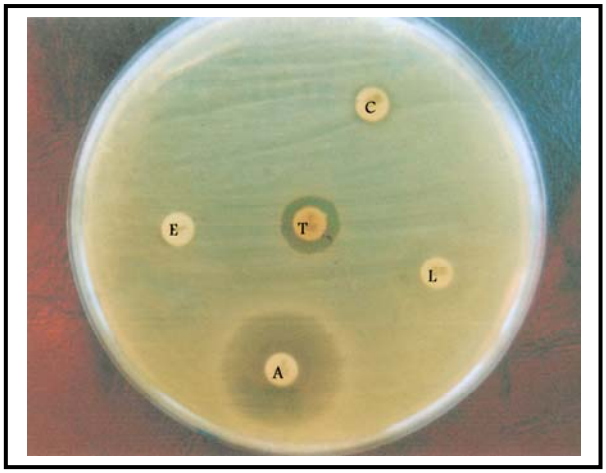


Figure (3-7): The sensitivity test of some tested commercially antibiotics against *Proteus* spp. (isolate no.15), where:

E- Erythromycin. **C-** Chloramphincol. **T-**Tetracycline**.**

L-Lincomycin. **A-** Ampicillin**.**

From Table (3-5) and Figure (3-6), it is evident that the highest percentage of resistance of the bacteria it's towards the antibiotics: Erythromycin, Chloramphincol and Tetracyclin with 86.9 %, 82.6 % and

73.9 % respectively. While the least it's toward Ciprofloxacin and Gentamycin with 8.6 % and 4.3 % respectively.

In this study the most isolates of *Proteus* spp. were sensitive toward Gentamycin and this antibiotic was the best to be selected to affect the isolated *Proteus* spp. from patients with U.T.I. and urinary stones. This bactericidal, antibiotics belong to aminoglycoside group which acts by inhibition of the process of protein synthesis in bacteria via breaking up polysomes of the bacteria and their separation in to monosomes [133,143].

The result of this study goes with the ones showed by Ling *et.al.*[192] and Gurctal [193] they found that percentage of resistance against Gentamycin was 5 % and 6 % respectively.

Mean while these results are not in agreement with Kahlmeter [194] and Karlowsky's results [17] which showed that the percentages were 26.96 % and 21 % respectively. Results also showed that Ciprofloxacin which is one of the quinolenes has an active effect on isolates of *Proteus* spp., since the percentage of resistance of these isolates against this antibiotic is 8.6 %, only which is in line with the results showed by Karlowisky *et.al*. [17] which was 9.1 % for *Proteus* spp. isolated from patients with U.T.I.. The results of the present study are in an agreement with the result showed by [12] which was 8.06 %.

Zhanel *et.al*.[195] in Canada and Obi *et.al*.[196] in Zimbabwe found that all *Proteus* spp. under their studies were sensitive to Ciprofloxacin. This leads them to conclude that this antibiotics could be considered an active and efficient drug for the curative treatment of U.T.I.

The percentage of resistance of the isolated *Proteus* spp. against Tetracycline in this study was 73.9 %. This goes with the results of [12] and Ali *et.al*. in the city of Mousl [197] which was 74.3 % and 78 % respectively. But this result is not in agreement with the result of

Al-Marjani [198] and Ling *et.al.*[192] who found the percentage of resistance against Tetracycline was 96.8 % and 97 % respectively.

Regarding the antibiotics Erythromycin and Chloramphincol, the resistance of the isolated *Proteus* spp. in this study against these antibiotics were the highest as they 86.9 % and 82.6 % respectively. One can conclude that these antibiotics are not suitable in the treatment of patients with U.T.I., and these results were in line with [12] showed as the percentage of resistance was 88.6 %, while the results were not in line with Ling *et.al.*[192] showed the percentage of resistance against Chloramphincol was 56 % .

Lima *et.al.*[199] referred to the careful selection and better use of antibiotics in developed countries that lead to the lower percentage of resistance against antibiotics, while in less developed and developing countries there is uncontrolled and random of antibiotics that lead to higher percentage of resistance against the widely used antibiotics.

The results of the present study showed the importance of selection the suitable antibiotics to be used according to the culture and sensitivity tests, with a clear explanation for patients in real need for the antibiotics regarding the dose and the duration of treatment. All isolated other than 22 are of multi-drug resistant bacteria this sort of resistance is considered as a dangerous problem on the health of human beings which is increasing with time that lead to increase in suffering from U.T.I. [200]. Beside what is written before this problem stimulates for the continuous search about new antibiotics to be used effectively against the infections by *Proteus* spp. [151, 201].

3.2.3. Bacterial sensitivity against the prepared compounds

The results of three methods were used in this study to determine the inhibiting power of prepared compounds against the isolated *Proteus*

spp., were observed and different according to the following technique was used:

3.2.3.1. The disc diffusion method

The prepared compounds showed inhibitory effect in a concentration of 25 mg/mL, where they have the highest inhibition ability for the compounds (7, 8, 9, 10, 11, 12, 13, 14, 15.1, 15.2, 15.3, 15.4, 15.5, 15.6) against *Proteus* spp. by the inhibition zone diameter between (20-25) mm., and the concentration of other compounds (1, 2) showed inhibition zone diameter between (12-18) mm. Also some concentrations of the compounds (3, 4, 6) showed only small inhibition zone against *Proteus* spp. was between (3-9) mm.. But for the concentration of compound no.5 showed no inhibition zone (no inhibitory activity) against the *Proteus* spp.

The compounds (7, 8, 9, 10, 11, 12, 13, 14) with high inhibitory effect against isolated *Proteus* spp., because the fact that have urea or thiourea functional group act as competitive inhibition against urease that have consist of the same group, and also there compound have more important heterocyclic as (benzothiazoles, oxadiazoles, thiadiazoles and triazoles) included biological activity more than another groups and also there compounds consist of donating groups are found in their structure as (phenyl group, $-NH_2$).

The compounds (15.1, 15.2, 15.3, 15.4, 15.5, 15.6) are considered as Schiff bases and their have antibacterial effects are highest because these compounds consist of (C=N) group which is peculiar to the schiff bases, and this group increases the activity of these compounds against the growth of *Proteus* spp., Table (3-6) showed the inhibition zone diameter of prepared compounds against its bacteria:

Table (3-6): The inhibition zone diameter of prepared compounds against *Proteus* spp. in different concentrations (mg/mL)

No.	0.1mg/mL	1 mg/mL	10mg/mL	25mg/mL
1	-	-	+	++
2	-	+	++	++
3	-	-	-	+
4	-	+	+	+
5	-	-	-	-
6	-	-	-	+
7	-	-	+	+++
8	-	-	++	++
9	-	-	+	+++
10	-	+	++	+++
11	-	-	+	++
12	+	+	++	+++
13	-	-	+	++
14	-	+	++	+++
15.1	-	-	+	+++
15.2	-	+	++	+++
15.3	-	-	+++	+++
15.4	-	-	+	+++
15.5	-	-	++	+++
15.6	-	-	+	+++

Where: +++ = (20-25) mm.

++ = (12-18) mm.

\+ = (3-9) mm.,

\- = No inhibition.

From Table (3-6) shows when increase the concentration of these compounds increase the inhibitory effect against *Proteus* spp. especially in 25 mg/dL conc., the following Figure (3-8) showed the some of these compounds in disc diffusion method against *Proteus* spp.

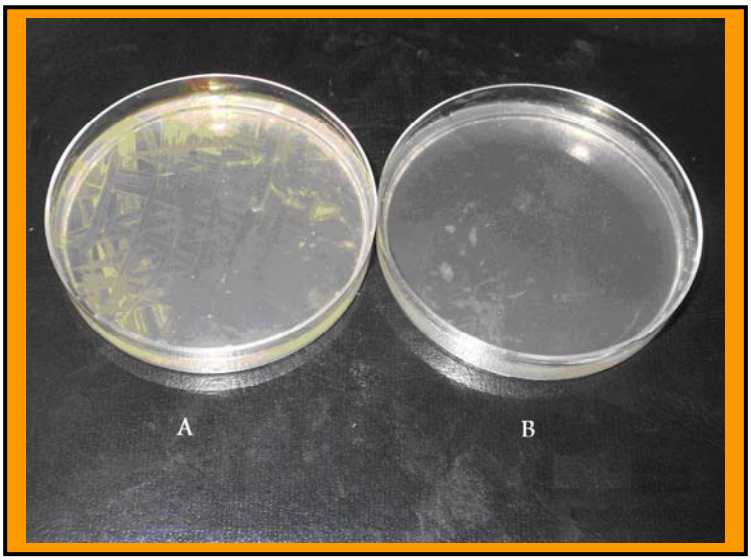
Figure (3-8): Inhibition activity of some prepared compounds against *Proteus* spp. using disc diffusion method, in 25 mg/dL conc.

A-Compound.no.1**, B**-Compound no.2

C-Compound no.3, **D**- Compound no.4**.**

3.2.3.2. The pour - plate method

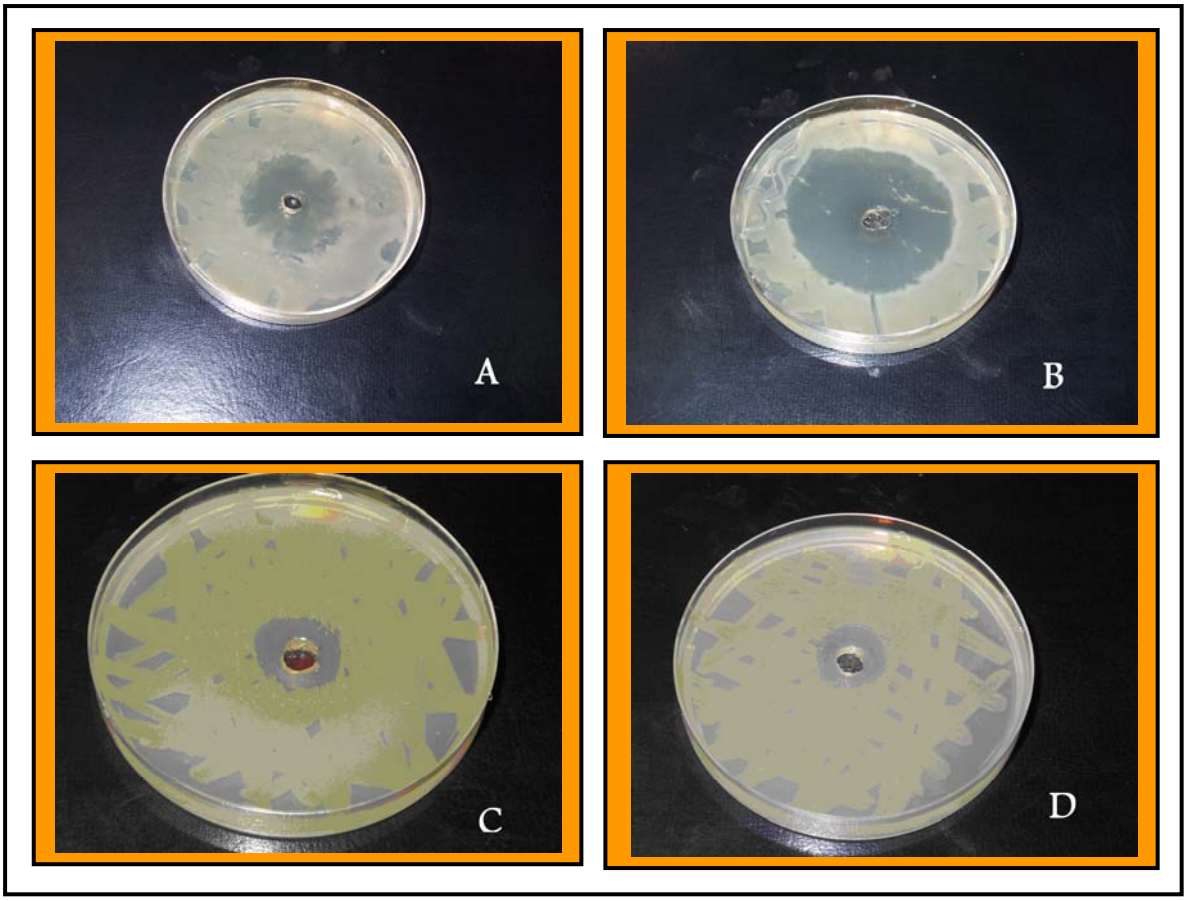
This method depending on the volume of prepared compounds solutions was added to M.H.A with cultured by isolated *Proteus* spp. [167]. From the series of concentration of the compounds and by general law of dilution, only volumes that range as (0.75, 1, 1.25, 2.5) mL of these compounds show inhibitory activity against *Proteus* spp., the 2.5 mL volume was added gave the highest inhibitory activity in this method and for most prepared compounds. The Figure (3-9), showed the inhibition of some prepared compound against *Proteus* spp.



Figure(3-9):Inhibition activity of some prepared compounds against *Proteus* spp. using Pour - plate method,{A-Bacteriostatic of growth**, B-** Bactericidal of growth**}.**

3.2.3.3. The well-diffusion method

The amount of prepared compounds was added to the well in M.H.A. [168] that culture by isolated *Proteus* spp. in this study, showed inhibitory effect against its bacteria by diffusion on agar in a concentration 25 mg/mL. The prepared compounds (7, 8, 9, 10, 11, 12, 13, 14, 15.1, 15.2, 15.3, 15.4, 15.5, 15.6) shows the highest inhibition zone diameter between (25-30) mm., other compounds (1, 2) observed inhibition zone diameter between (17-23) mm. and some of the prepared compounds as (3, 4, 6) showed small inhibition zone against gram-negative *Proteus* spp. was between (4-11) mm.. But no inhibition zone from the concentration of compound no.5 against isolated *Proteus* spp., and the following Figure (3-10) showed the inhibition zone diameter of the some prepared compounds in this method.



Figure(3-10):The inhibition zone of growth of some prepared compounds against *Proteus* spp., using well-diffusion method, in 25 mg/dL conc.{A-Compound no.2, B-Compound no.12, C-Compound no.3, D-Compound no.6}.

From the results of this method, the biological activity of a prepared compound in this method is similar to the biological effect in disc diffusion method, but in higher values of the inhibition zone diameter. Because in the well-diffusion method concentration of prepared compounds as the higher in liquid state, therefore diffusion of these compounds more than in solid state as in disc diffusion method.

Also from all results in this study for commercially antibiotics and new prepared compounds in sensitivity test, showed the *Proteus* spp. have the ability to develop resistance to their chemotherapeutic agents. Such strains which are resistant cause major health and economic problems in the level of the world [150],

for this reason searching for new antimicrobial agents are a continuous process and great efforts have been employed to find new antibiotics or new heterocyclic compounds with good antimicrobial activity which be used as chemotherapeutic (antimicrobial) agents.

3.2.4. Chemical composition of stone samples

Qualitative chemical analysis has been used in this study to investigate the presence of carbonate, phosphate, ammonium, calcium, and magnesium ions for 13 stones that were removed from bladder and kidney of 23 selected patient's samples that are infected by *Proteus* bacteria.

The results obtained from this study have indicated that phosphate, ammonium, and magnesium ions which called struvite stones which are commonly found more than other infected types of stones, followed by stones of carbonate, phosphate and calcium ions which called apatite carbonate stones, and the less type ratio was for carbonate, ammonium, magnesium, phosphate and calcium ions as well this type is called mixed stones.

Eight, four and one stones of struvite, apatite carbonate and mixed types were found in the stones of this study, and the percentages for chemical composition of latter stones types were 61.5 %, 30.7 % and 7.7 % respectively.

These results are in an agreement with Martinez *et.al*.[202] who found that the formation percentage of struvite stone was 57.5 %, apatite stone 28.2 % and mixed stones 9.1 %, and the others of oxalate ions was the 5.1 %.

Many former studies indicated that stones are removed from patients suffering from urinary tract infection, are belonging to struvite and apatite [203].

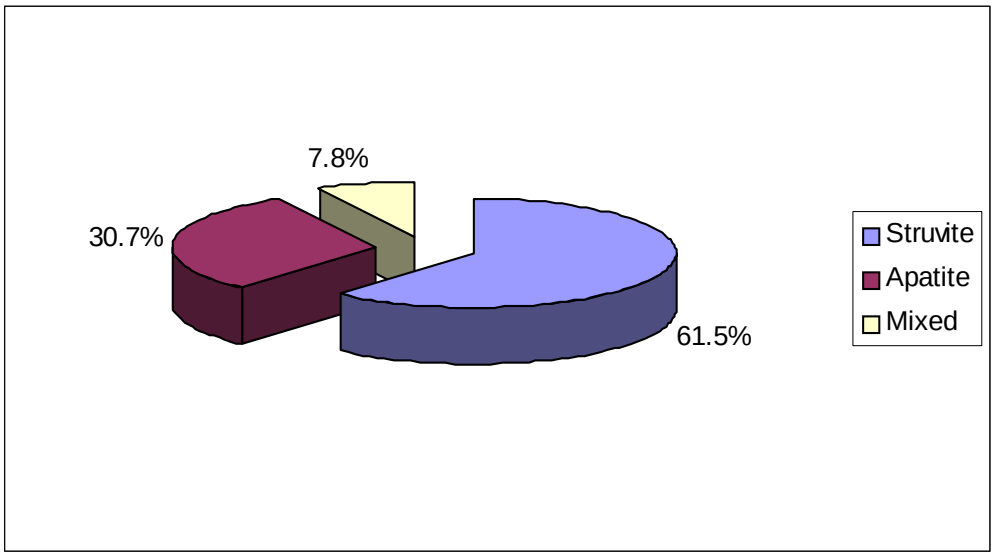


Figure (3-11): The percentage of urinary stones formation in patients U.T.I. according to qualitative chemical analysis.

3.2.5. The biochemical tests

3.2.5.1. Blood urea

Table (3-7) showed the mean value of blood urea in patients with U.T.I. and /or urinary stone is (31.65 ± 15.30 mg/dL), while in control samples is (22.51 ± 3.25 mg/dL). Statistically there is a significant difference observed ($P< 0.05$) in blood urea of patients when compared to that of control group, and this is also showed in Figure (3-12).

Table (3-7): Biostatistical calculations of Z-test for blood urea level (mg/dL) in patients and control groups.

Group	No.	Mean (mg/dL)	S.D.	Range (mg/dL)		P-value
				Min.	Max.	
Patient	200	31.65	15.30	18 - 61		< 0.05 (significant)
Control	100	22.51	3.25	16 - 28		

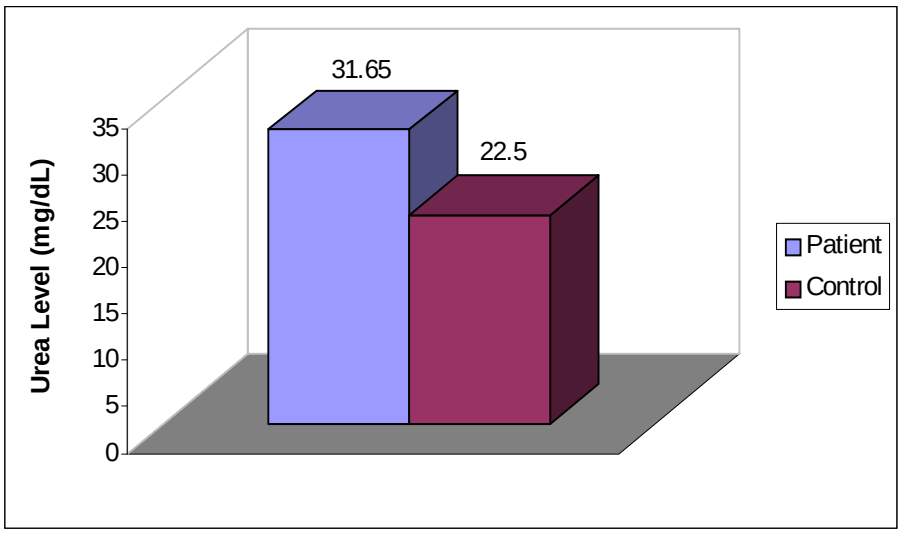


Figure (3-12): The urea level (mg/dL) in serum for patients and control groups.

The result of the present study is in agreement with [204], who found an elevation of urea level in patients with U.T.I. and / or renal stone because of renal dysfunction, since the measurement of blood urea concentration have considerable clinical value though it is a poorer index of filtration rate than the serum creatinine level. So the estimation of blood urea has a great implication in the management of patients with renal stone or U.T.I. because higher than 50 mg/dL implies impairment of glumerular filtration [204].

3.2.5.2. Uric acid

Table (3-8) showed the mean value of serum uric acid in patients with U.T.I. and /or urinary stone is (6.28 ± 1.75 mg/dL), while in control samples is (3.93 ± 0.36 mg/dL). Statistically there is a significant difference observed ($P< 0.05$) in serum uric acid of patient when compared to that of control groups, and this is also showed in Figure (3-13).

Table (3-8): Biostatistical calculations of Z-test for serum uric acid level (mg/dL) in patients and control groups.

Group	No.	Mean (mg/dL)	S.D.	Range (mg/dL)		P-value
				Min	Max	
Patient	200	6.28	1.75	4.3 - 9.5		< 0.05 (significant)
Control	100	3.93	0.36	2.8 - 4.2		

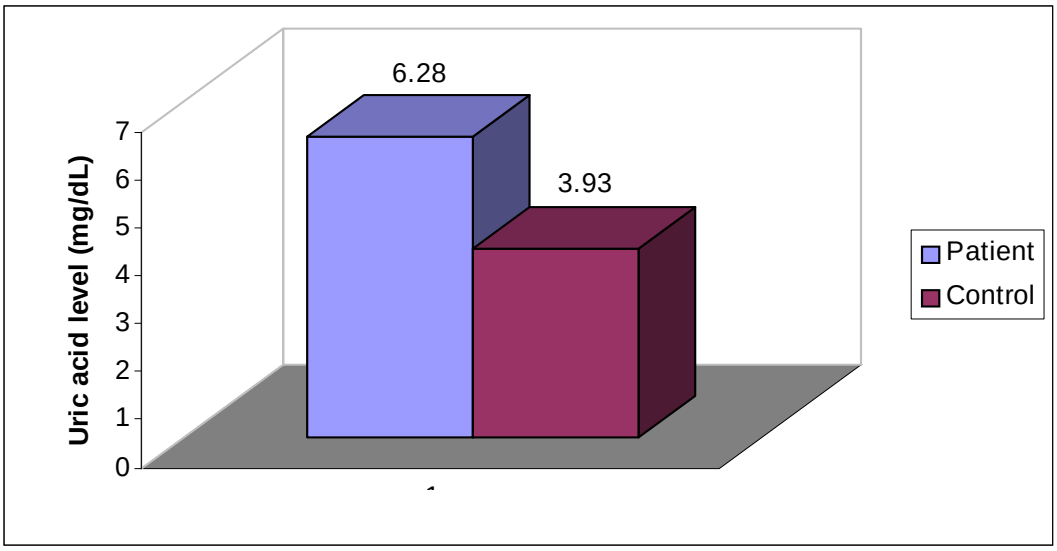


Figure (3-13): The uric acid level in (mg/dL) for patients and control groups.

The result of the present study is in agreement with [205], which explains the relationship between the hyperurecemia and renal function defects, since the elevated level of uric acid in blood is potentially nephrotoxic, on the other hand there is a good evidence of some varieties of renal disease may be associated with unusual degree of hyperurecemia that is complicated by secondary gout [205].

3.2.5.3. Serum glucose

Table (3-9) showed the mean value of serum glucose in patients with U.T.I. and/ or renal stone is (160.75 ± 38.36 mg/dL), while in control samples is (95.76 ± 7.51 mg/dL). Statistically there is no significant difference observed (P > 0.05) in serum glucose of patient when compared to that of control groups, and this is also showed in Figure (3-14).

Table (3-9): Biostatistical calculations of Z-test for serum glucose level (mg/dL) in patient and control groups.

Group	No.	Mean (mg/dL)	S.D.	Range (mg/dL)		P-value
				Min	Max	
Patient	200	137.85	38.36	110 - 170		> 0.05 (**no significant**)
Control	100	100.76	7.51	85 - 130		

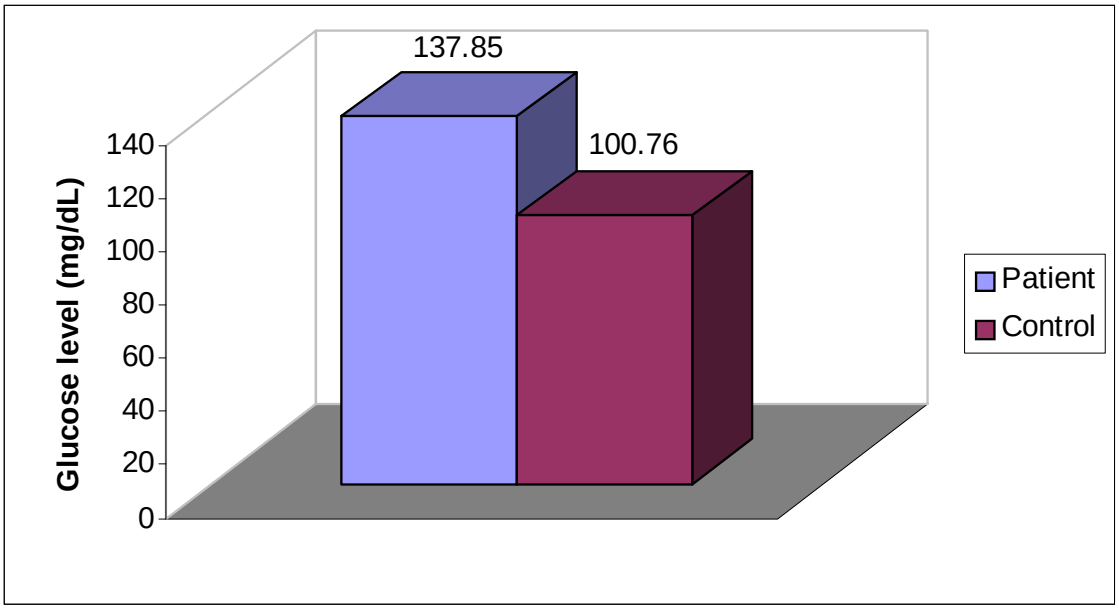


Figure (3-14): The glucose level in (mg/dL) for patient and control groups.

The result of this study is an agreement with [206], who found the presence of elevation of glucose level in U.T.I. and renal stone patients, but with no significance when compared with control group. It can be explained by the fact that the kidney synthesizes the glucose from amino acids by gluconeogenesis like liver, and releases it into the blood stream.

3.2.5.4. Serum creatinine

Table (3-10) showed the mean value of serum creatinine in patients with U.T.I. and /or urinary stone is (1.45 ± 0.73 mg/dL), while in control samples is (0.71 ± 0.18 mg/dL). Statistically there is a significant difference observed (P< 0.05) in serum creatinine of patient when compared to that of control groups, and this is also showed in Figure (3-15).

Table (3-10): Biostatistical calculations of Z-test for serum creatinine level (mg/dL) in patients and control groups.

Group	No.	Mean (mg/dL)	S.D.	Range (mg/dL)		P-value
				Min	Max	
Patient	200	1.45	0.73	0.6 - 2.7		< 0.05 (significant)
Control	100	0.71	0.18	0.5 - 0.9		

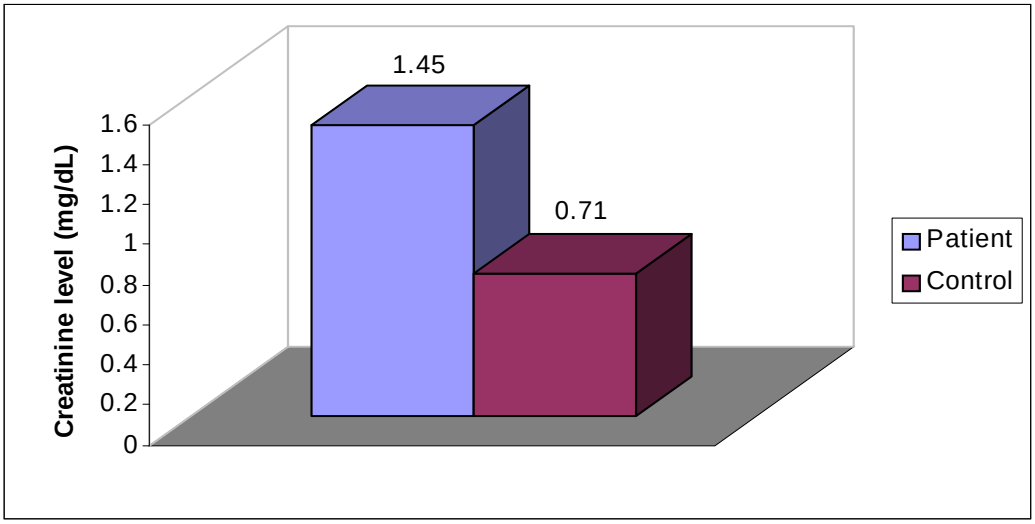


Figure (3-15): The creatinine level in (mg/dL) for patients and control group.

The result of this study is in agreement with [207], which states the presence of elevation of creatinine concentration in U.T.I. and renal stone patients explained by the fact that the creatinine is excreted by glumerular filtration, changes in serum creatinine concentration are almost always a reflection of changes in the G.F.R. as a measurement of renal function [207]. So higher results of serum creatinine in patients of this study than in the controls could well be explained by defect in renal function due to the destructive effect of renal stones and U.T.I. on the tissues of the kidney and other organs of the urinary system.

3.2.5.5. Serum calcium

Table (3-11) showed the mean value of serum calcium in patients with U.T.I. and /or urinary stone is (10.7 ± 8.24 mg/dL), while in control samples is (7.9 ± 6.35 mg/dL). Statistically there is a significant difference observed ($P< 0.05$) in serum calcium of patient when compared to that of control groups, and this is also showed in Figure (3-16).

Table (3-11): Biostatistical calculations of Z-test for serum calcium level (mg/dL) in patient and control groups.

Group	No.	Mean (mg/dL)	S.D.	Range (mg/dL)		P-value
				Min	Max	
Patients	200	10.7	8.24	10.9 – 14.4		< 0.05 (significant)
Control	100	7.9	6.35	6.2 – 9.8		

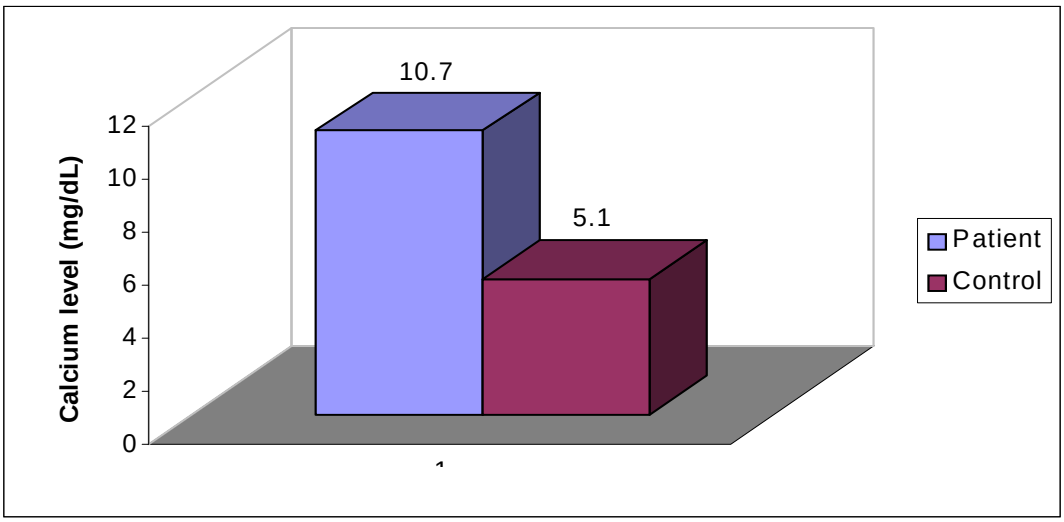


Figure (3-16): The calcium level in (mg/dL) for patient and control groups.

The result of this study is in agreement with [208], which states that the presence of elevation of calcium level in U.T.I. and renal stone patients explained by the disturbance of calcium metabolism in urinary stone (renal stone formation is one of the clinical manifestation of hypercalcemia).

2. Materials and Methods

2.1. Chemicals and Instruments

2.1.1. Chemicals

The chemicals used in this work were listed in Table (2-1):

Table (2-1): The chemicals used in this work with Manufacturer's Company and country.

Reagents	Company supplied (country)
Ammonium hydroxide	BDH(England)
Ammonium acetate	Fluka(Switzerland)
Ammonium carbonate	Fluka(Switzerland)
Ammonium chloride	BDH(England)
Ammonium molybdate	Fluka(Switzerland)
Ammonium solution	Fluka(Switzerland)
Ammonium sulphate	Fluka(Switzerland)
Anhydrous sodium carbonate	BDH(England)
Benzyldehyde	BDH(England)
Calcium carbonate	BDH(England)
Calcium chloride	BDH(England)
Calcium hydroxide	BDH(England)
Calcium oxalate	BDH(England)
Chloroform	BDH(England)
Concentrated nitric acid	BDH(England)
Crystal-violet	BDH(England)
Dimethylsulphoxide(DMSO)	Merck (Germany)

---Continued

Reagents	Company supplied (country)
Ethanol (Absolute)	BDH (England)
Ethyl acetate	Fluka(Switzerland)
Ethanol (95%)	BDH(England)
Glacial acetic acid	BDH(England)
Hydrochloric acid	BDH(England)
Hydrous ammonium oxalate	Fluka(Switzerland)
Hydrous calcium chloride	BDH(England)
Hydrous magnesium chloride	BDH(England)
Hydrous potassium ferrocyanide	BDH(England)
Iodine (I_2)	Fluka(Switzerland)
KH_2PO_4	BDH(England)
Magnesium chloride	BDH(England)
Methanol	Fluka (Switzerland)
Mercury (I) nitrate	Merck (Germany)
Mercury (II) chloride	Merck (Germany)
Na_2HPO_4	BDH(England)
Ortho-Toluidine	Fluka(Switzerland)
Phenol	Merck (Germany)
Potassium hydroxide	BDH(England)
Potassium iodide	Fluka(Switzerland)
Pure mercury	Merck (Germany)
Sodium hydroxide	BDH(England)
Thiourea	BDH(England)
Urea	Fluka(Switzerland)

2.1.2. Instruments

The instruments used in this work were listed in Table (2-2):

Table (2-2): Instruments used in this work with their remarks.

Instrument	Remarks
Autoclave	Sturdy SA- 300 VL (Taiwan)
Balance	Sartorius portable (Germany)
Centrifuge	Haraeus sepatech labofuga A
Compound light microscope	Olympus (Japan)
F.T.I.R.	Shemadzo spectrophotometer (8300).Japan.
Incubator	Gallenkamp (England)
Magnetic stirrer with hot plate	Jlassco (India).
Melting point apparatus, using hot stage	Gallenkamp M.F.B.
Millipore filter paper, (size 11.0 cm), also no.1.	Whatman sterile membrane filter paper
Oven	Memrert 854 Schwabach (Germany)
pH-meter	Expandable ion analyzer
UV-Visible spectrophotometer	Shemadzo -1650 PC
Vacuum pump	Schuco.Inc (England)
Vortex	Griffin and George Ltd.
Water bath (0-100) $^{\circ}$C.	Precisterm-Tafesa-W .Germany

2.1.3. Cultures media

The cultures media used in this work were listed in Table (2-3):

Table (2-3): Cultures media used in this work with their company country supplied and using.

Media	Company supplied (country)	Using
Agar- Agar (AA)	Mumbai (India)	For routine bacteriological work.
Blood Agar (BA)	Mumbai (India)	For isolation and cultivation of many fastidious pathogenic microorganism.
MacConkeys Agar (MA)	Mumbai (India)	For selective isolation and differentiation of coliform organisms and other enteric pathogens.
Mueller Hinton Agar (MHA)	Mumbai (India)	For determination of susceptibility of microorganism to antimicrobial agents.
Nutrient Agar (NA)	Mumbai (India)	For general culture media for isolation the microorganism.
Nutrient Broth (NB)	Mumbai (India)	For activation the microorganism before tested.
Urea Broth Base (UBB)	Mumbai (India)	For the identification of bacteria on the basis of urea utilization.

2.1.4. Antibiotics discs

The antibiotics discs used in this work were listed in Table (2-4):

Table (2-4): The antibiotics discs used in this work with their standard inhibition zones [155,156].

Antibiotics	Symbol	Remark	Conc. of antibiotic in disc (µg)	Inhibition zone (mm).		
				R (or <)	I	S (or >)
Ampicillin	A	India	10	11	12-13	14
Carbenicillin	Cb	India	100	13	14-16	17
Cefotaxiime	Ce	India	30	14	15-22	23
Chloramphenicol	C	India	30	12	13-17	18
Ciprofloxacin	Cf	India	5	15	16-20	21
Erythromycin	E	India	15	13	14-17	18
Gentamycin	G	India	10	12	13-14	15
Lincomycin	L	India	2	9	10-14	15
Tetracycline	T	India	30	14	15-18	19

2.2. Methods

2.2.1. Samples collection

From November 2005 to March 2006 urine and blood specimens were collected from 200 patients definitely diagnosed with U.T.I. and/or urinary calculi. Those patients attending (Diwaniya teaching hospital, teaching hospital for Pediatrics and Gynecology, from health centers and private clinics) in Al-Diwaniya Governorate were included in the study.

On the other hand, 100 samples were also collected from persons do not suffering from U.T.I. were taken as a control group. A case sheet for all samples was filled with information as a questionnaire.

2.2.1.1. Collection of serum

Three milliliter of venous blood was obtained from each patient as well as healthy individual and sent for biochemical tests (B.U., S.Cr., U.A., R.B.S. and Ca^{+2} ions.). Sera were separated by centrifugation of blood (3000 r.p.m. for 10 minutes), and stored in the refrigerator at -20 ^{0}C [157].

2.2.1.2. Collection of urine

Midstream urine samples were collected from patient and from healthy, who were instructed on how to collect the midstream urine into sterile bottles. The samples were then transported to the laboratory with ice packs in sterile containers. They were then kept in the refrigerator at 4 ^{0}C until analysis and transplantation, which was done within 24 hour [157].

2.2.1.3. Collection of stones

Arrangements were made with surgeons doing operations for urinary stones' aiming at obtains 13 stones removed from the urinary tract (7 stones from the kidney and 6 from the bladder) of patients. Stones were collected at the operating theaters in clean glass containers and carried to the laboratory for stone analysis to reveal the chemical composition of each stone.[158].

2.2.2. Preparation of chemical compounds

A series of a novel compounds (urea, thiourea and Schiff bases) derivatives were prepared and their biological activities were examined.

2.2.2.1. N-4-aminobenzylurea (0.1 M)

N-4-aminobenzylurea was prepared by dissolving 1.65 gm of N-4-aminobenzylamine and 0.6 gm of urea in 10 mL of absolute ethanol, then one drop of concentrated HCl was added in a round bottom flask. The mixture was refluxed for 5 hr. Ice bath was used to separate the precipitate. The mixture was filtered and purified by dissolving it in an absolute ethanol and then recrystallize by addition of distilled water. The precipitate is dark brown in color as a product.

2.2.2.2. N-4-aminobenzylthiourea (0.1 M)

N-4-aminobenzylthiourea was prepared by dissolving 1.81 gm of N-4-aminobenzylamine and 0.76 gm of thiourea in 10 mL of absolute ethanol, then one drop of concentrated HCl was added in a round bottom flask. The mixture was refluxed for 5 hr.. Ice bath was used to separate the precipitate. The mixture was filtered and purified by dissolving it in an absolute ethanol, and then recrystallize by addition of distilled water, the precipitate is deep violet in color as a product.

2.2.2.3. N-pyrimidine-6-ylurea (0.1 M)

N-pyrimidine-6-ylurea was prepared by dissolving 1.52 gm of N-pyrimidine-6-ylamine and 0.6 gm of urea in 10 mL of absolute ethanol, then two drop of concentrated HCl was added in a round bottom flask. The mixture was refluxed for 8 hr. Ice bath was used to separate the precipitate. The mixture was filtered and purified by dissolving it in an absolute ethanol, and then recrystallize by addition of distilled water, the precipitate is brown in color as a product.

2.2.2.4. N-pyrimidine-6-ylthiourea(0.1 M)

N-pyrimidine-6-ylthiourea was prepared by dissolving 1.68 gm of N-4-aminobenzylamine and 0.76 gm of thiourea in 10 mL of absolute ethanol, then two drop of concentrated HCl was added in a round bottom flask. The mixture was refluxed for 8 hr. Ice bath was used to separate the precipitate. The mixture was filtered and purified by dissolving it in an absolute ethanol and then recrystallize by addition of distilled water. The precipitate is light brown in color as a product.

2.2.2.5. N-pyridine-4-ylurea (0.1 M)

N-pyridine-4-ylurea was prepared by dissolving 1.51 gm of N-pyridine-4-ylamine and 0.6 gm of urea in 10 mL of absolute ethanol, then two drop of concentrated HCl was added in a round bottom flask. The mixture was refluxed for 6 hr. Ice bath was used to separate the precipitate. The mixture was filtered and purified by dissolving it in an absolute ethanol, then recrystallize by addition of distilled water to form pure crystals as a product.

2.2.2.6. N-pyridine-4-ylthiourea (0.1 M)

N-pyridine-4-ylthiourea was prepared by dissolving 1.67 gm of N-pyridine-4-ylamine and 0.76 gm of thiourea in 10 mL of absolute ethanol, then two drop of concentrated HCl was added in a round bottom flask. The mixture was refluxed for 6 hr. Ice bath was used to separate the precipitate. The mixture was filtered and purified by dissolving it in an absolute ethanol, then recrystallize by addition of distilled water to form pure crystals.

2.2.2.7. N-benzothiazole-2-ylurea (0.1 M)

N-benzothiazole-2-ylurea was prepared by dissolving 1.93 gm of 2-aminobenzothiazole and 0.6 gm of urea in 10 mL of absolute ethanol, then two drop of concentrated HCl was added in a round bottom flask. The mixture was refluxed for 5 hr. Ice bath was used to separate the precipitate. The mixture was filtered and purified by dissolving it in an absolute ethanol, then recrystallize by addition of distilled water to form pure grey crystals.

2.2.2.8. N-benzothiazole-2-ylthiourea (0.1 M)

N-benzothiazole-2-ylthiourea was prepared by dissolving 2.09 gm of 2-aminobenzothiazole and 0.76 gm of thiourea in 10 mL of absolute ethanol, then two drop of concentrated HCl was added in a round bottom flask. The mixture was refluxed for 5 hr. Ice bath was used to separate the precipitate. The mixture was filtered and purified by dissolving it in an absolute ethanol, then recrystallize by addition of distilled water to form pure reddish-brown crystals.

2.2.2.9. [N-(5-phenyl-1,3,4-oxadiazole)-2-yl] urea(0.1 M)

[N-(5-phenyl-1,3,4-oxadiazole)-2-yl]urea was prepared by dissolving 2.04 gm of 2-amino-5-phenyl-1,3,4-oxadiazole and 0.6 gm of urea in 10 mL of absolute ethanol, then two drop of concentrated HCl was added in a round bottom flask. The mixture was refluxed for 5 hr. Ice bath was used to separate the precipitate. The mixture was filtered and purified by dissolving it in an absolute ethanol, then recrystallize by addition of distilled water to form pure orange crystals.

2.2.2.10. [N-(5-phenyl-1,3,4-oxadiazole)-2-yl] thiourea(0.1 M)

[N-(5-phenyl-1,3,4-oxadiazole)-2-yl]thiourea was prepared by dissolving 2.2 gm of 2-amino-5-phenyl-1,3,4-oxadiazole and 0.76 gm of thiourea in 10 mL of absolute ethanol, then two drop of concentrated HCl was added in a round bottom flask. The mixture was refluxed for 5 hr. Ice bath was used to separate the precipitate. The mixture was filtered and purified by dissolving it in an absolute ethanol, then recrystallize by addition of distilled water to form pure light-orange crystals.

2.2.2.11. [N-(5-phenyl-1,3,4-thiadiazole)-2-yl] urea(0.1 M)

[N-(5-phenyl-1,3,4-thiadiazole)-2-yl]urea was prepared by dissolving 2.2 gm of 2-amino-5-phenyl-1,3,4-thiadiazole and 0.6 gm of urea in 10 mL of absolute ethanol, then two drop of concentrated HCl was added in a round bottom flask. The mixture was refluxed for 5 hr. Ice bath was used to separate the precipitate. The mixture was filtered and purified by dissolving it in an absolute ethanol, then recrystallize by addition of distilled water to form pure crystals.

2.2.2.12.[N-(5-phenyl-1,3,4-thiadiazole)-2-yl] thiourea(0.1 M)

[N-(5-phenyl-1,3,4-thiadiazole)-2-yl]thiourea was prepared by dissolving 2.36 gm of 2-amino-5-phenyl-1,3,4-thiadiazole and 0.76 gm of thiourea in 10 mL of absolute ethanol, then two drop of concentrated HCl was added in a round bottom flask. The mixture was refluxed for 5 hr. Ice bath was used to separate the precipitate. The mixture was filtered and purified by dissolving it in an absolute ethanol, then recrystallize by addition of distilled water to form pure crystals.

2.2.2.13. [N-(5-phenyl-1,3,4-triazole)-2-yl] urea(0.1 M)

[N-(5-phenyl-1,3,4-triazole)-2-yl]urea was prepared by dissolving 2.03 gm of 2-amino-5-phenyl-1,3,4-triazole and 0.6 gm of urea in 10 mL of absolute ethanol, then two drop of concentrated HCl was added in a round bottom flask. The mixture was refluxed for 5 hr. Ice bath was used to separate the precipitate. The mixture was filtered and purified by dissolving it in an absolute ethanol, then recrystallize by addition of distilled water to form pure crystals.

2.2.2.14. [N-(5-phenyl-1,3,4-triazole)-2-yl] thiourea(0.1 M)

[N-(5-phenyl-1,3,4-triazole)-2-yl]thiourea was prepared by dissolving 2.19 gm of 2-amino-5-phenyl-1,3,4-triazole and 0.76 gm of thiourea in 10 mL of absolute ethanol, then two drop of concentrated HCl was added in a round bottom flask. The mixture was refluxed for 5 hr. Ice bath was used to separate the precipitate. The mixture was filtered and purified by dissolving it in an absolute ethanol, then recrystallize by addition of distilled water to form pure crystals.

2.2.2.15. Preparation of Schiff-bases

2.2.2.15.1.[benzylidene-6-aminopyrimidine]carboamide

[benzylidene-6-aminopyrimidine]carboamide was prepared by mixed 0.01 mole of benzyldehyde and 0.01 mole of N-pyrimidine-6-ylurea in a round bottom flask, 10 mL of absolute ethanol was added to the mixture, and two drops of glacial acetic acid were added, then mixture was refluxed for 6 hr..Ice bath was used to separate the product; it was filtered and washed by absolute ethanol. The product is orange in color.

2.2.2.15.2. [benzylidene-6-aminopyrimidine] carbothioamide

[benzylidene-6-aminopyrimidine]carbothioamide was prepared by mixed 0.01 mole of benzyldehyde and 0.01 mole of N-pyrimidine-6-ylthiourea in a round bottom flask, 10 mL of absolute ethanol was added to the mixture, and two drops of glacial acetic acid were added, then the mixture was refluxed for 6 hr. Ice bath was used to separate the product, it was filtered and washed by absolute ethanol. The product is pale orange in color.

2.2.2.15.3. [benzylidene-4-aminopyridine] carboamide

[benzylidene-4-aminopyridine]carboamide was prepared by mixed 0.01 mole of benzyldehyde and 0.01 mole of N-pyridine-4-ylurea were mixed in a round bottom flask . 10 mL of absolute ethanol was added to the mixture, and two drops of glacial acetic acid were added, then the mixture was refluxed for 5 hr. Ice bath was used to separate the product, it was filtered and washed by absolute ethanol. The product is yellow in color.

2.2.2.15.4. [benzylidene-4-aminopyridine] carbothioamide

[benzylidene-4-aminopyridine]carbothioamide was prepared by mixed 0.01 mole of benzyldehyde and 0.01 mole of N-pyridine-4-ylthiourea were mixed in a round bottom flask. 10 mL of absolute ethanol was added to the mixture, and two drops of glacial acetic acid were added, then the mixture was refluxed for 5 hr. Ice bath was used to separate the product, it was filtered and washed by absolute ethanol. The product is deep-yellow in color.

2.2.2.15.5. [benzylidene-2-aminobenzothiazol] carboamide

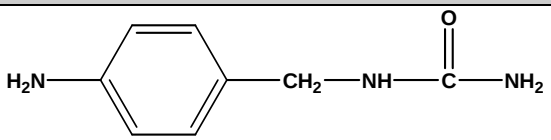
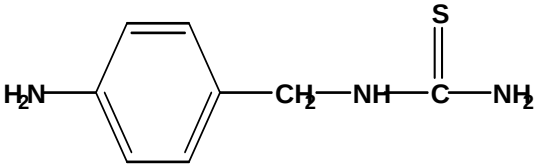
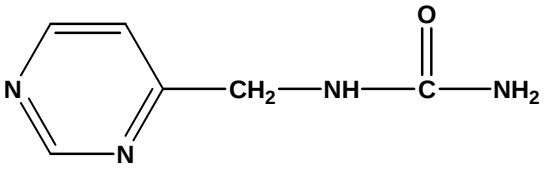
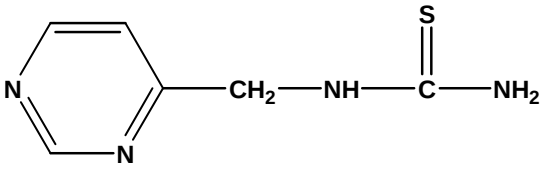
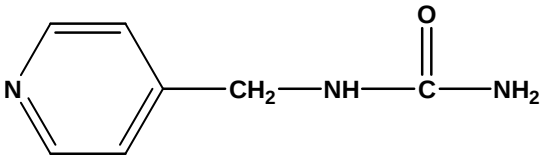
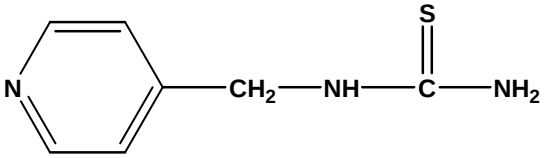
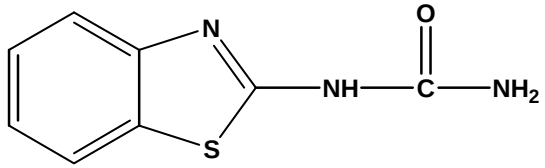
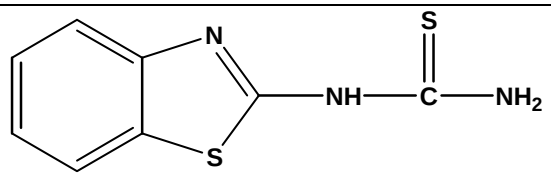
[benzylidene-2-aminobenzothiazol]carboamide was prepared by mixed 0.01 mole of benzyldehyde and 0.01 mole of N-benzothiazole-2-ylurea were mixed in a round bottom flask. 10 mL of absolute ethanol was added to the mixture, and two drops of glacial acetic acid were added, then the mixture was refluxed for 5 hr. Ice bath was used to separate the product; it was filtered and washed by absolute ethanol. The product is brown crystals in color.

2.2.2.15.6. [benzylidene-2-aminobenzothiazol]carbothioamide

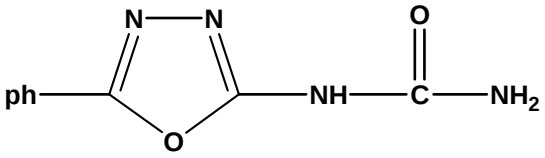
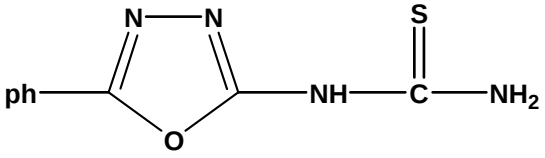
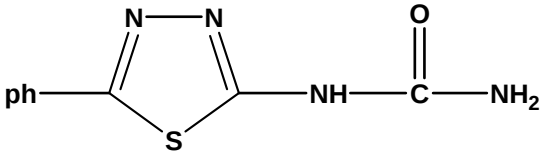
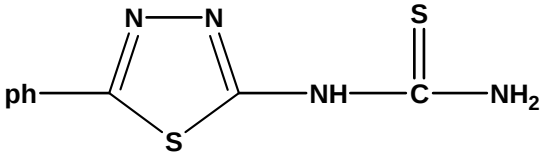
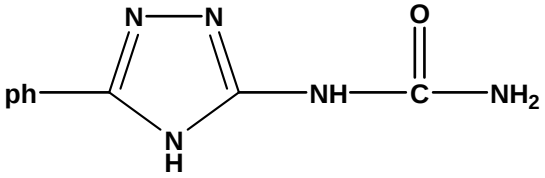
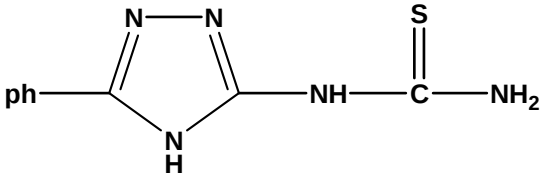
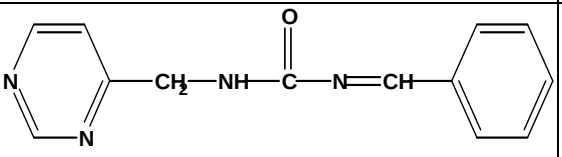
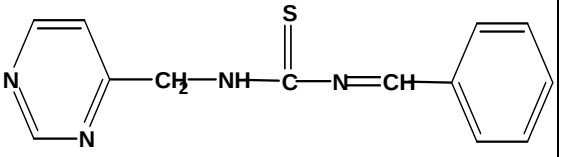
[benzylidene-2-aminobenzothiazol]carbothioamide was prepared by mixed 0.01 mole of benzyldehyde and 0.01 mole of N-benzothiazole-2-ylthiourea were mixed in a round bottom flask. 10 mL of absolute ethanol was added to the mixture, and two drops of glacial acetic acid were added, then mixture was refluxed for 5 hr. Ice bath was used to separate the product. It was filtered and washed by absolute ethanol. The product is pale brown crystals in color.

All of these prepared compounds purities were monitored by T.L.C.; it was used with chloroform and ethyl acetate in ratio (1:1) as eluent .The names and structures of these compounds are shown in the following Table (2-5):

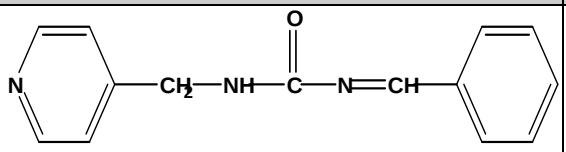
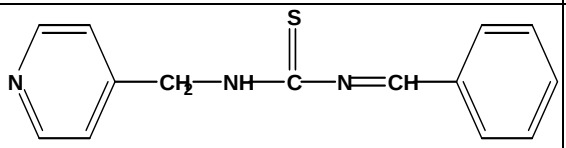
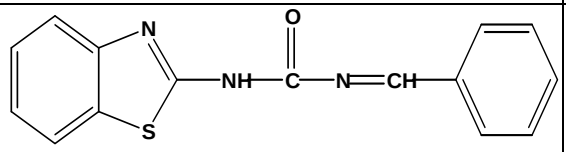
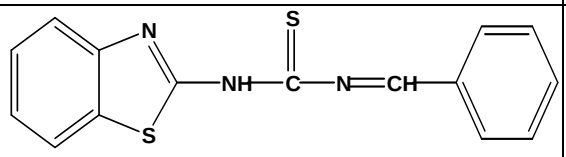
Table (2-5): The chemical structure and name of prepared compounds.

No.	Structure	The name of compound
1.		N-4-aminobenzylurea
2.		N-4-aminobenzylthiourea
3.		N-pyrimidine-6-ylurea
4.		N-pyrimidine-6-ylthiourea
5.		N-pyridine-4-ylurea
6.		N-pyridine-4-ylthiourea
7.		N-benzothiazole-2-ylurea
8.		N-benzothiazole-2-ylthiourea

---Continued

No.	Structure	The name of compound
9.		[N-(5-phenyl-1,3,4-oxadiazol)-2-yl]urea
10.		[N-(5-phenyl-1,3,4-oxadiazol)-2-yl]thiourea
11.		[N-(5-phenyl-1,3,4-thiadiazol)-2-yl]urea
12.		N-(5-phenyl-1,3,4-thiadiazol)-2-yl]thiourea
13.		[N-(3-phenyl-1,3,4-triazol)-5-yl]urea
14.		[N-(3-phenyl-1,3,4-triazol)-5-yl]thiourea
15.1		[benzylidene)-6-amino pyrimidine]carboamide
15.2		[benzylidene)-6-amino pyrimidine]carbothioamide

---Continued

No.	Structure	The name of compound
15.3		[benzylidene)-4-amino pyridine]carboamide
15.4		[benzylidene)-4-amino pyridine]carbothioamide
15.5		[benzylidene)-2-amino benzothiazol] carboamide
15.6		[benzylidene)-2-amino benzothiazol]carbothioamide

2.2.3. Cultures media

The following media were prepared according to instructions of manufacturer, as (Nutrient agar, Nutrient broth, MacConkey agar, Blood agar, Mueller Hinton agar, Urea broth base and Agar–Agar). These media in powder form, and known amount of the specific powders were dissolved in D.W. [159].

2.2.4. Sterilization methods

A. **Autoclaving**

Media and solutions were sterilized by autoclaving at 121 ^{0}C, 15 Ibs.pressure for 15 minutes[160].

B. **Oven sterilization**

Glasswares were sterilized using electric oven at 180 ^{0}C for 3 hrs.

2.2.5. Maintenance of bacterial isolates

Maintenance of bacterial isolates was performed according to Maniatis *et.al.* [161] as following:

- **Short term storage**

  Bacterial isolates were maintained for period of few weeks on MacConkey agar plates. The plates were tightly wrapped in parafilm and stored at 4 ^{0}C.

- **Medium term storage**

  Bacterial isolates were maintained in stab culture for period of few months. Such cultures were prepared in screw-capped bottles containing (5-8 mL) of agar medium and stored at 4 ^{0}C.

- **Long term storage**

  Bacterial can be stored for many years in nutrient broth containing 15 % glycerol at low temperature without significant loss of viability. This was done by adding (1.5 mL) of sterilized glycerol to an exponential growth of bacterial isolates in a screw-capped bottle with final volume (10 mL) and stored at -20 ^{0}C.

2.2.6. Identification of bacterial isolates

This is achieved by following steps for identification the bacterial isolates by appearance of colonies (culture morphology) and by using Api 20E system for this study [162].

2.2.6.1. Primary identification

Colonies of isolated bacteria were identified primarily depending on culture characters as seen on the MacConkey agar, and to notice: swarming phenomenon clearly on the blood agar [160].

2.2.6.2. Direct microscopic examination

The bacteria within each colony were identified by taking a swab using a sterile loop to spread it on sterile slide, then fixation and staining were used by Gram stain.

The Gram is helpful for distinguishing different bacterial types in a sample and determining their predominance [162].The primary stain in the Gram stain is crystal-violet, which is applied to the fixed smear and then rinsed off with water .Gram's iodine is then added to the smear and rinsed off with water. The smear is decolorized with 95 % ethanol, and counter stained with safranin. The Gram stain does not only provide information about Gram reaction, but also about cell size, shape, and arrangement [162].

2.2.6.3. Api 20 E system

The Api 20E system as a standardized characterization system for Enterobacteriaceae and other non-fastidious Gram-negative rods. The system consists of 20 microtubes containing dehydrated substrates. These tests are inoculated with a bacterial suspension which reconstitutes the media. Then after inoculation, strips are incubated in plastic trays at 35 ^{0}C for 24 hr. and then examined for color changes as either a positive or negative test) [162]. The color change results of the Api 20E method are presented in Table (2-6):

Table (2-6): The biochemical tests of Api 20E method [162]

Name	Symbol	Result	
		Positive	Negative
β-galactosidase production	**ONPG**	**Yellow**	**Colorless**
Decarboxylation of arginine	**ADH**	**Purple**	**Yellow**
Decarboxylation of lysine	**LDC**	**Purple**	**Yellow**
Decarboxylation of orinithine	**ODC**	**Purple**	**Yellow**
Citrate utilization	**CIT**	**Blue**	**Green to Yellow**
Hydrogen sulfide production	**H_2S**	**Blackening**	**No Blackening**
Urea hydrolysis	**URE**	**Red**	**Yellow**
Tryptophan deaminase	**TDA**	**Brown**	**Yellow to Orange**
Indole production from tryptophan	**IND**	**Red ring**	**Yellow ring**
Voges- Proskauer	**VP**	**Red**	**Colorless**
Gelatin utilization	**GEL**	**No Blackening**	**Blackening**
Fermentation of glucose	**GLU**	**Yellow**	**Blue to Green**
Fermentation of manitol	**MAN**	**Yellow**	**Blue to Green**
Fermentation of inositol	**INO**	**Yellow**	**Blue to Green**
Fermentation of sorbitol	**SOR**	**Yellow**	**Blue to Green**
Fermentation of rhamnose	**RHA**	**Yellow**	**Blue to Green**
Fermentation of sucrose	**SAC**	**Yellow**	**Blue to Green**
Fermentation of melibiose	**MEL**	**Yellow**	**Blue to Green**
Fermentation of amicdaline	**AMY**	**Yellow**	**Blue to Green**
Fermentation of arabinose	**ARA**	**Yellow**	**Blue to Green**

The metabolic properties of isolates are also used. They are unique for each species. One of the most important tests for *Proteus* spp. was used in this study as a urease production test [163], according to the following steps:

1-The tube of urea broth base (containing urea and pH indicator as phenol red that is yellow-orange at the initial pH of 6.8 but change to pinkish-red at pH 8.4) was inoculated by the taken isolate using a sterile cotton swab.

2- The tube was incubated at 35 ^{0}C for 24 hr. before examination for color change to distinguish species of *Proteus* [164]. As in following Figure (2-1): [162]

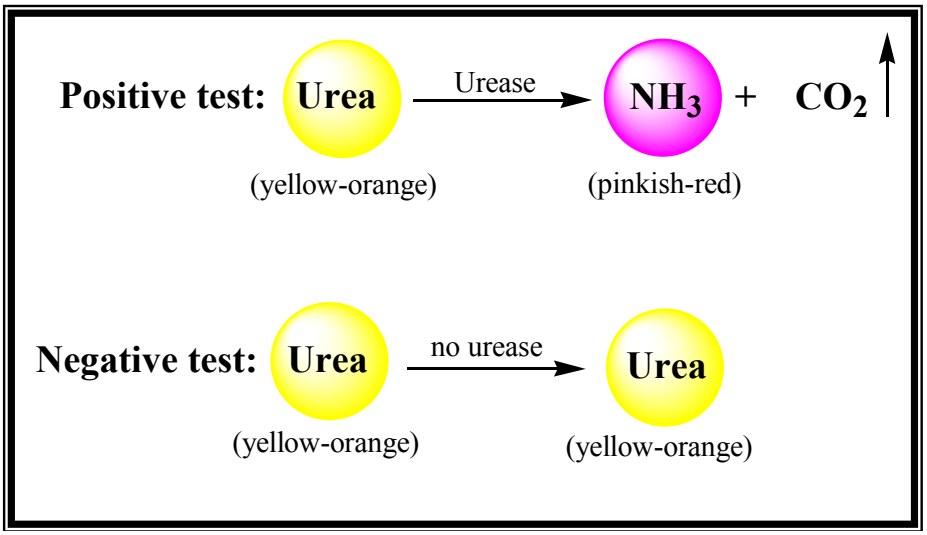


Figure (2-1) : Urea utilization test results [162].

2.2.7. Antibiotics sensitivity tests

2.2.7.1. Bacterial sensitivity tests against commonly used antibiotics

Disc diffusion method was used to determine the inhibitory effects of 9 types of commonly used antibiotics were illustrated in Table (2-4), and used against the isolated bacteria as in the following steps [162]:

1- The plates of Mueller Hinton agar was inoculated by *Proteus* spp. then spread it on surface of medium by three ways (carpet streaking method).

2- These plates left 10-15 minutes from culture, and then the antibiotics discs were placed over the medium by sterilized forceps with compression of discs. Distances were left between disc and another, and also between disc and plate margin to avoid interference between zones of inhibition.

3- These plates were incubated at 37 ^{0}C for 24 hr.

4- Inhibition diameter was measured for each disc using a ruler, and these measured in millimeter (mm), and comparison these results with standard measurements [156].The *Proteus* spp. was considered either , S; I; or R, according to diameter of zone of inhibition.

2.2.7.2. Bacterial sensitivity tests against the prepared compounds

Three methods were used to determine the inhibiting activity of prepared compounds against *Proteus* spp.:

A. Disc diffusion method

The experiment was carried according to Bauer *et.al.* [165], and [166] as follows:

A.1. Preparation of chemical compounds discs

The following concentrations (0.1, 1, 10 and 25) mg/mL for each prepared compound that dissolves in DMSO solvent were illustrated in Table (2-5)**.** Then these compounds were filtered. For each concentration above, 100 discs were made by the use of a punch from the filter paper about 6 mm. in diameter, and then put each disc in one of known concentration of the compound in sterilize vial. Vials were mixed so the discs will be of equal and even concentration to that of the prepared compounds, then discs were dried in oven.

A.2. Culture of bacteria

The plates of Mueller Hinton agar was inoculated by *Proteus* bacterial isolates , by taking a swab and put it in bacterial solution isolate ,and then spread it on surface of medium by three ways (carpet streaking method), and left for 10-15 minutes.

A.3. Discs spread over plate

Prepared discs in (2.2.6.2.1.1.) were placed over the medium by sterilized forceps with compression of discs. Distances were left between disc and another, and also between disc and plate margin to avoid interference between zones of inhibition. Tablets on plate were marked according to the concentrations, and then these plates were incubated at 37 ^{0}C for 24 hr.

A.4. Measurement of zone of inhibition

Zone of Inhibition is an area around the disc where there is no bacterial growth [162].Inhibition diameter was measured for each disc using a ruler, and these measured in millimeter .Also the *Proteus* spp. was considered either, S ; I ; or R , according to diameter of zone of inhibition.

B. Pour- plate method [167]

This method depends upon the variation in volumes of a fixed concentration which is 10 mg/mL of stock solution. A series of concentrations were made from the already prepared compounds with arranged from (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5) mg/mL , and the volume needed from these compounds was calculated according to the general law of dilution.

The known amount of prepared compounds was added to the medium as mixed directly after pouring into the sterile Petri dish. The dish was kept at 15-20 min. at room temperature to get solid, and each dish was labeled with a permanent marker for each volume.

The plate of (Mueller Hinton agar + sol. of prepared compound) was inoculated by *Proteus* bacterial isolates , by taking a swab and put it in bacterial solution isolate ,and then spread it on surface of medium by three Ways (carpet streaking method), and then these plates were incubated at 37 ^{0}C for 24 hr.. Bacterial growth was noticed on each dish. Te results were compared and added with other dishes of different volumes.

C. Well-diffusion method [168, 169]

This method depends upon the variation in concentration of prepared compounds. A series of concentrations were made from the already prepared compounds with arranged from (0.1, 1, 10 and 25) mg/mL. The plates of Mueller Hinton agar was inoculated by *Proteus* bacterial isolates , by taking a swab and put it in bacterial solution isolate ,and then spread it on surface of medium by three ways (carpet streaking method), and left for 10-15 minutes . In each medium five pores were made by the use of a sterile dry rod with a diameter of 6 mm. , these pores were made with equal spaces were left between pores and another, and also between pores and plate margin by use of a prepared diagram on a paper to be put under the dish.

Then the solutions of different concentrations of the prepared compounds were added in an a fixed amount of 0.5 mL from each conc. in one pore , and then these plates were incubated at 37 ^{0}C for 24 hr. and the last step the inhibition diameter was measured for each pore using a ruler ,and these measured in millimeter. The *Proteus* spp. was considered either, S ; I ; or R , according to diameter of zone of inhibition.

2.2.8. The chemical composition of urinary stones

2.2.8.1. Preparation of solutions

In order to analyze the chemical composition of the stones collected for this study, the following solutions were prepared in steps as mentioned by [170]:

1- Lime solution (0.5 M)

This solution was prepared by dissolving 5 gm of calcium hydroxide $Ca(OH)_2$ in 100 mL of D.W., left to stand for 24 hr., and the supernatant clear solution was used in the chemical analysis.

2- Sodium carbonate (0.5 M)

This solution was prepared by dissolving 53 gm of anhydrous sodium carbonate in D.W., mixed well, then the volume was completed to 1 L by D.W.

3- Hydrochloric acid (0.5 M)

This solution was prepared from mixing 4.5 mL of concentrated HCl with in D.W. , mixed well, then the volume was completed to 100 mL by D.W.

4- Ammonium molybdate (1 M)

This solution was prepared by dissolving 44.2 gm of ammonium molybdate $(NH_4)_6Mo_7O_{27}.4H_2O$ with in a mixture of 60 mL concentrated ammonia and 40 mL of D.W., then 120 gm of ammonium nitrate was added. After completing solubility, the volume of sol. was completed to 1 L by D.W. Prior to its use HNO_3 was added in order to make the reaction in acidic media.

5- Ammonium acetate (0.5 M)

This solution was prepared by dissolving 46.3 gm of ammonium acetate in D.W., mixed well, then the volume was completed to 100 mL by D.W.

6- Mono-hydrous sodium phosphate (0.033 M)

This solution was prepared by dissolving 4.2 gm of mono-hydrous sodium phosphate Na_2HPO_4 in D.W., mixed well, then the volume was completed to 1 L by D.W.

7- Ammonium chloride (1 M)

This solution was prepared by dissolving 53.5 gm of ammonium chloride NH_4Cl in D.W., mixed well, then the volume was completed to 1 L by D.W.

8- Mercury (I) nitrate (1 M)

This solution was prepared by dissolving 28.1 gm of hydrous mercury (I) nitrate $Hg_2(NO_3)_2.2H_2O$ with in a cooled mixture of 500 mL of D.W., and 10 mL of concentrated nitric acid ., the volume of sol. was completed to 1 L by D.W., then adding one drop of pure mercury to prevent the oxidation process.

9- Nessler's solution

(Solution-A) This solution was prepared by dissolving 10 gm of potassium iodide KI in 100 mL of D.W.

(Solution-B) This solution was prepared by dissolving (6 gm) of mercury (II) chloride in 100 mL of D.W.

(Solution-C) This solution was prepared by dissolving (45 gm) of potassium hydroxide in D.W. then the volume was completed to 80 mL by D.W.

(sol.-B) was added to the (sol.-A) as drop wise until it formed a stable complex , and then (sol.-C) was added to the new mixture , then the volume was completed to 200 mL by D.W., and this mixture was left to 24 hr. to use the supernatant sol.

10- Ammonium carbonate solution (1 M)

This solution was prepared by dissolving 96.1 gm of ammonium carbonate $(NH_4)_2CO_3$ in D.W., mixed well, then the volume was completed to 1 L by D.W.

11- Acetic acid solution (2 M)

This solution was prepared from mixing 114 mL of glacial HCl with in D.W., mixed well, then the volume was completed to 1 L by D.W.

12- Ammonium sulphate solution (1 M)

This solution was prepared by dissolving 96.1 gm of ammonium sulphate $(NH_4)_2SO_4$ in D.W., mixed well, then the volume was completed to 1 L by D.W.

13- Ammonium oxalate solution (1 %)

This solution was prepared by dissolving 25 gm of hydrous ammonium oxalate $(COONH_4)_2.H_2O$ in 900 mL D.W., mixed well, then the volume was completed to 1 L by D.W.

14- Potassium ferro cyanide solution (0.5 M)

This solution was prepared by dissolving 10.5 gm of hydrous potassium ferro cyanide $K_4[Fe(CN)_6].3H_2O$ in D.W., mixed well, then the volume was completed to1 L by D.W.

15- Calcium chloride solution (0.5 M)

This solution was prepared by dissolving 109.5 gm of hydrous calcium chloride $CaCl_2.6H_2O$ in D.W., mixed well, then the volume was completed to 1 L by D.W.

16- Magnesium chloride solution (0.5 M)

This solution was prepared by dissolving 101.7 gm of hydrous magnesium chloride $MgCl_2.6H_2O$ in D.W., mixed well, then the volume was completed to 1 L by D.W.

17- Sodium hydroxide solution (2 M)

This solution was prepared by dissolving 80 gm of sodium hydroxide NaOH in 80 mL of D.W. by mixing cautiously, mixed well, then the volume was completed to (1 L) by D.W.

18- Litmus paper (red color).

2.2.8.2. Preparation of stone samples

Each stone collected from the urinary tract of patients with U.T.I. was grinded, then concentrated nitric acid was added, and the formed solution was heated till boiling. Then by the use of filter papers of the type whatman no.1 the solution was filtered. Each filtrate was used for the detection of ions.

2.2.8.3. Detection of ions in urinary stones

The following procedures were used according to [170,171], as follows:

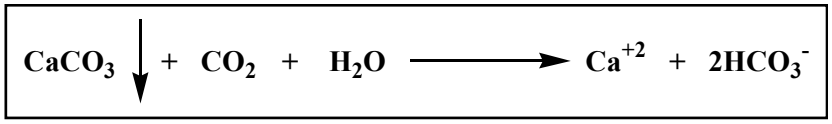
1- Carbonate ion ($CO3^{-2}$)

A small amount from the powder of grinded stone was taken by clean spatula into a test tube. In another test tube, an equal amount of calcium carbonate ($CaCO_3$) was used as control. Then to each test tube the following steps were followed:

A- Diluted HCl was added with heating, the appearance of gas bubbles is an indication of the presence of carbonate ion, as in the following equation:

B- The gas produced was passed rapidly through the lime water. The turbidity of lime water is an indication of the presence carbonate ion, as in the following equation:

C- The continuous passage of the gas for long period, the disappearance of the turbidity is an indication of the presence of carbonate ion, as in the following equation:

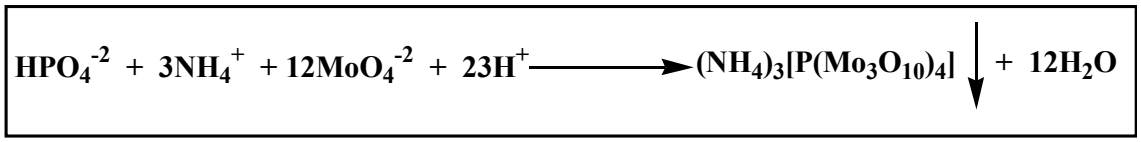


2- Phosphate ion ($PO4^{-3}$)

The 0.5 mL of the stone filtrate was taken in a test tube, and a similar volume of monohybrids sodium phosphate Na_2HPO_4 0.033 M was taken in another test test as control. Then to each test tube the following steps were taken:

A- Concentrated HNO_3 was added with heating.

B- (2-3) mL of ammonium molybdate was added with heating (not over 40 ^{0}C) .If the formation pale-yellow ppt. is an indication of the presence of phosphate ion, as in the following equation :



3- Ammonium ion (NH_4^+)

The 0.5 mL of the stone filtrate was taken in a test tube, and a similar volume of ammonium chloride 1 M was taken in a another test tube as control. Then to each test tube the following steps were taken:

A- Sodium hydroxide NaOH was added .If the formation of ammonia gas is an indication of the presence of ammonium ion, as in the following equation:

$$NH_4 + OH^- \longrightarrow NH_3 \uparrow + H_2O$$

To know the ascending ammonia gas, one of these methods was used:

1- Change the color of reddish litmus paper to blue color.

2- The special odor of this gas.

B- Drops of Nessler's solution were added to the solution of grinded stone with heating, the appearance of brown color ppt. is a surly indication of the presence of ammonium ion. , as in the following equation:

$$NH_4^+ + 2K_2HgI_4 + OH^- \longrightarrow Hg.Hg(NH_3)I \downarrow + 7KI + H_2O$$

4- Calcium ion (Ca^{+2})

Two mL of the stone filtrate was put in a test tube, and a similar volume of calcium chloride 0.5 M was put in a another test tube as control. Then to each test tube the following steps were taken:

A- Drops of ammonium oxalate were added, the appearance of white color ppt. from calcium oxalate is an indication of the presence of calcium ion. , as in the following equation:

$$Ca^{+2} + (COO)_2^{-2} \longrightarrow Ca(COO)_2 \downarrow$$

B- Filtration process was done on the ppt. by using filter paper of the type whatman (no.1), and then saving the filtrate to the experiment to detect magnesium ion, but the ppt. washed by heated D.W.

C-To dissolve the ppt., drops of conc. HCl, sodium hydroxide, excess of ammonium chloride and potassium Ferro cyanide were added respectively. If the appearance is white ppt., it is an indication of the presence of calcium ion, as in the following equation:

$$Ca^{+2} + NH_4Cl + K_4Fe(CN)_6 \longrightarrow CaNH_4KFe(CN)_6 \downarrow + 3K^+$$

5- Magnesium ion (Mg^{+2})

In this test the following steps were taken:

A- Ten drops were added of dihydrogen potassium phosphate KH_2PO_4 10 % M to both of filtrate from (step-B) in the detection of calcium ion, and to the magnesium chloride sol. that found in another test tube as control.

B- From step-A the pH of sol. is basic, if the appearance is white ppt., it is an indication of the presence of magnesium ion, as in the following equation:

$$Mg^{+2} + HPO_4^{-2} \longrightarrow MgHPO_4 \downarrow$$

2.2.9. Analytical methods for determination of serum biochemical tests

2.2.9.1. Determination of blood urea

The concentration of urea was measured by enzymatic method [172] with commercially a variable kit (BioMerieux France). The principle of this method is shown by the following equation:-

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

In an alkaline medium, the ammonium ions react with the salicylate and hypochlorite to form a green colored indophenols (2,2-dicarbxylindophenol) was determined spectrophotometrically at λ=580 nm , according to the following equation :

$$\text{The concentration of Urea (mg/dL)} = \frac{\text{Abs. of sample}}{\text{Abs. of standard}} \text{ X n}$$

n = 50 mg/dL concentration of standard urea.

2.2.9.2. Determination of uric acid

The concentration of uric acid was measured by enzymatic method [172] with commercially a variable kit (Biomaghreb Ariana).The principle of this method is that uric acid is oxidized by uricase to allantoine and hydrogen peroxide, according to the following equation:-

$$\text{Uric acid} + O_2 + H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone peroxidase} + \text{2,4-dichlorophenolsulfonate} \rightarrow \text{Ouinoneimine} + 4H_2O$$

Uric acid was determined spectrophotometrically at λ=510 nm, according to the following equation:

$$\text{The concentration of Uric acid (mg/dL)} = \frac{\textbf{Abs. of sample}}{\textbf{Abs. of standard}} \textbf{ X n}$$

n = 6 mg/dL concentration of standard uric acid.

2.2.9.3. Determination of serum glucose

The concentration of glucose was measured by enzymatic method [173] with commercially a variable kit (BioMerieux France). The principle of this method is that glucose is determined by enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed and reacts with phenol and 4-aminophenazone to form a red – violet quinoneimine dye an indicator, as the following equation:-

$$\textbf{Glucose} + \textbf{O}_2 + \textbf{H}_2\textbf{O} \xrightarrow{\text{Glucose oxidase}} \textbf{Gluconic acid} + \textbf{H}_2\textbf{O}_2$$

$$2\textbf{H}_2\textbf{O}_2 + \textbf{4-aminophenazone} + \textbf{phenol} \xrightarrow{\text{Peroxidase}} \textbf{Ouinoneimine} + 4\textbf{H}_2\textbf{O}$$

Glucose was determined spectrophotometrically at λ=500 nm, according to the following equation:

$$\text{The concentration of glucose (mg/dL)} = \frac{\textbf{Abs. of sample}}{\textbf{Abs. of standard}} \textbf{ X n}$$

n = 100 mg/dL concentration of standard glucose.

2.2.9.4. Determination of serum creatinine

The concentration of creatinine was measured by enzymatic method [174], with commercially a variable kit (BioMerieux France). The principle of this method is creatin kinase (CK) utilizes creatin phosphate as substrate to act as the initial catalyst for a series of reactions resulting in the formation of NADPH. The NADPH produced is proportional to (CK) activity and is used to reduce nitroblue tetrazolium (NBT), in the presence of diaphorase, to give the blue-violet color of diformazan as the following equations:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{Glucose-6-P} + \text{ADP}$$

$$\text{Glucose-6-P} + \text{NADP}^{+} \xrightarrow{\text{G-6-P-DH}} \text{Gluconate-6-P} + \text{NADPH}$$

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{Creatine} + \text{ATP}$$

Creatinine was determined spectrophotometrically at λ=560 nm, according to the following equation:

$$\text{The concentration of creatinine (mg/dL)} = \frac{\textbf{Abs. of sample}}{\textbf{Abs. of standard}} \textbf{ X n}$$

n = 2 mg/dL concentration of standard creatinine.

2.2.9.5. Determination of serum calcium Ca^{+2}

The concentration of calcium was measured by enzymatic method [175] with commercially a variable kit (BioMerieux France). The principle of this method is that colorimetric determination of calcium,

without deproteinization, using O-cresophthalein complex one. Interface due to Mg^{2+} ions is eliminated by 8-hydroxquinolune up to 10 mg/dL.

The concentration of calcium was determined spectrophotometrically at λ = 572 nm. according to the following equation:-

$$\text{The concentration of calcium (mg/dL)} = \frac{\textbf{Abs. of sample}}{\textbf{Abs. of standard}} \textbf{ X n}$$

n = 10 mg/dL concentration of standard calcium

2.2.10. Statistical analysis

Statistical analysis was performed using statistical software. For descriptive statistics, (mean value ± 2 standard deviation) were given. Z-test was employed to examine the differences between patients (U.T.I.) and control groups, and patient with (U.T.I.) for various biochemical and microbiological parameters, with a probability value of ($P< 0.05$) considered significance at 95 % confidence limit.

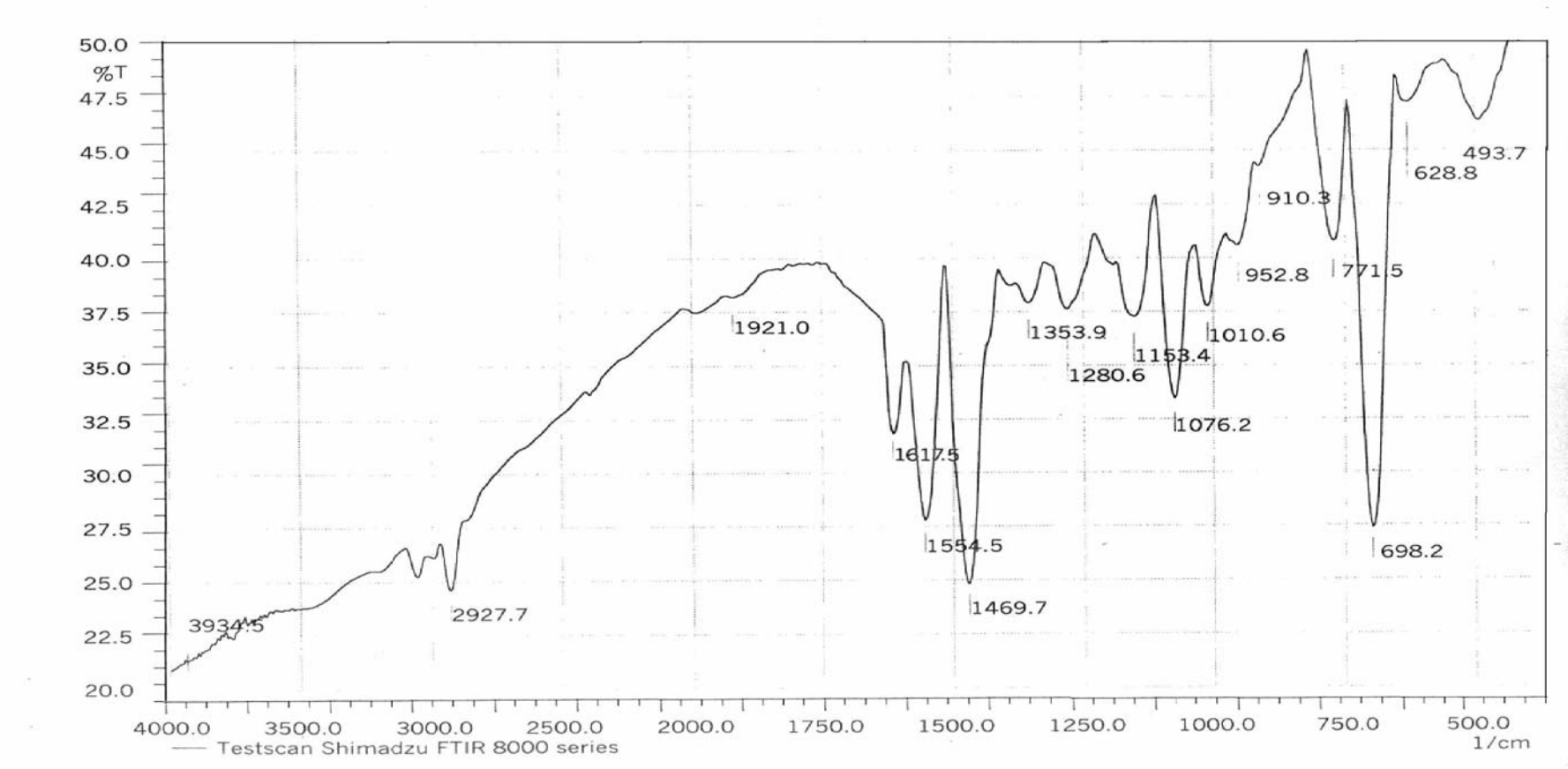
Conclusions

- From the preparation of the 20 novel compounds, it is noticed that schiff bases are more active than urea and thiourea derivatives compounds due to the presence of (C=N) group that have a role in their biologic activity against isolated *Proteus* spp. from patients with U.T.I. and renal stone.
- N-pyridine-4-ylurea does not have any biologic inhibitory effect against *Proteus* **s**pp., While [N-(5-phenyl – 1,3,4-thiadizole)-2-yl] thiourea has the highest biologic inhibitory effect among them *in vitro* in comparison with Gentamycin .
- The commercially used antibiotics Erythromycin, Chloramphincol and Tetracycline were not effective against isolated *Proteus* spp., but the Gentamycin and Ciprofloxacin are the best antibiotics used to the U.T.I. treatment and the management of urinary stone formation.
- For drug-sensitivity testing, this study shows that using well-diffusion method is a better method in comparison to other methods used in this study.
- From the analysis of chemical composition of urinary stones it is showed that the struvite stones were the commonest type among these stones, followed by apatite carbonate and lastly by mixed stone.
- The vast majority of *Proteus* spp. that is isolated in this study was *Proteus mirabilis* in comparison to other spp. was *Proteus vulgaris*.
- It is noticed that there is a significant rise in the levels of blood urea, uric acid, creatinine and calcium in patients as compared with control groups, but not in R.B.S. of this study.

Recommendations

- Further studies can be carried out: The synthesis and studying the activities of other new compounds with different groups against *Proteus* spp.
- Studying the urease extracted from *Proteus* bacteria as an enzyme activity by *in vitro* and *in vivo*. As kinetic study of the activity of urease by application of different concentrations of these prepared compounds as Ki, V_{max} and K_m inhibition percentage by calculation using Line weaver- Burk equation.
- Testing the prepared compounds against other urease producing bacteria that cause human infection.
- Determination of the toxic dose (LD_{50}) of prepared compounds on the laboratory animals before their application as alternative drugs.
- Studying other biochemical tests such as (Serum Cholesterol, Serum Protein, Na^+ ion and K^+ ion) to elucidate their relation to U.T.I. due to *Proteus* spp. infection.
- Studying the histopathologic changes in urinary tract of rats infected by *Proteus* spp. and to follow the effects of the prepared compounds in them.

Contents



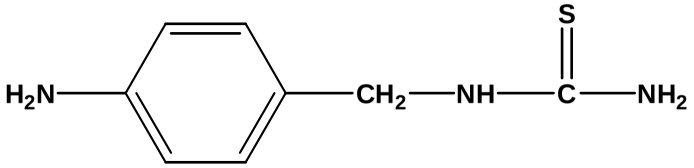
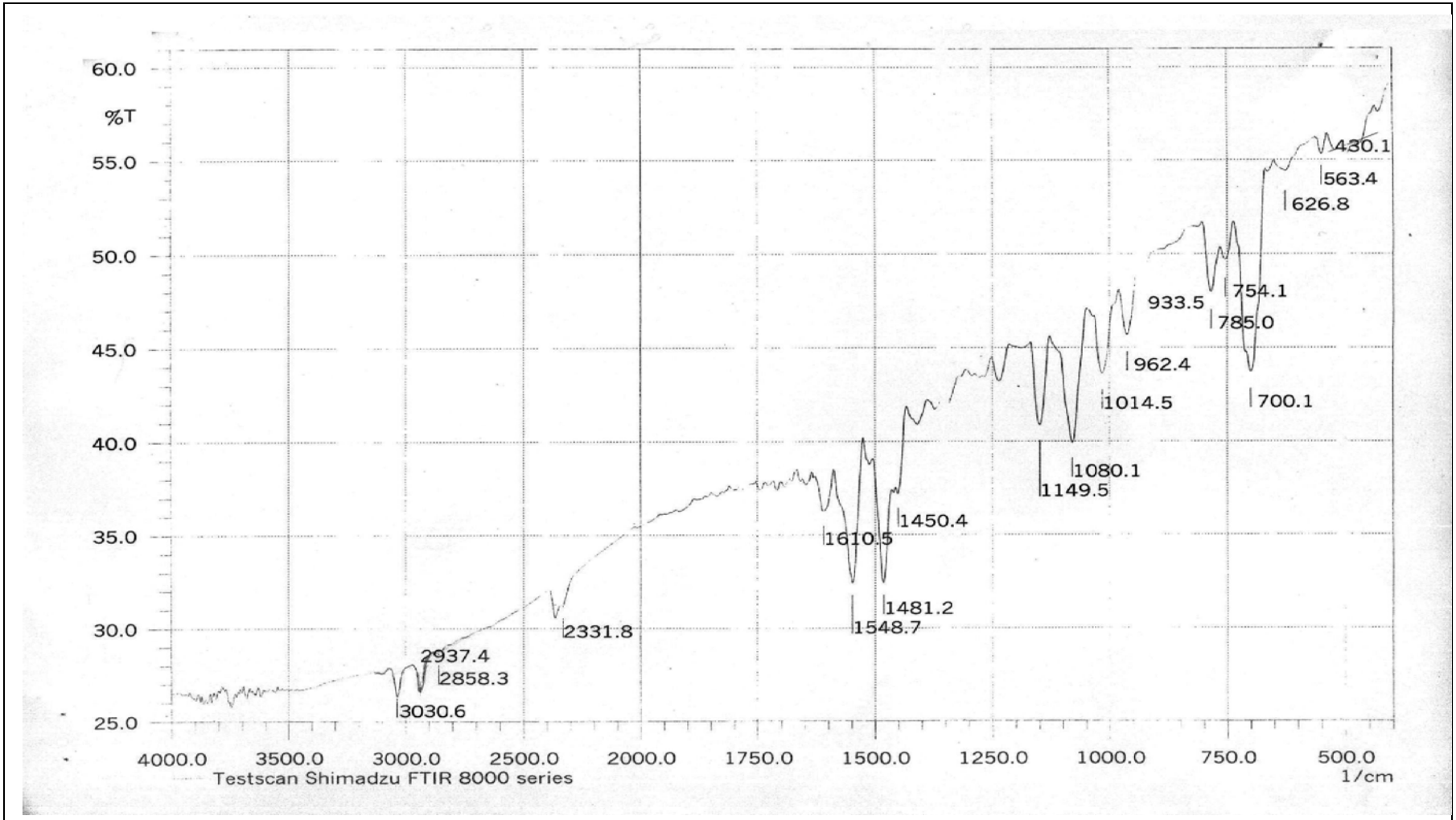
Melting point: 177-179 °C	
Yield: 67.45 %	

Figure (2): Structure, Physical Properties and F.T.I.R. spectrum of N-4-aminobenzylthiourea



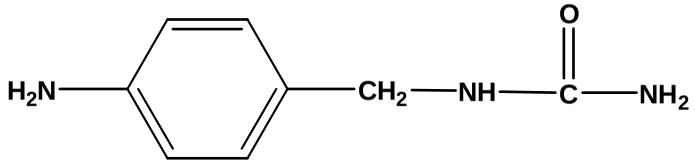
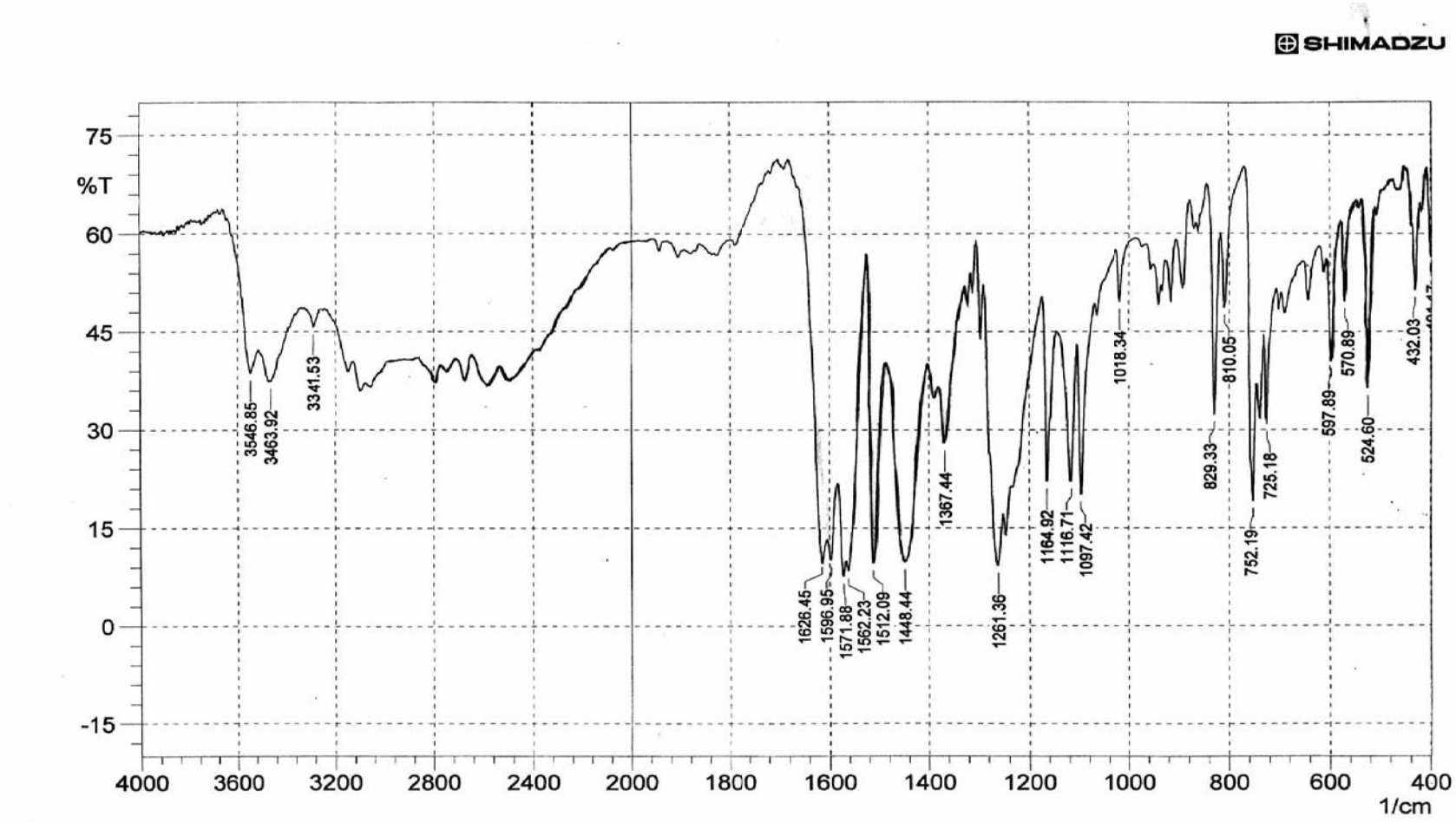
Melting point: 169-171 °C	
Yield: 71.22%	

Figure (1): Structure, Physical Properties and F.T.I.R. spectrum of N-4-aminobenzylurea



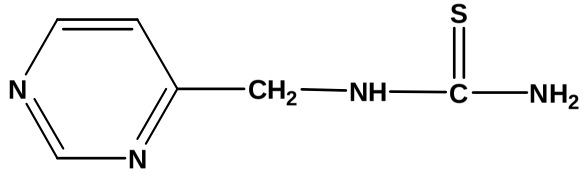
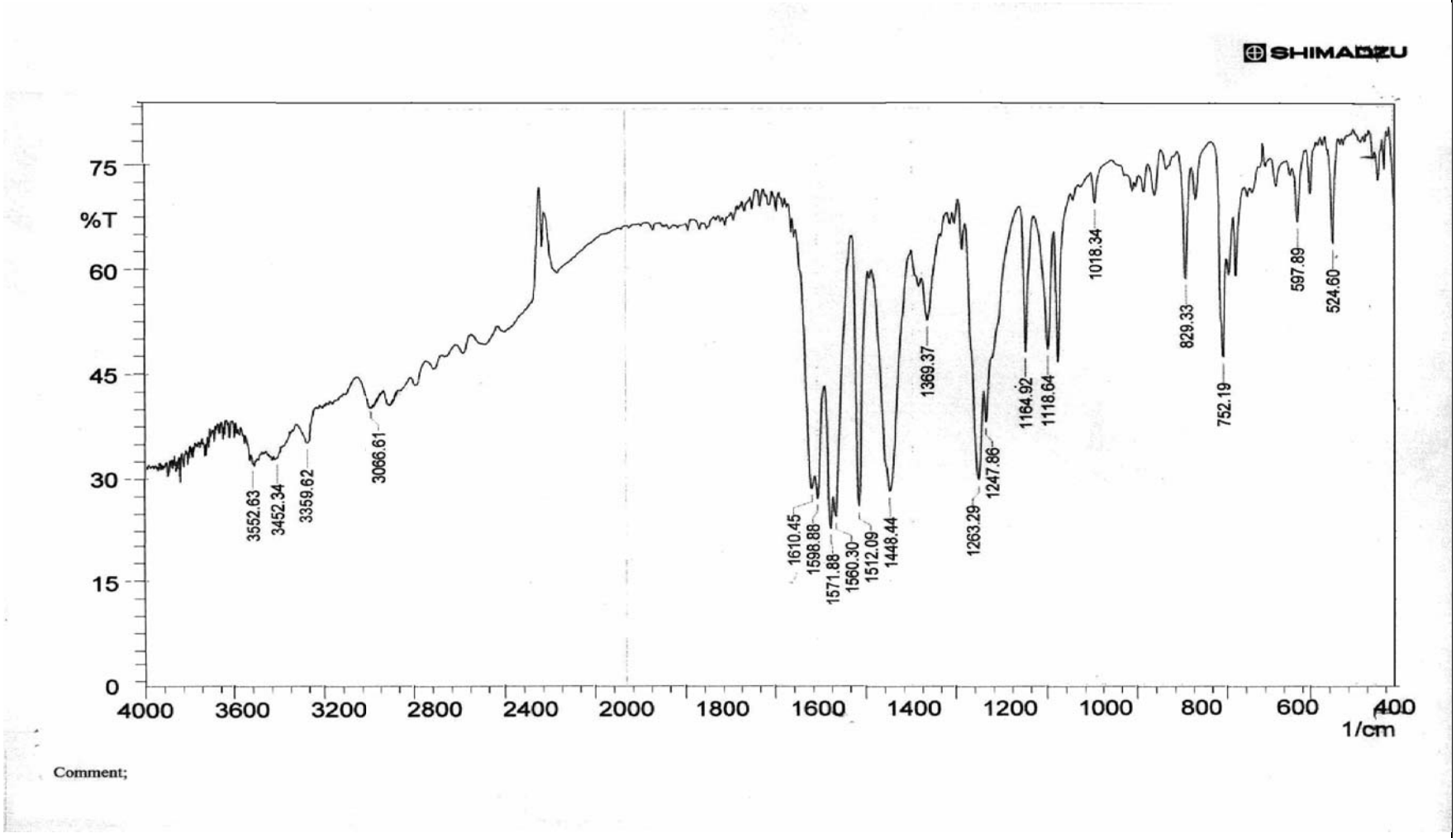
Melting point: 295-297 ^{o}C	
Yield: 80.88%	

Figure (4): Structure, Physical Properties and F.T.I.R. spectrum of N-pyrimidine-6-ylthiourea



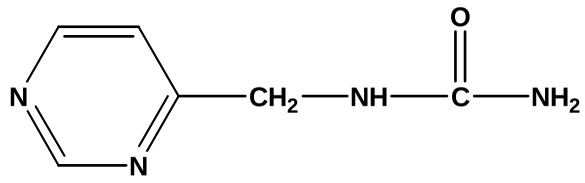
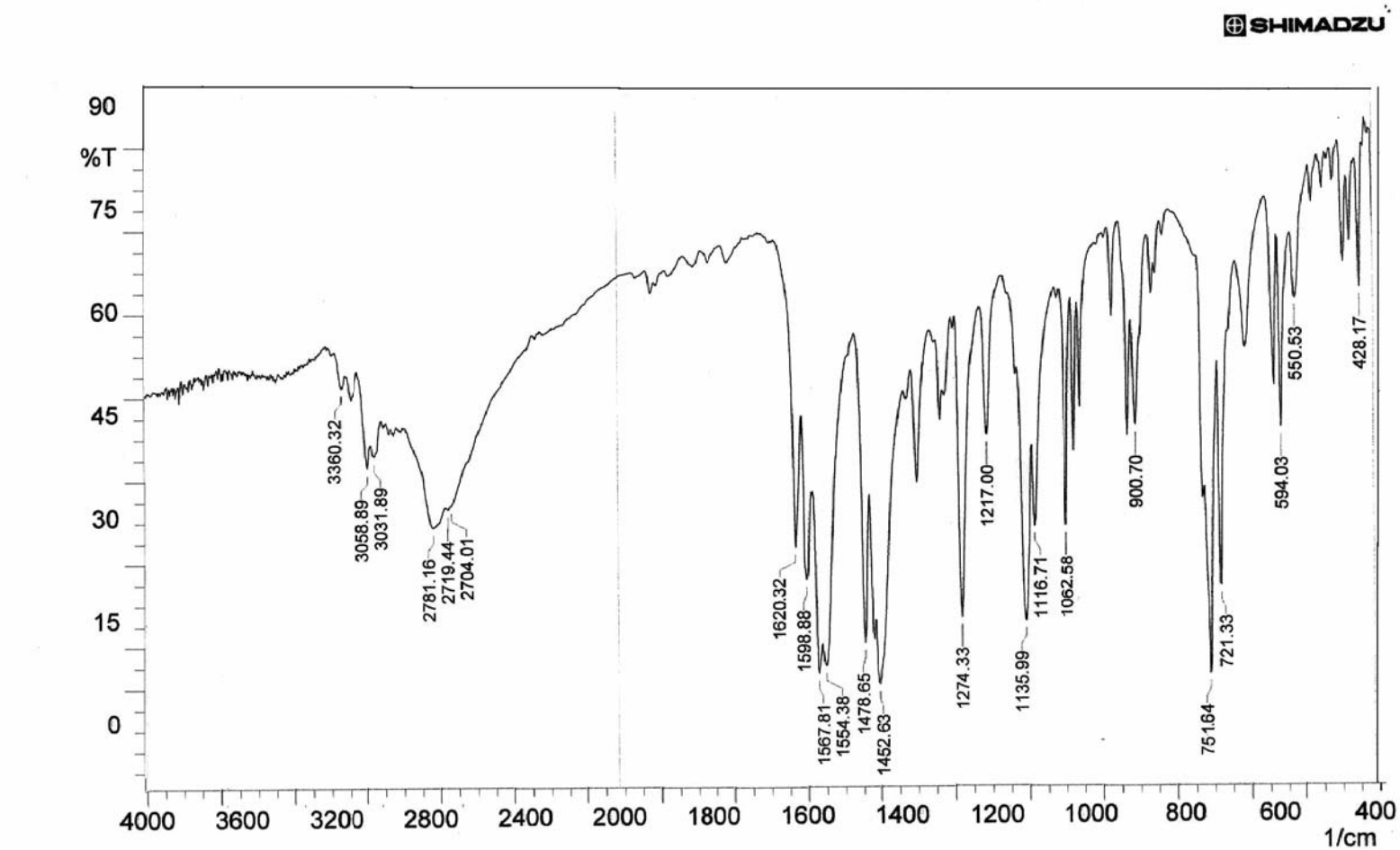
Melting point: 287-289 ^{o}C	
Yield: 86.75 %	

Figure (3) : Structure, Physical Properties and F.T.I.R. spectrum of N-pyrimidine-6-ylurea



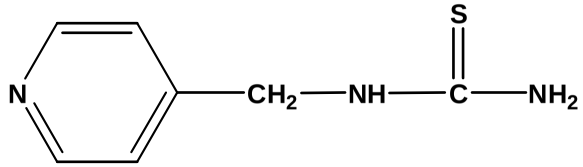
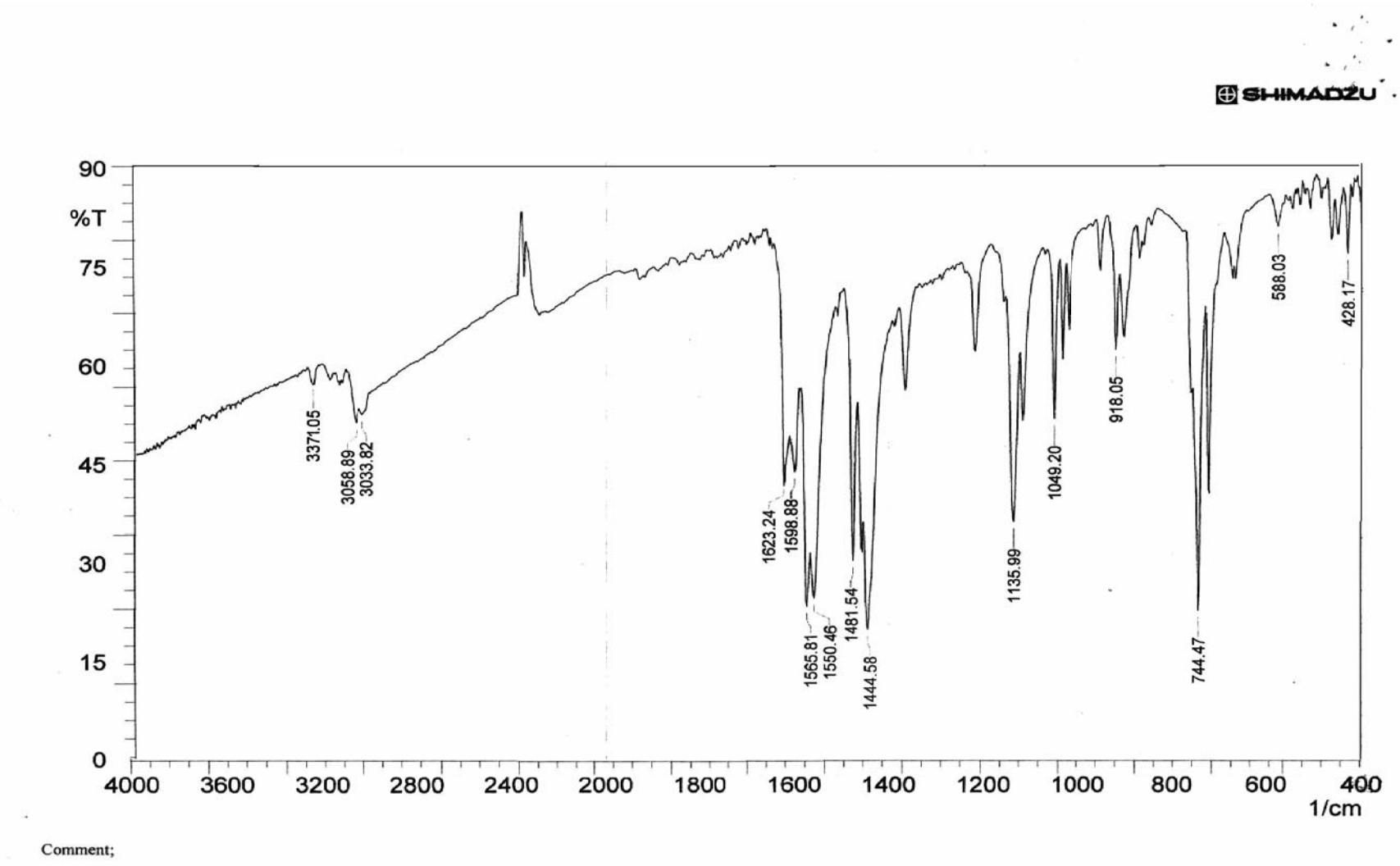
Melting point: 238-240 ^{o}C	
Yield: 90.12 %	

Figure (6): Structure, Physical Properties and F.T.I.R. spectrum of N-pyridine-4-ylthiourea



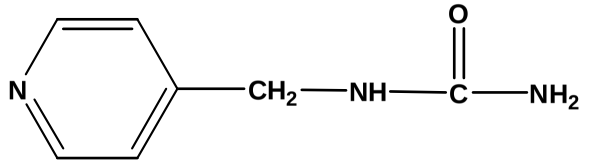
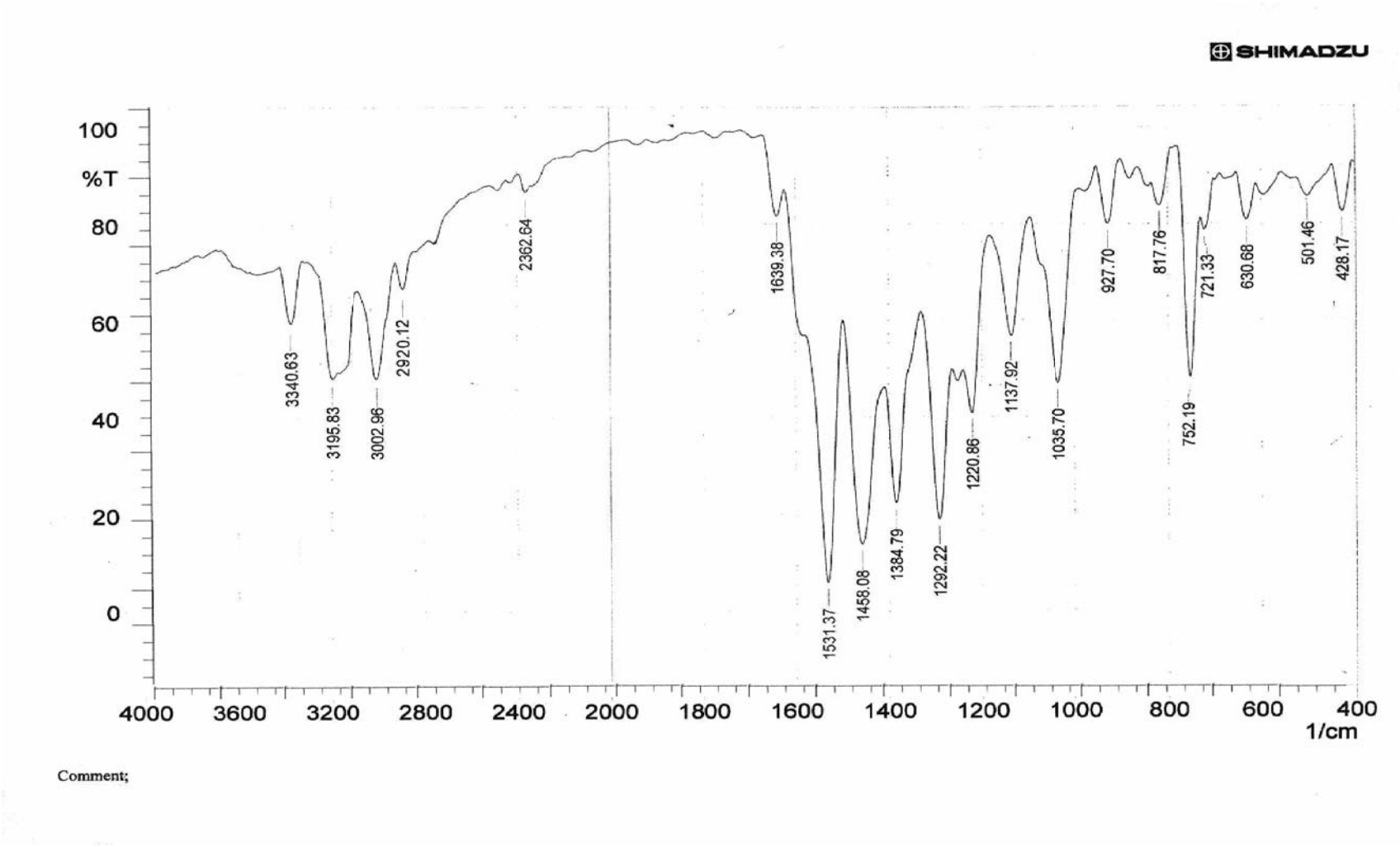
Melting point: 232-234 ^{o}C	
Yield: 82.56 %	

Figure (5): Structure, Physical Properties and F.T.I.R. spectrum of N-pyridine-4-ylurea



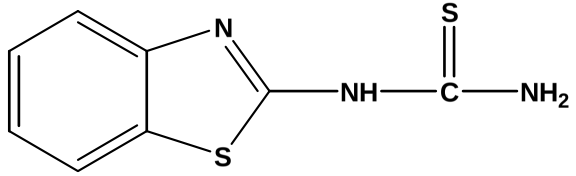
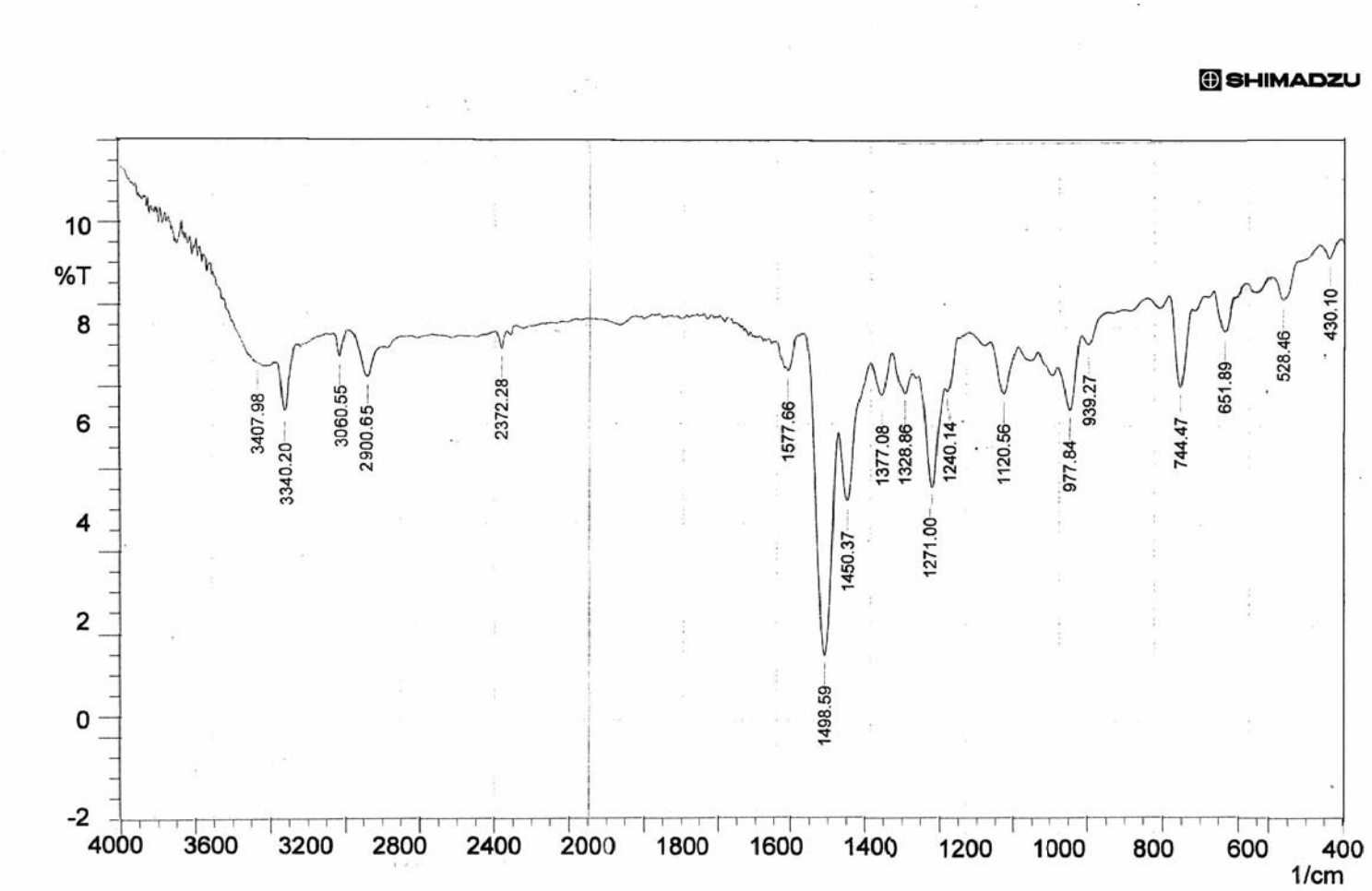
Melting point: >300 ^{o}C	
Yield: 69.71 %	

Figure (8): Structure, Physical Properties and F.T.I.R. spectrum of N-benzothiazole-2-ylthiourea



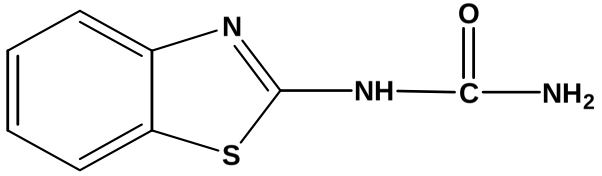
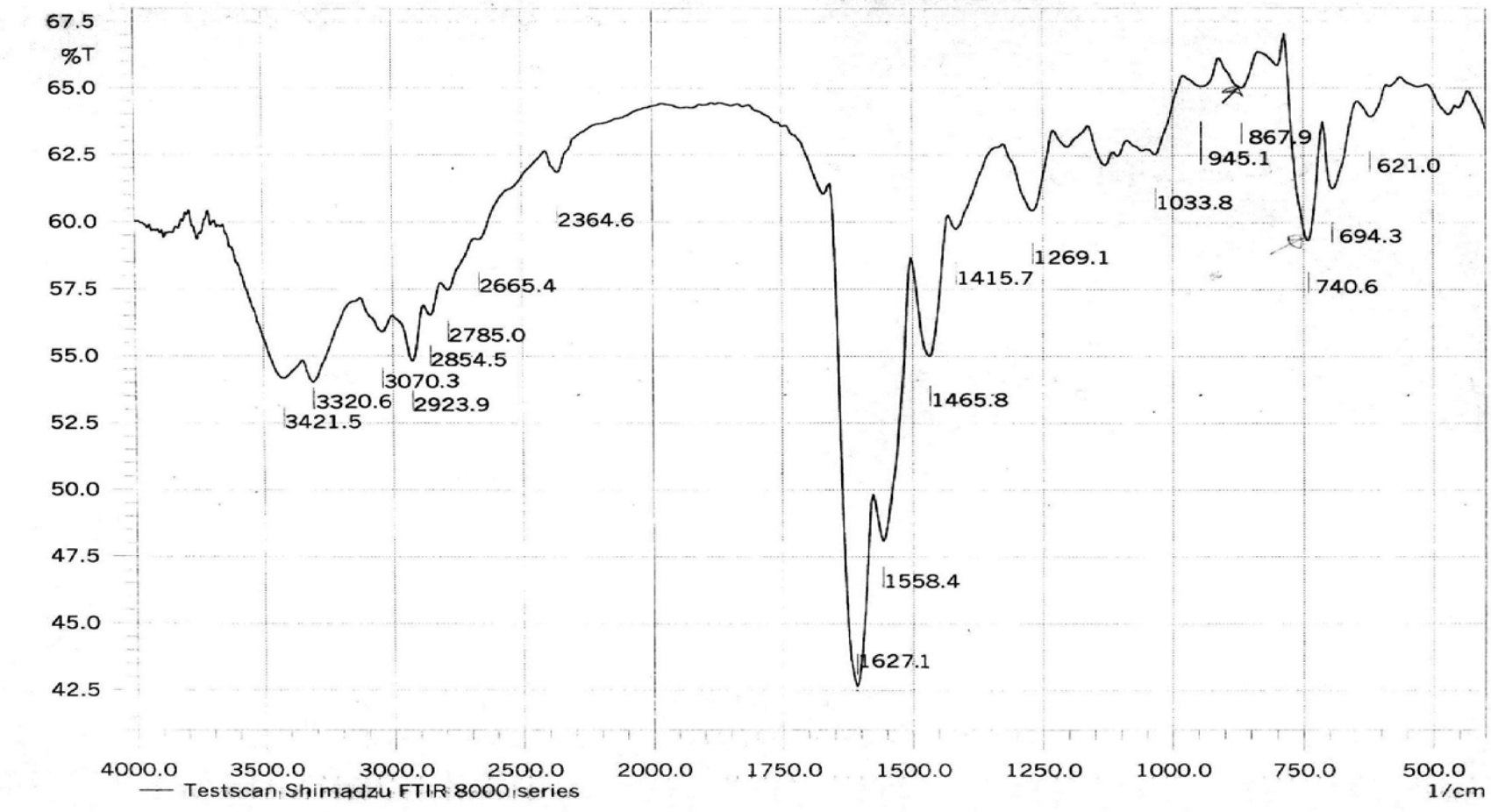
Melting point: >300 °C	
Yield: 75.99 %	

Figure (7): Structure, Physical Properties and F.T.IR spectrum of N-benzothiazole-2-ylurea



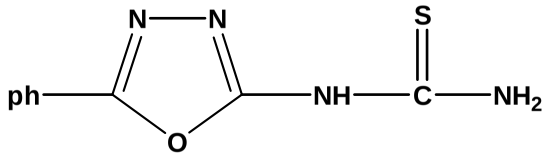
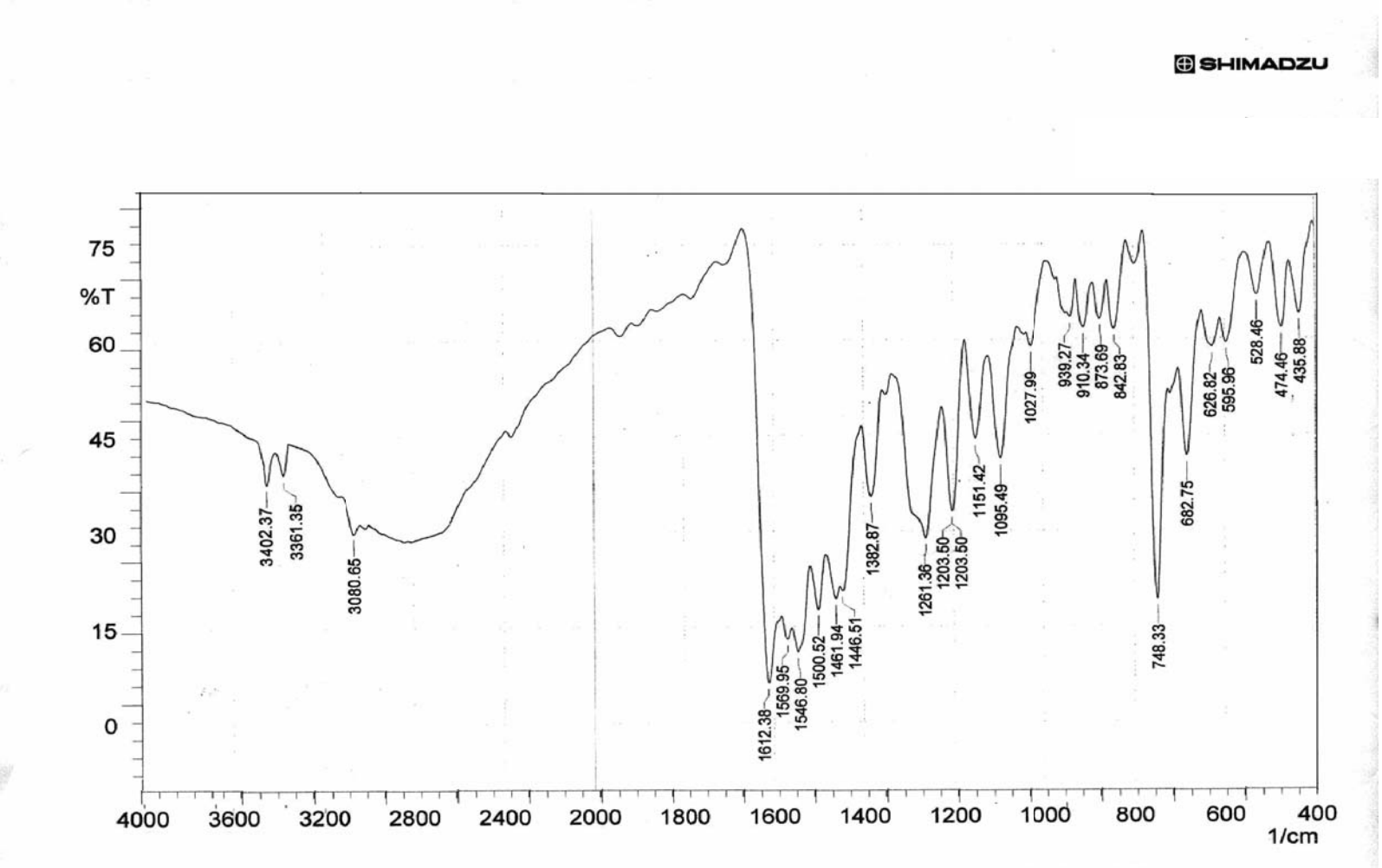
Melting point: 224-226 °C	
Yield: 93 %	

Figure (10): Structure, Physical Properties and F.T.I.R. spectrum of [N-(5-phenyl-1,3,4-oxadiazole)-2-yl]thiourea



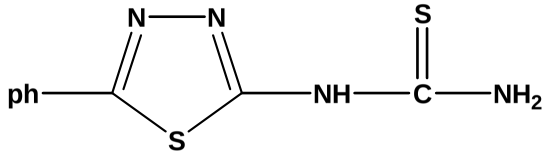
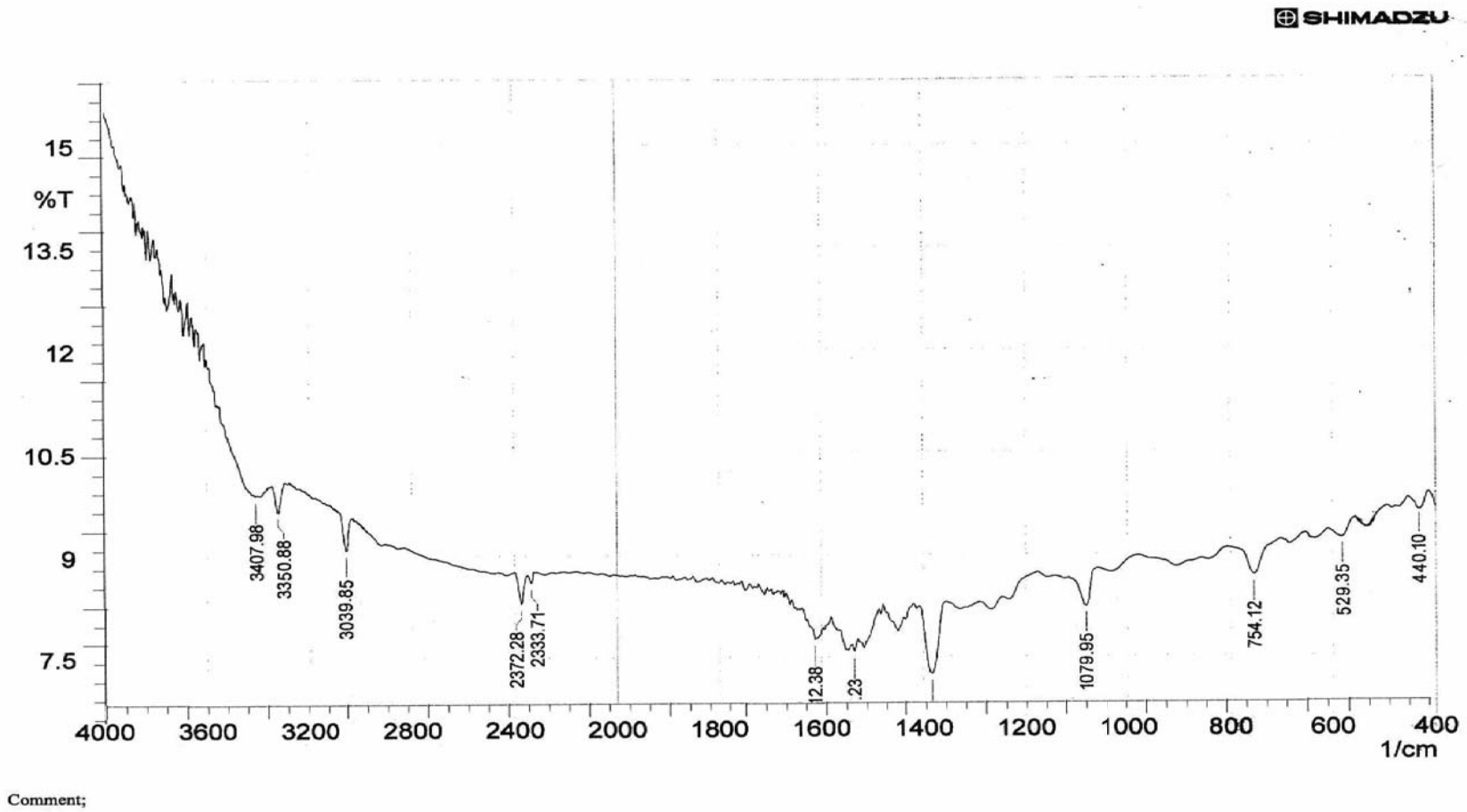
Melting point: 263-265 ^{o}C	
Yield: 56.35 %	

Figure (12): Structure, Physical Properties and F.T.I.R. spectrum of [N-(5-phenyl-1,3,4-thiadiazole)-2-yl]thiourea



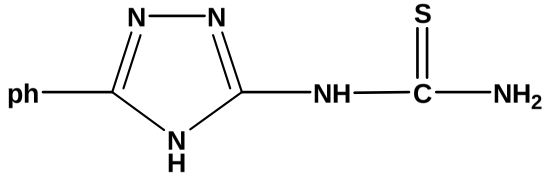
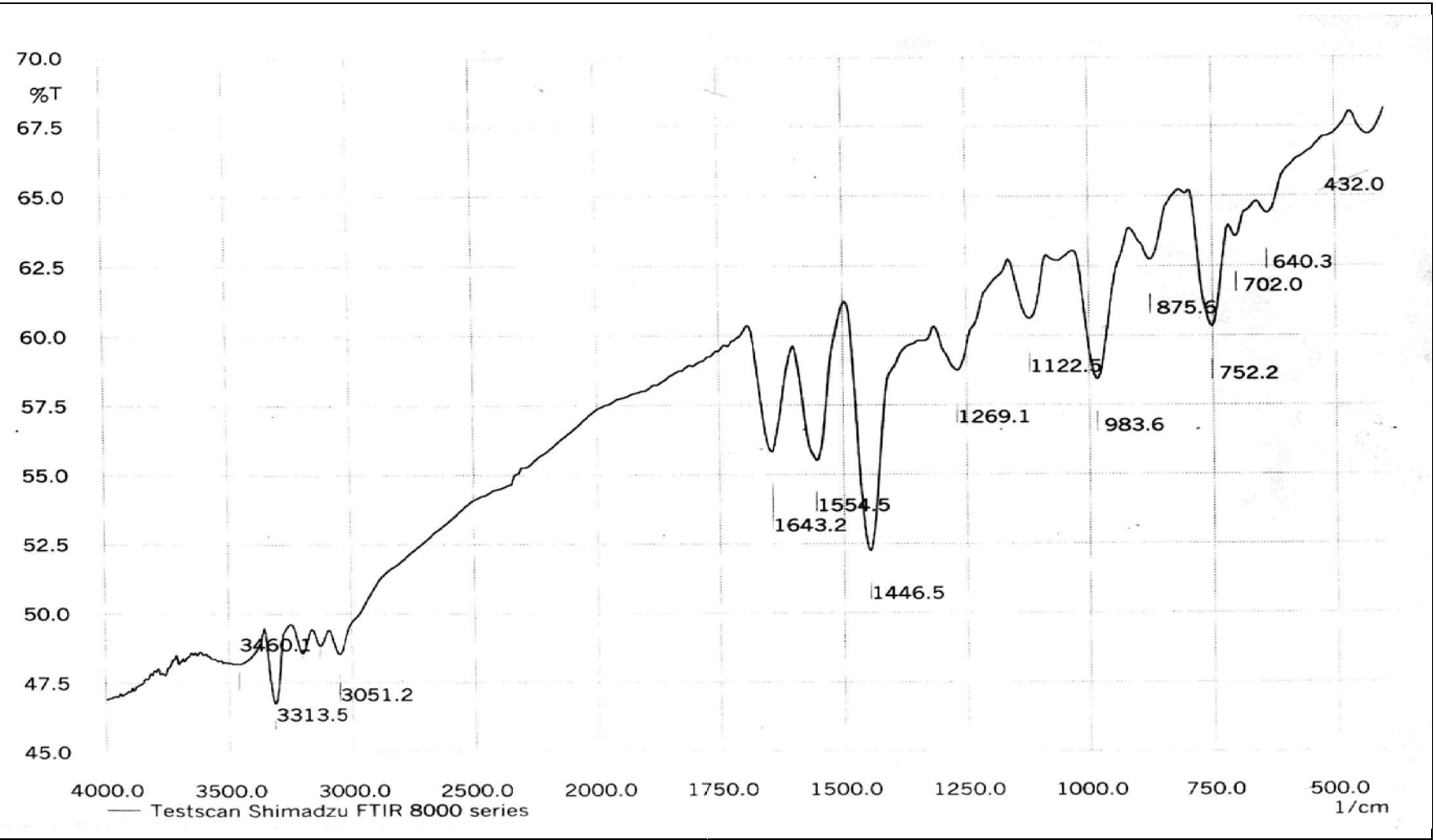
Melting point: 350 ^{o}C	
Yield: 78.90 %	

Figure (14): Structure, Physical Properties and F.T.I.R. spectrum of [N-(5-phenyl-1,3,4-triazole)-2-yl]thiourea



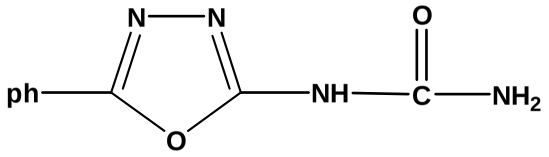
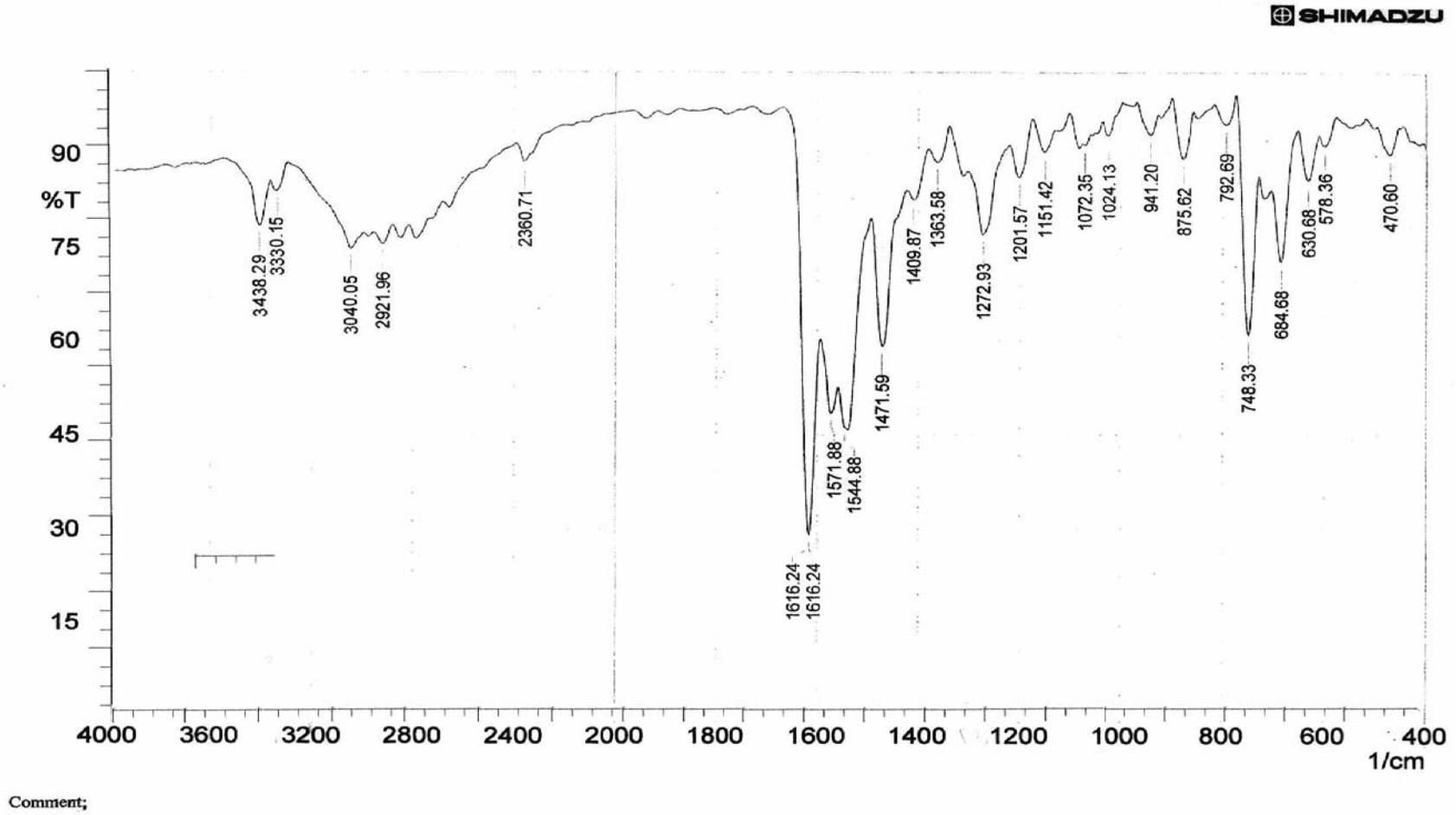
Melting point: 216-218 ^{o}C	
Yield: 90.6 %	

Figure (9): Structure, Physical Properties and F.T.I.R. spectrum of [N-(5-phenyl-1,3,4-oxadiazole)-2-yl]urea]



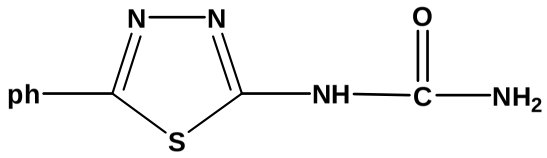
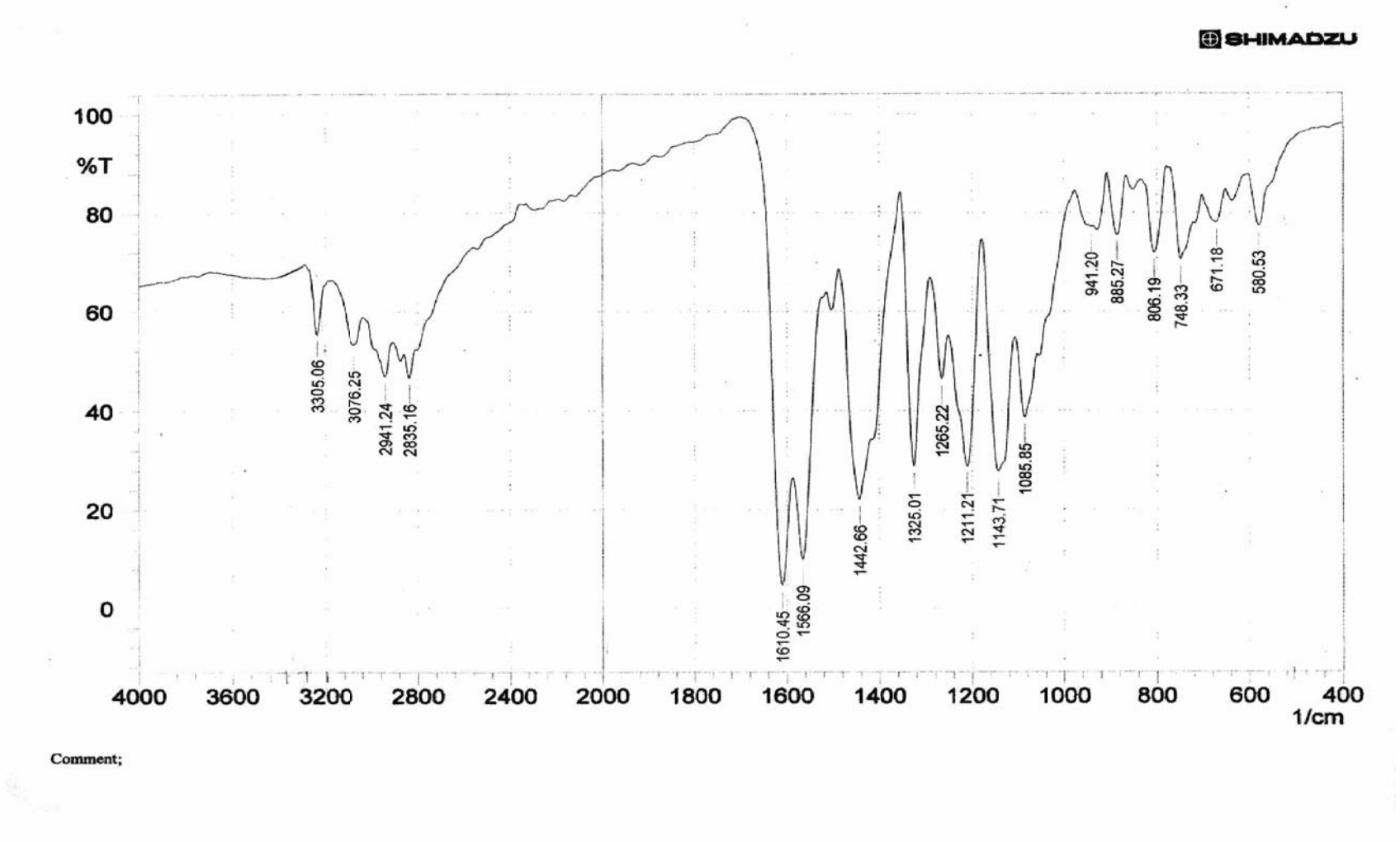
Melting point: 255-257 ^{o}C	
Yield: 77.95 %	

Figure (11): Structure, Physical Properties and F.T.I.R. spectrum of [N-(5-phenyl-1,3,4-thiadiazole)-2-yl]urea



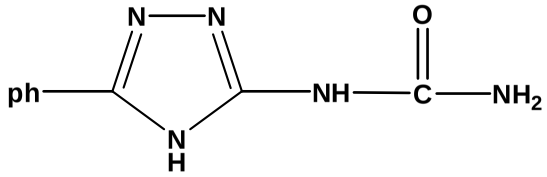
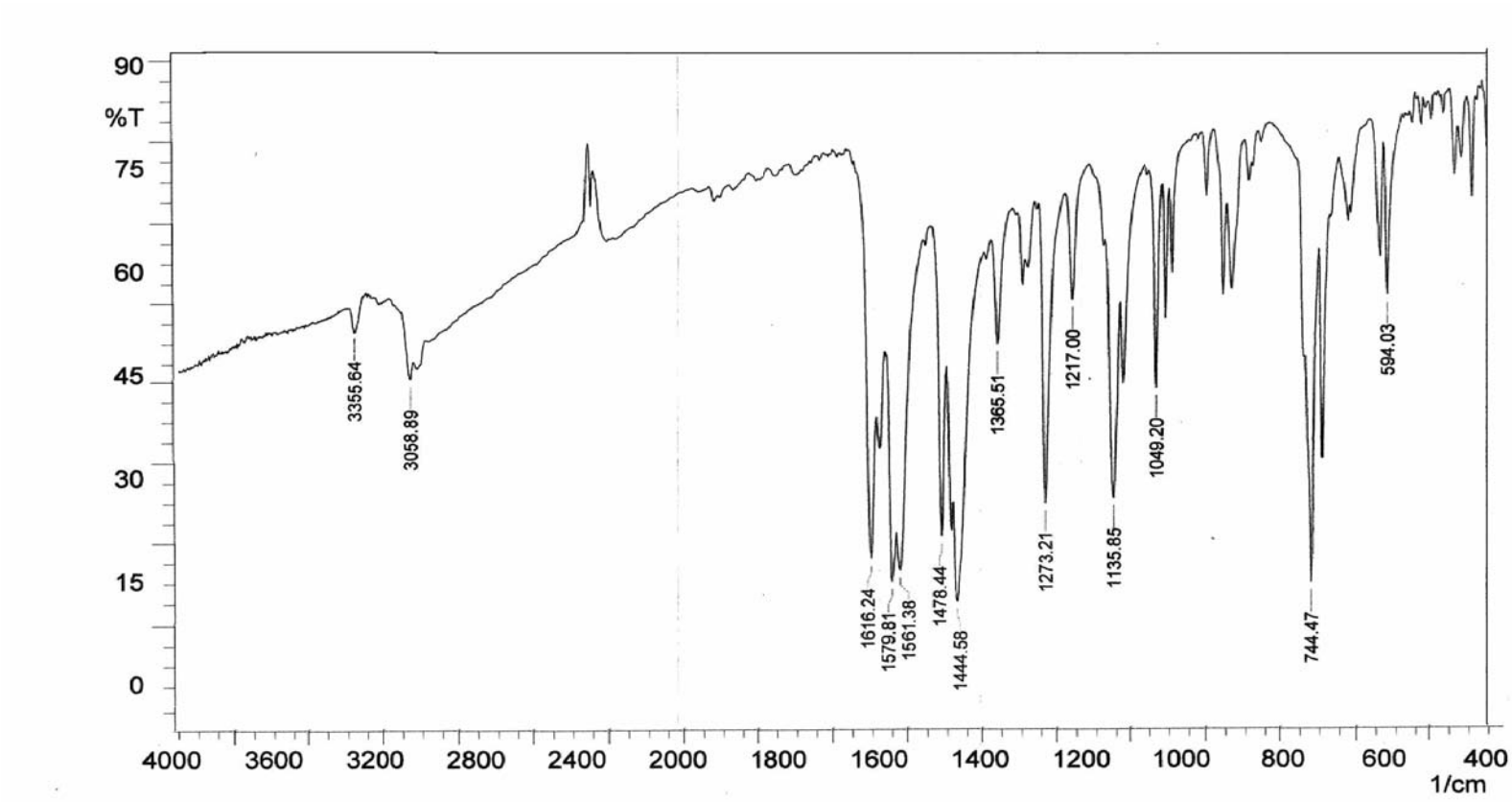
Melting point: 350 ^{o}C	
Yield: 95.56 %	

Figure (13): Structure, Physical Properties and F.T.I.R. spectrum of [N-(5-phenyl-1,3,4-triazole)-2-yl]urea



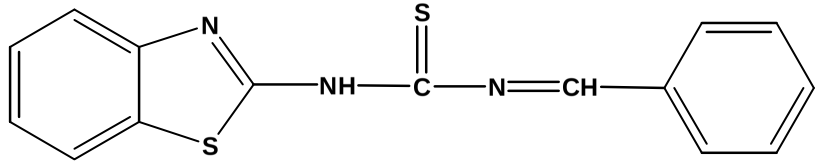
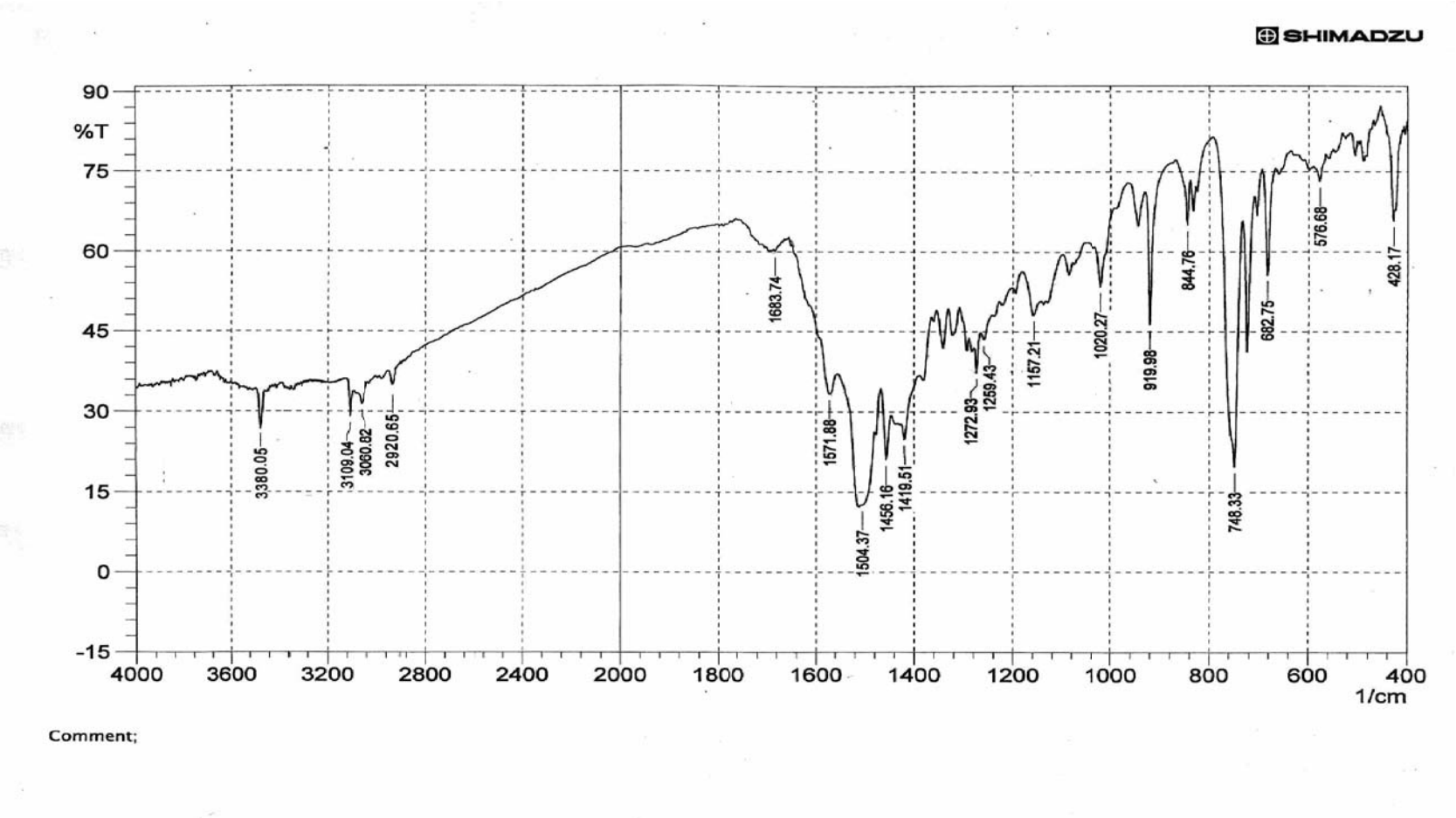
Melting point: >300 ^{o}C	
Yield: 80.75 %	

Figure (20): Structure, Physical Properties and F.T.I.R. spectrum of [benzylidene-2-minobenzothiazol]carbothioamide



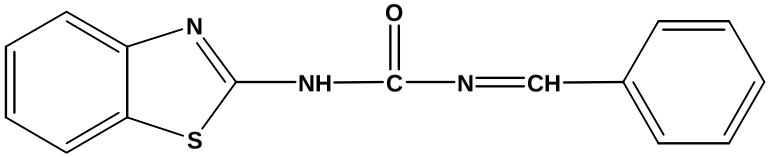
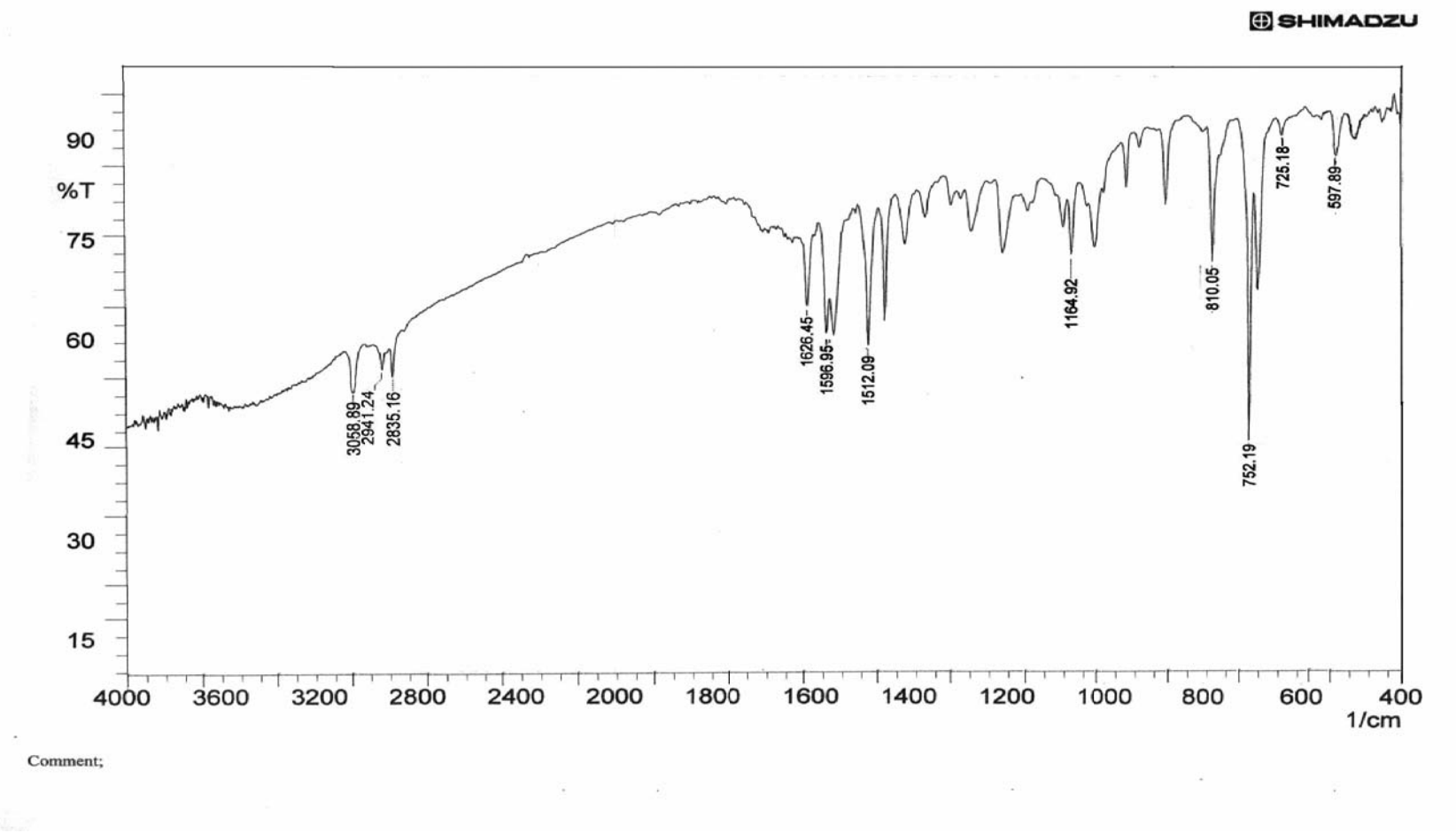
Melting point:>300 ^{o}**C**	
Yield: 83.71 %	

Figure (19): Structure, Physical Properties and F.T.I.R. spectrum of [benzylidene-2-aminobenzothiazol]carboamide



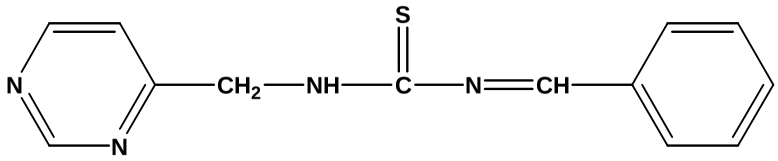
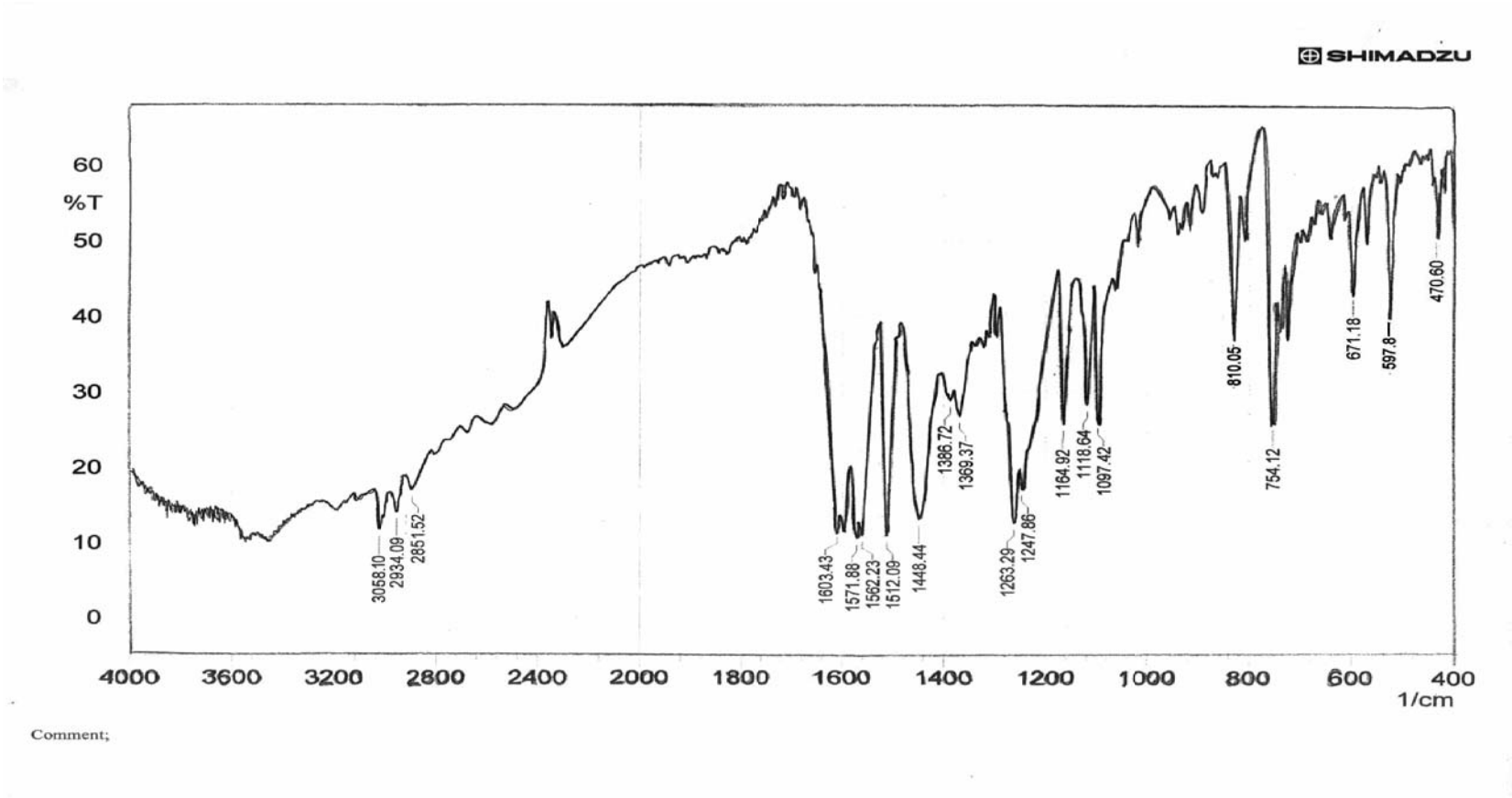
Melting point: 295-297 ^{o}C	
Yield: 64 %	

Figure (16): Structure, Physical Properties and F.T.I.R. spectrum of [benzylidene-6-aminopyrimidine]carbothioamide



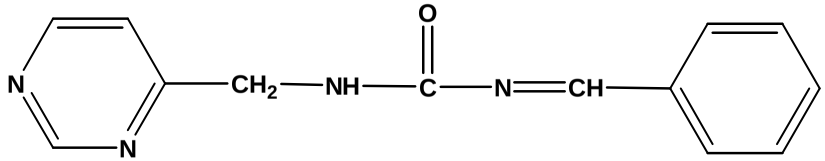
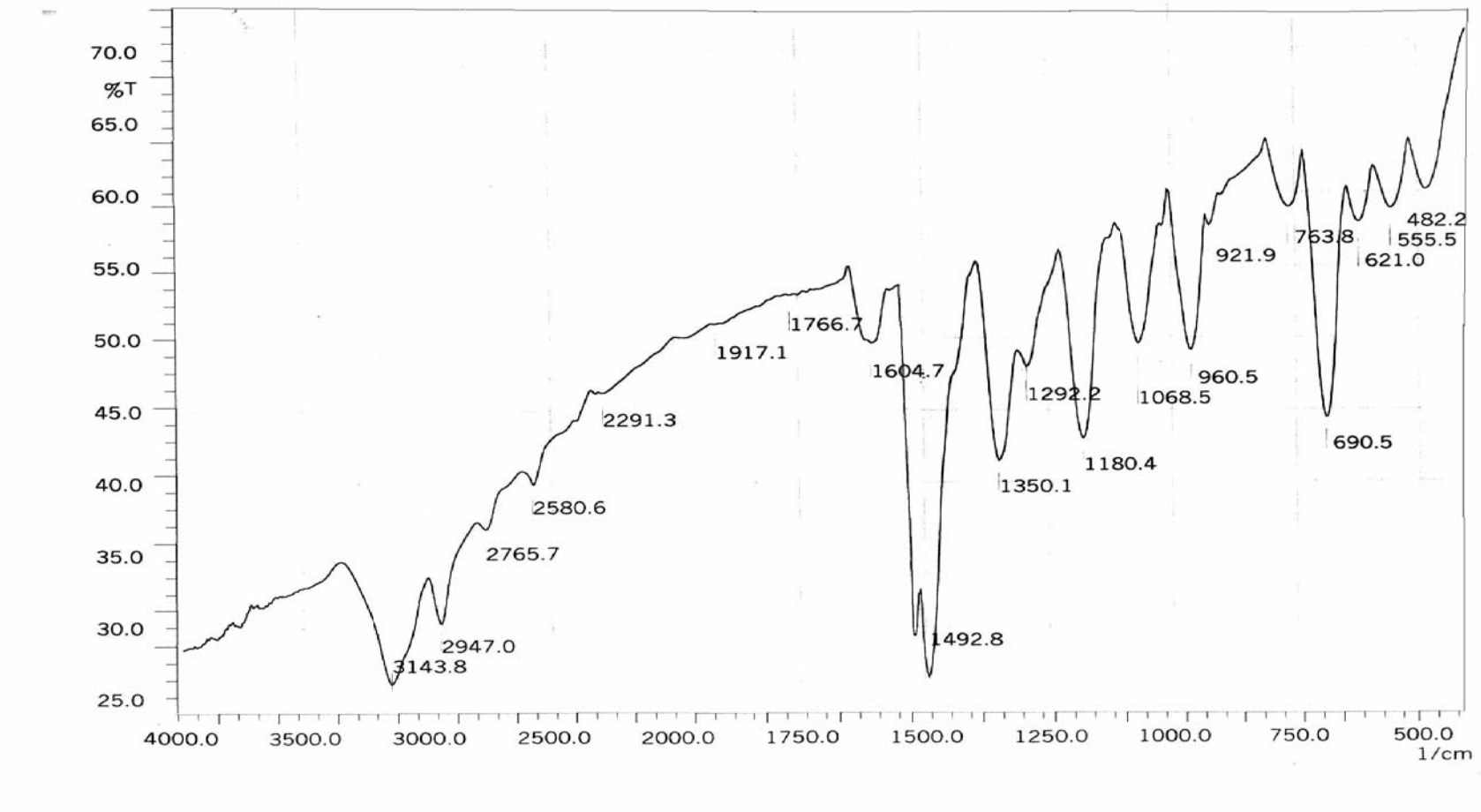
Melting point: 277-279 ^{o}C	
Yield: 92.26 %	

Figure (15): Structure, Physical Properties and F.T.I.R. spectrum of [benzylidene-6-aminopyrimidine]carboamide



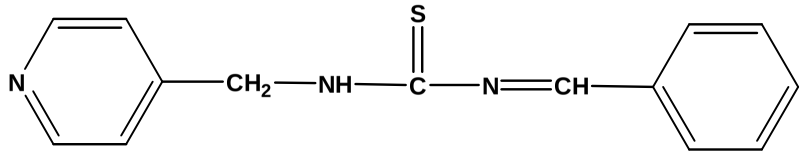
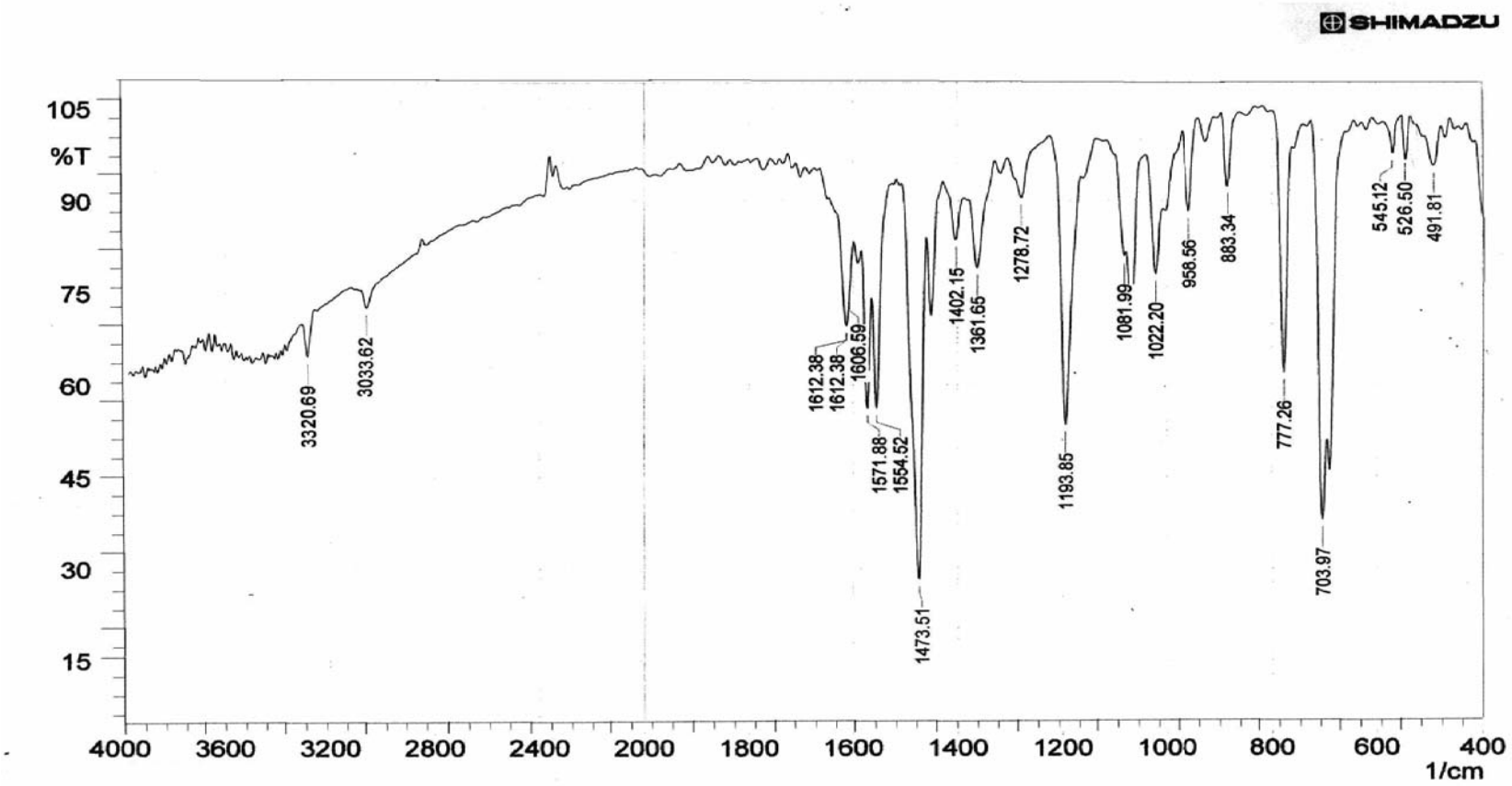
Melting point: 298-300 ^{o}C	
Yield: 52.78 %	

Figure (18): Structure, Physical Properties and F.T.I.R. spectrum of [benzylidene-4-aminopyridine]carbothioamide



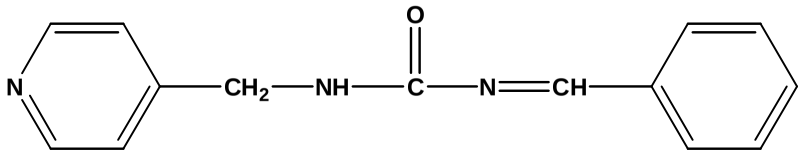
Melting point:295-297 ^{o}C	
Yield: 61.34 %	

Figure (17): Structure, Physical Properties and F.T.I.R. spectrum of [benzylidene-4-aminopyridine]carboamide

List of Figures

List of Tables

1. Swierzko, A.S., Kirikae, T., Hirata,M., Cedazynsk, M., Ziolkowski , A ., Hirai, Y .,Kusumoto, S., Yokochi, T. and Nakano, M. "Biological activities of lipopolysaccharides of *Proteus* species " , *J. Med. Microbiology.*, **49**: 127-138, (2000).

2. Quinu, P.J., Carter, M.E., and Markey, B.K., (1998). Clinical Veterinary & Microbiology. Mosby. Philadelphia.

3. Grunberg, R.N., Changes in urinary pathogens and their antibiotic sensitivities., *J.Antimicrob. Chemother*., **33**, suppl A: 1-8, (1994).

4. Gilbert, P.N., Kohlhepp, S.T., Salma, K.A., Grunkemeier, G., Lewis,G., Dioorkin, R.J., Slanghter, S.E. and Leggett, J.E. Phenotypic resistance of selected Enterobacteriaceae, and *Pseudomonas aeruginosa* after single and multiple *in vitro* exposures to ciprofloxacin, levofloxacin and trovafloxacin., *J. Antimicrob. Agents Chemotherapy*, **45** (3): 883-892, (2001).

5. Rice, L.B., Willey, S.H., Papanicolaou, G.A., Mederiro, A.A. and Jacoby, G.A. Outbreak of ceftazidim resistance caused by extended spectrum β-lactamases at Massaachusetts chronic-care facility. *J.Antimicrob.Agents chemother*., **34**: 2193-2199, (1990).

6. Cherian, B.P., Manjunath, M., Pinto Pereira, L.M. and Prabhakar, P. Extended-spectrum β-lactamase producing Enterobacteriaceae in a tertiary care hospital in Trinidad and Tobago. *West Indian. Med.J.* **52** (1): 3-31, (2003).

7. Zunino, P., Piccini, C. and Legnani-Fajard, C.Groth, Cellular differentiation and virulance factor expression by *Proteus mirabilis in vitro* and *in vivo*. *J. Med. Microbiol*.**48**: 527-534, (1999).

8. Mobley, H.L.T. and Hausinger, R.P. Microbial urease: Significance regulation and molecular characterization. *Microbiological reviews* **53**(1): 85-108, (1989).

9. Woodhead, M., Fleming, D. and Wise, R. Antibiotics resistance and clinical outcomes. *B.M.J.* **328** : 1297-1300, (2004).

10.Musher, D.M.., Griffith, D.P. and Yamn, D. Role of urease in Pyelonephritis resulting from urinary tract infection with *Proteus*. The *J. of Infectious Diseases*. **131**(2): 177-181, (1975).

11.Kaijser, B. and Larsson, P. Experimental acute pyelonephritis caused by *Enterobacteria* in Animals. *A. Review. J. Urol*. **127**: 786-790, (1973).

12. Sa'eed, M.A., " Extraction and characterization of urease of *Proteus* spp. Isolated from patients with U.T.I. in Yemen "Ph.D. Thesis, Al-Mustansyria University, (2004).

13. Noor, R.H. " Synthesis of urea analogues and applications of these compounds as urease inhibitors " M.Sc., Thesis, Al-Nahrain University, (2006).

14.O'hara, G.M., Brenner, F.M. and Miller J.M., classification, identification and clinical significance of *Proteus, Providencia* and *Morganella*. *Clinical Microbiology Reviews*.**13** (4):534-546, (2000).

15.Mobley, H.L.T., and Belas, R. Swarming and pathogenicity of *Proteus mirabilis* in the urinary tract trends in Microbiology.**3**(7): 280-284, (1995).

16.Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.A. and Williams, S.T."Bergy's Manual of Derminatives Bacteriology". (9^{th}) ed. (1994).Williams and Wilkins.

17.Karlowsky, J.A., Jones, M.E., Thornsberry, C. Friedland, I.R. and Sahm, D.F. Trends in antimicrobial susceptibility among Enterobacteriaceae Isolated from hospitalized patients in united states from 1998 to 2001. *Antimicrobial agents and chemotherapy.* **47**(5): 1672-1680, (2003).

18.Toth, V. and Emody, L., *Proteus* virulence: involvement of the pore Forming alpha-hemolysin. *Acta. Microbiot. Immunol. Hung* .**47**(4):457-470, (2000).

19. Baron, E., Pealler, M.A., Tenover, F.C. and Yokken , R.H. "Commensal and Pathogenic Microorganism in Manual of Clinical Microbiology". (7^{th}) ed.(1999). ASM press Washington, U.S.A.

20. Buchaman, R.E., and Gibbaus, N.E. "Berge's Manual of Determinative Bacteriology". 8^{th} ed., (1994). Williams and Willkins Company. Baltimore.

21. Farmer, J.. "Enterobacteriaceae and Identification in Manual of Clinical Microbiology". 7^{th} ed., (1999) .A.S.M. press. Washington, U.S.A.

22. Gue, M., Duport, V., Dufour, A. and Sire, O. Bacterial swarming: a biochemical time-resolved FT.IR- ATR study of *Proteus mirabilis* Swarm-cell differentiation. *Biochemistry*. **40**(39): 11938 - 11945, (2001).

23. Liaw, S.J., Lai, H.C., Ho, S.W., Luh, K.T. and Wang, W.B. Characterization of P-nitrophenylglycerol- resistant *Proteus mirabilis* super-swarming mutants. *J. med. Microbiology*. **50**:1039-1048, (2001).

24. Liaw, S.J., Lai, H.C., HO, S.W., Luh, K.T. and Wany, W.B. Role of RSMA in the regulation of swarming mobility and virulence factor expression in *Proteus mirabilis*. *J. of Med. Microbiology*. **52**:19-28, (2003).

25. Jones, B.D., Virginia, L.C., Johnson, P.E. and Modley, H.L.T. Construction of a Urease- Negative Mutants of *Proteus mirabilis*. *Infect. and Immun*. **58** : 1120-1123, (1999).

26. Liaw, S.J., Lai, H.C., HO, S.W. and Wany, W.B. Inhibition of virulence factor expression and swarming differentiation in *Proteus mirabilis* by P- nitrophenylglycerol *J.med. microbiology* **49**: 725-731 (2000).

27. Mandell , G.L., Bennet, J.E. and Dolin, R. " Principles and Practice of Infections Diseases". 4^{th} ed., (1995). Churchill Livingstone, London.

28.Brenner, D.J., Fanning A.G., Hickman F.W., and Wing H.W.E. Deoxyribonucleic and related ness of *Proteus* and *Providencia* species. *Int. J. syst. Bacterial*. **28:** 269-282, (1978).

29.Penner, J.L. Genus XI. *Proteus*, pp.:491-494. In N.R. skrieg And J.G. Holt(ed).,(1984).. Bergey's manual of systematic bacteriology **vol.1**.The Williams and Wilkins Co., Baltimore, Md.

30.Senior B.W. the ability of a *Proteus mirabilis* strain to invase the blood stream is independent of its proticine, production proticine Sensitivity type. *J. Med. Microbiol*. **46** :407-412,(1997).

31. Warren , J.W. Clinical presentation and epidemiology of urinary tract infection ,pp.: 3-27. In H.L.T. Mobley and J.W. Warren(ed.), (1996). U.T.I., Molecular pathogenesis and clinical management.ASM press, Washington D.C.

32. Panner, J.L. The general *Proteus*, *Providencia* and *Morganella*,pp.: 2849-2853. In A. Balows, H.G. Truper, (1992) vol.3. Springer-verlag KG, Berlin, Germany.

33.Warren, J.W.D., Damron, J.H.T., Tenny, I.M., Hoopes, B., Deforege, H.L. and Muncie, J.R. Fever bacteremia and death as complication of bacteruria in women with long-term urethra catheters., *J. Infect. Dis.* **155** : 1151- 1158, (1987).

34.Warren, J.W., Tenny, J.H. . Hoopes, J.M Muicie, H.J. and Anthony,W.C., A prospective microbiologic study of bacteruria in patient with chronic indwelling urethral catheters., *J. Infect. Dis.* **146**: 719-723, (1982).

35.Senior, B.W., Voros, S., *Proteus* profile typing- a new method at typing *Morganella* morganil strains. *J.Med. Microbiol*. **24**: 175- 180, (1990).

36.Tipple, M.A., Jarvis, W.R. and Martone, W.J. "Bacteremia and Fugemia. In Hospital acquired infection in the pediatric patient" (Donowitz, L.G., ed.), (1988) Williams and Wikins, Baltimore, Hong Kong, London, Sydney. pp.: 47-54.

37. Ayliffe, G.A., Collins, B.J. and Tylor, L.J.(1982) Hospital acquired Infection : Principles and prevention .Britol, London, Boston.

38. Pandit, D.V., Core, M.A., Saile S.N. and Deodhar, L.P. Laboratory data from the surveillance of a burns ward for the detection of hospital infection (abs.) *Burns* , **19(1)** , 52-55, (1993).

39. Chan, R.K., Lye, W.C., Lee, E.J. and Kumara, S.G. Nosocomial Urinary tract infection: intemicrobiological study (abs.) *Ann. Acad. Med. Singapore*, **22(6)** , 837 , (1973).

40. Adler , J. L. Burke, J.P., Martin , D.F., Finland , M. *Proteus* infections in a general hospital .2 . Some clinical and epidemiological characteristic with analysis of 71 cases of *Proteus* bacteria, *Anal of internal medicine*, **75** : 531-536 , (1971).

41. Senior B.W. Investigation of the types and characteristics of the Proteolytic enzymes formed by diverse strains of *Proteus* species bacterial pathogencity. *J.Med.Microbiol*,**48** :623-628(1999).

42. Rubin, R.H., Tolkoff- Rubin, N. E. and cotran, R.S. Urinary Tract infection, pyelone phrities and reflux nephropathy.pp.: 1085-1143.in B.M. Brenner and F.C. Rector (ed.), (1986), the kidney. The W.B. Saunders Co., philed elphia, Pa.

43. Engler, H.D., Troy, K. and Botton, E.J. Bacteremia and Subscutaneous a bscess caused by *Proteus penneri* in a neutropenic host. *J. Clin. Microbiol*., **28**,:1645-1646, (1990).

44. Krajden, S., Fuksa, M., Petrea, C., Crisp, L.J. and Panner, J.L. Expanded clinical spectrum of infection caused by *Proteus Panneri. J.clin. Microbiol*.**25** : 578-579,(1987).

45. Wilson, C., Corbett, M.and Ebringer, T. Increase isolation of *Proteus mirabilis* species from rheumatoid arthritis patients and healthy contrls. *Br. J. rhematol*, **29** (suppl 2):99,(1982).

46. Dumanski, A.J., Hedelin, A., Edin-Liljegren, D., Beauchermin, and R.J.C. Mclean. Unique ability of *Proteus mirabilis* capsule to enhance mineral growth in infection urinary calculi. *Infection Immune*. **62** : 2998- 3003 (1994) .

47. Senior B.W., McBride, P.D.P, Morley, K.D. and Kerr, M.A. The detection of raised level of IGM to *Proteus mirabilis* in sera From patients with rheumatoid arthritis. *J. Med. Microbiol*. **43**: 176-184 (1995).

48. Clapham, L.R.,Mclean, J.C. Nickel, J. and Costerton, J.W. The influence of bacteria on struvite crystal habit and its importance in urinary stone formation. *J. crystal Growth*. **104** :475-484, (1990).

49. Torzewska, A., Staczek, P., Rozalski, A. Crystallization of Urine mineral components may depend on the chemical nature of *Proteus* endotoxine polysaccharides. *J. of Med. Microbial*. **52** (6): 471-477, (2003).

50. Itami, N., Akustu, Y., Yasoshima, K. and Yamamato, K. Renal Tubular Acidosis and U.T.I. *J. Pediatric*.**117**:156, (1990).

51. Kaitwatcharachi, C., Vasuvattakul, S., Yenchitsomeans P. *et. al.*, Distal Renal Tubular Acidosis and high urine carbon dioxide tension in patients with south East Asian Ovolocytosis. *Am.J. of Kidney Dis.* **33** : 1147-1151, (1999).

52. Van-Deale, P.L., Quero-Guillen, J.C., lesterhuis W. and Van-Der-Menlen, J. Hyperammonemia in Hydronephrosis . *Ned. Tijdschr- Geneesked.* **142**: 2414-2415 (1998).(Abstract).

53. Peerbooms, P.G.M., Verwej, A. M.J.J. and Maclean, D.M. Uropathogenic properties of *Proteus mirabilis* and *Proteus vulgaris*. *J. Med. Microbiol*. **19**:55-60 (1985).

54. Senior , B.W. and Hughes, C. Production & Properties of Hemolysins from clinical isolates of the proteeae. *J. Med. Microbiology.* **24** :17-25 (1987).

55.Belas, R. Expression of multiple flagellum coding genes of *Proteus mirabilis*. *J. Bacteriol*. **176** :7169 - 7181, (1994).

56.Silverblatt, F.J. Host-parasite interaction in the rat renal pelvis: a Possible role of pilli in the pathogenesis of pyelonephritis, *J. Exp. Med*. **140** :1696-1711,(1992).

57.Raid, G. and Sobel, J.D. Bacterial adherence in the pathogenesis of urinary tract infections: *Rev. Infect .Dis*. **9**:470-87, (1987).

58.Cellinl,L.R., Piccolomini, N. Allocati, S. and Ravvagnann, G. Adhesive properties of *Proteus* Genus related antimicrobial agent's resistance. *Microbiological.* **10**: 291-299(1987).

59.Rietschel, E.T., T.Kirikae, F.U., Schdae, U., Mamat, G., Schmidt, H., Loppnow, A.J. and Brade, H. Bacterial endotoxine: moleculer Relationship of structure to activity and function *FASEB J*. **8**: 217-225, (1994).

60.Vaara, M., and Viljanen, B. Binding of the polymyxine B Nonapeptide to gram negative bacteria. *Antimicrob. Agents Chemother.* **27**: 548-554, (1985).

61.Coyne, C.P., and Fenwich, B.W. Inhibition of Lipopolysaccharide Induced macrophage rumor necrosis factor synthesis by polymyxine (B) sulfate. *A.M.J. Vet Res*. **54**:305-314, (1993).

62.Chippendale, G.R., Warren, J.W. and Mobley, H.L.T.In ternalization of *Proteus mirabilis* by human renal epithelial cells. *Infect .Immune*. **62**: 3115-3121, (1994).

63.Allison, C., N. Coleman, P.L. Jones, and Hughes. Ability of *Proteus mirabilis* to invade human Urothelial cell in coupled to motility and Swarming differentiation. *Infect. Immune.* **60**:4740-4746, (1992).

64.Beynon, L.M.D.J.Dumanski, R.J.C. Melean, L.L.Maclean J.C.Richareds, and M.B.Perry. Capsule structure of *Proteus mirabilis*(ATCC 49565).*J. Bacteriol*. **174**:2172-2177(1992).

65. Assher, A.W.(1980), "The challenge of urinary tract infection: the Problem", Academic press Inc, London, LTD.

66. Kumazawa, J.; and Shingawa, N. The microbiocenosis of the urinary Tract during treatment with fluorinated quinolones. *Jpn.J.Antibio*., **47** (10): 1259-1260, (1994).

67. O'Gradyfwl (1980),"Factors of affecting urinary tract infection" In: tutorial in post graduated medicine urology by Chisholm G.D.

68. Lohr, J.A. "The foreskin and urinary tract infection" *J. pediatrics*, **114 (3)**:502- 504, (1989).

69. Santoro, J. and Kaye,D.,"recurrent UTI pathogenesis and management" Med. *Clin. Noth. Am*., **62**: 1005-1006, (1987).

70. Anderson, W.A.D.; and Thomas, M.S.," Synopsis of pathology" 9th ed., (1976), the C.V. Mosby Company.

71. Andrews, R.K., Blakeley, R.L. and zerner, B. (1984). Urea and Urease "Advances in Inorganic Biochemistry"(Ed. by: Eichhorn, G.L. and Marzilli, L.G.).6, pp.:245-283. Elsevier Science Publishing Inc., New York.

72. Mobley, H.L.T.(1992). "Urease, Microbial, Encyclopedia of Gastroenterology". Suppl., **187**, pp.: 39-46.

73. Glupczyrzski, Y. Microbiological and serological diagnostic tests for Helicobacter pylori. An over view. *Acta.Gastroentrol.Beig*., **61**: 326-321, (1998).

74. Tokunaga, Y., Shirahase, H.,Yamamoto, E., Gouda Y., Kanaji, K. and Ohsumi, K. Semi quantitative evaluation for diagnosis of *Helicobacter pylori* infection in relation to histological changes. *Am. J. Gastroenterol.*, **93**: 26-29, (1998).

75. Phillips, A., Pretorius, G.H. and DU-Toit, P.J. A survey of yeast Urease and characterization of partially purified Rhodospoddiurn Paludigenum urease. *FEMS. Microbial. Lett*., **63**:21-25,(1991).

76.Polacco, J.C. and Havir, E.A. Comparisons of soy bean urease isolated from seed and tissue culture. *J. biol. Chem*.., **254**: 1707-1715, (1979).

77.Deiluva, A.M., Markley, K., and Davies, R.E. The absence of Gastric urease in gram- free animals. *Biochem. Biophys, Acta*.,**51**:646-650 (1968).

78.Bailey, C.J. and Boulter, D. Urease a Typical seed Protein of the lequminosae. "Chemotaxonomy of the leguminosae"(Ed. By Harborne, J.B., Boulter, D. and Turner, B.L.), (1971). pp.: 485-502. Academic press, London.

79.Du-Toit, PJ., Van-Aswcgen, C.H., Nel, J.A., Steyn, P.L., Ligthelm, A.J. and De- Pless, D.J. Invitro effects of urease production Bacteria involved with the pathogenesis of infection-induced Urolithiasis on renal urokinase and sialidase activity. *Uro. Research*. **23**, pp.: 335-338, (1995).

80.Reithal F.J. Urease the enzyme, ed. By Boyer, P.D., Lardy, H. and *Mybock, K.*, **4**: 17- 21, (1971).

81.Booth, J. and Vishniae, H. Urease testing and yeast taxonomy. Can. *J. Microbiol*., **33**: 396- 404, (1987).

82.McCoy, D.D. Cetin, A. and Hausinger, R.P. Characterization of Sporsarcina urea. *Arch. Microbiol*., **157**: 411-416, (1992).

83.Mclaen, R. J., Cheng, K.J. Gould, W.D., Nickel, J.C. and costertion J.W. H.istochemical and biochemical urease localization in the Periplasm and outer membrane of the two *Proteus mirabilis* strain. Can. *J. Microbiol*., **32**: 772-778, (1988).

84.Dunn, B.E., Vakil, N. B., Sclineider, B.G., Miller, M.M., Zizter J.B., Peutz, T. and Phadnis, S.H. localization of *Helicobacter Pylori* urease and heat protein in human gastric biopsies. *Infect. Immun*. **65**: 1181-1188, (1997).

85.Dixon, N.E., Riddles, P.W., Gazzola, R.C., Blakeley, R. L. and Zerner, B. Jack bean urease (E.C.3.5.1.5).On the mechanism of action of urease on urea, form amide, acetamide, N-Methylurea, and Related compounds. *Can. J. Biochem*., **58**:1335-1344,(1980).

86.Mobley, H.L.T., Island, M.D. and Hausinger,R.P. Molecular biology of microbial urease. *Microbiol Review*, **59**: 450- 481, (1995).

87.Jabri, F., Carr, M.13., Hausinger, R.P. and Karplus, P.A. The crystal structure of urease from *Klibsiella aerogenes*. *Sci*., **268**: 998-1004 (1995).

88.Benini, S., Rypniewski, W.R., Wilson, K.S.,Miletti, S., Ciurli, S. and Mangani, S. the complex of Bacillus pasteurii urease with acetohydroxamate anion from X-Ray at 1.55 A Resolution, *JBIC*., **5**: 112- 118, (2000).

89.Zerner, B., Recent advances in the chemistry of an old enzyme, Urease. *Bio.Org. Chem*., **19**:116-131, (1991).

90.Richard-Houpt, T. and Houpt, K.H., Transfer of urea nitrogen Across the rumen wall, *Am. J. physiol*., **211**:296-303,(1963).

91.Khanna, M., Pearson, M.A. and Hausinger, R.P., 70 years of crystalline Urease: *Academic chem.. Review*, **30**:327-330, (1990).

92.Hu, L.T., Nichlon, E.B., Jones, B.D., Lyncli, M.J. and Mobley, H.L., *Morganella morganii* urease: Purification, characterization and a solution of gene sequences, *J. Bacterial*., **172(6)**:307-308,(1990).

93.NaKarnura, H., Yoshiyama, H., Takeuclii, H., Mizote, T., Okita, K., and Nakazawa, T., Urease play an important role in the Chemo tactic motility of *Helicobacter pylori* in a viscous environment, *Infect. Immun*., **66**:4832- 4837, (1998).

94.Sobel, J.D. and Kaye D, (2006) "Urinary Tract Infection".

95.Stamm, E.W.; and Turck. M., (1987). "Principle of Internal Medicine" Harrison's, Martin, J. Fauce, A.S.

96.Haslett, C.; Chilvers, E.R.; Hunter, J. A.A., and Boon, N.A. Davidson's principles and practice of medicine.(18th)ed. (1999) U.K.

97.Mimis, C.A, playfair, H., roitt, I.M. and Wakelin, D. (1995). "Medical Microbiology". Mosby. Times mirror intern. Publisher Ltd. Hong Kong.

98.Macleod, J. and Edwards, C. "Disease of the Kidney and Genito Urinary system in: Davidson's principles and practice of Medicine" (17th) ed. (1995), P: 650. ELBS and Bouchier, I.

99.Goven, A.T.; Macfarlane, P.S.; Challender, R.. "Genito Urinary system in: pathology illustrated". (2nd) ed., (1988), P: 629, Churchill Livingstone.

100.Neu, H.C." Therapy and prophylaxis of Bacterial infection". pp.:478-493. In Harrison's principles of internal Medicine by Wilson J.D.; Braundwold, E.; Martin, J.B.; Fauci, A.S.; and Root, R.(12th)ed. (1991), Mc-Graw. Hill. New York.

101.Stamm, E.W., Hooton, T.M., Johnson, J.R. ***et. al***. U.T.I. from Pathogenesis to treatment. *J. Infect. Dis*. **159**: 400-405, (1989).

102.Sligh, J.D., and Timbury, M.C.(1994).Notes on Medical Bacteriology. Churchill Livingstone. Inc. New York.

103.Mandell, G.L., Bennett, J.E. and Dolin, T.R., "Urinary Tract Infections During Pregnancy", *American Academy of Family Physicians*.(2006).

104.Schaffer, J.A., Cystitis and pyelonephritis in: The biologic And clinical basis of infection disease Youmans, G.P.;Paterson, P.Y.; and sommer, H.M.P:418(1985).

105.Li, X.; Zhao, H.; Lockatell, V.; Drachenberg, C.B.; Lohnson, D.E.; Mobley, H.L.T. Visulalizaion of *Proteus mirabilis* Within the matrix of urease-induced bladder stone during Experimental urinary tract infection. *Infection and Immunity Jan*. **45(2)**:389-394, (2002-a).

106.Li,X.; Lockatell, V.; Jahnson, D.E.; Mobley, H.L.T. Identification of MRPI as the sole recombines that regulates the phase variation of MR/P fimbria, a bladder colonization factor of uropathogenic *Proteus mirabilis*. *Molecular Microbiology*. **45(3)**:865-874 (2002-b).

107. Li, X., Lockatel, C.V., Johnson, D.E., Lane, M.C., Warren, J.W., Mobley, H.L.T. Development of intranasal Vaccine to prevent Urinary tract infection by *Proteus mirabilis*. *Infect. Immun. Jan*. **72(1)**: 66-75(2004).

108. Dattelbaum; J.D.; Lockatell, C.V.; Johonson, D.E.; Mobley, H.L.T. Ure. R, the transcriptional activator of the *Proteus mirabilis* urease gene cluster, is required for urease activity And virulence in experimental urinary tract infection, infect. *Infect. Immun. Feb*. **71(2)**:1026-1030,(2003).

109. Li, X.; Mobley, H.L.T. Vaccines for *Proteus mirabilis* in urinary tract infection. *International J. of Antimicrobial agents*. **19**:461-465, (2002).

110.Kumamato, Y.; Tsukamoto T.; Hirose, T.; Yokoo, A.; Takahashi, T. *et. al.* Comparative studies on activities of antimicrobial agents against causative organisms, Isolated from patients with U.T.Is. Background of patients. *Jpn.J. Antibiotic*. **50**; pp.: 251-264 (1997).

111.Seftal, Z.; Madhun, T.; and Kim, E.D.Sexuaz function In Med. with Diabetes Association with Glycemic Control. *J.Urol*.**163**: 788-792, (2000).

112.Raka, L., Mulligi Osmani, G., Berisha, L., Begolli, L., Omeragiq, S., Parsons, L., Salfinger, M., Jaka, A., Kurti, A. and Jakupi, X. Etiology and susceptibility of urinary tract isolates in Kosovo. Int. *J. Antimicrob. Agents*. Mar. **23(suppl 1)**: 2-5 (2004).

113.Glennon, J.; Ryon, P.J.; Keane, V.T. and Rees, J.P. Circumcision and per urethral Carriage of *Proteus mirabilis* in Boys. *Arch. of Disease in childhood*. **63**:556-558, (1988).

114.Zhao, H.; Li, X., Johnson, D.E.; Mobley, H.L.T. Identification of protease and rpo N-associated genes of uropathogenic *Proteus mirabilis* by negative selection in a mouse model of ascending urinary tract infection. *microbiology*. Jan.**145(1)**:185-195, (1999).

115.Cohen, T.D.; Preminger, G.M. Struvite calculi. Semin. *Nephrol*., **16**:425-434 (1996).

116.Rassweiler, J.J.; Renner, C.; *Chaussy*, C.; Thuroff, S. Treatment Of renal stone by extracorporeal shockwave lithotripsy.: *An Update. Eur. Urol.* **39**:187-199, (2001).

117.Aswegan, C.; Dirksen, Van Sckalkwyk, J.; Toit, P.; Verster, I.; Franz, R.; and plessis, D. The effect of calcium and Magnesium Ions on Urkinase and Sialidase activity.*Res*. **20** , P.: 41 (1992).

118.Nancollas, G. The Kinetics of Crystal Growth and Renal stone Formation. In H. Fleisch, W.G. Roberston, L.H. smith, *et. al.* Urolithiasis research. New York: plenum:5-23(1976).

119.Cotran, R., Kumar, V., Robbins, S. and Schoen, F. "Pathologic Basis of Disease". 5th ed. (1996), W.B. Saunders Co. London. pp.: 930-986.

120.Disilverio, F.; Dangelo, A.; Gallucci, M.; Seccareccia, F. and Menotti, A. Incidence and Prediction of stone recurrence after lithotripsy In Idiopathic calcium patients: A multivariate Approach. *Eur. Urol*. **29**: 41-46, (1996).

121.Herring, L. Observation on the analysis of Ten Thousands Urinary Calculi. *J. Urol*. **88**: 545-562, (1962).

122.Stoller, M.; and Bolton, D.."Urinary Stone Disease", In E.A. Tanagho and J.W. Mc Aninch (eds.), General Urology. 14th ed. (1995), Appleton and Large. New York.

123.Ito, H.; Kotake, T.; Nomura, K.; and Masai, M. Clinical And Biochemical Features of Uric Acid Nephrolithiasis. *Eur. Urol*. **27**: 324-328 ,(1995).

124. Toit, P., Aswegan, C., Steyn, P., Pols, A., and Plessis, D.Effect of bacteria Involved with the Pathogenesis of Infection-Induced Urolithiasis on the Urokinase and Sialadase (Neuraminidase) Activity. *Urol. Res*. **20**:393-397(1992).

125. Schaecter, M., Medoff, G. and Elsenstein, B.I." Mechanisms of Microbial Disease". 2nd ed. (1993),Williams and Wilkins, U.S.A.

126. Ibrahim, S., Honig, P., Hunig, S.M., Gillespie, W. and Williams, R.L. Clinical Pharmacology and Regulatory Perspectives. *J. Clin. Pharmacol*. **40**:31-38 (2000).

127. Jawet, Levinson , W.(2002) "Medical Microbiology and Immunology" Examination and broad View.

128. Website: http://www.phs.co.uk/facts/, public Health Laboratory Service.

129. Laurence, D.R., Bennett, P.N. and Brown, M.J. "Clinical pharmacology" 8th ed. (1997)., Churchill Livingstone London.

130. Schroder, F.H. Comments on U.T.I. In: Recent Advances in the Treatment of U.T.I. Royal Society of Medicine Service, *International Congress and Symposium Series*. **97**:412-423, (1985).

131. The American FDA website **http:www.Fda.Gov/fdac/features/ antibio. Html.**

132. Donowitz, G.R. and Mandell, G.L.: Drug therapy: Beta-Lactam Antibiotics. (Two parts) *N. Engl. J. Med*. **318**:419-423, (1988).

133. Geo, F., Janet, S. and Stephen, A.; "Medical Microbiology" Jawetz, Melnick, and Adelberg's, 21st ed. (1998)., Asimon Singapore. pp.: 145 -150.

134. Gustaferro, C.A., Steckelberg, J.M., Cephalosporin antimicrobial agents and related compounds, *Mayo. Clin. Proc*. **66**:1073, (1991).

135.Brewer, N.S. and Hellinger, W.C. Antibiotic tolerance among Clinical isolates of bacteria, New Engl. *J. Med*., **345**:1042-1053, (1998).

136.Wilhelm, M.P., Vancomycin. Mayo. *Clin. Proc*. **66**:1165, (1991).

137.Pelczar, J., "Microbiology", 5th ed., (1986), McGraw-Hill, New Delhi.

138.Katzung, B.G.,"Basic and Clinical pharmacology",6thed.(1994), Appleton and Lange. New York.

139.Siegel , D.; Tetracyclines: New look at an old antibiotic, clinical Pharmacology, mechanism of action, and untoward effects. N. Y.State. *J. med*. **78**: 950, (1978).

140.Stryer, L., "Biochemistry" 4th ed. (2000) pp.: 875-877. W.H. Freeman and Company. New York.

141.Katzung, B.G. "Basic and Clinical Pharmacology". 8th ed. (2001) Lange Medical Books/ McGraw-Hill. New York.

142.Busse, H.J., Wostmann C. and Bakker, E.P. The bacterial action of streptomycin: membrane permeabilization caused by the insertion of mistranslated proteins into the cytoplasmic membrane of *Escherichia coli* and subsequent caging of the antibiotic inside the cell :degradation of these proteins. *J. Gener. Microb*., **138**: 551-561, (1992).

143.Mingeot-Leclerco, M.P.; Glupczynski, Y. and Tulkens, P.M. Mini Review: Aminoglycosides Activity and Resistance. *J. Antimicrob. Agents chemther*. **43**: 727- 737, (1999).

144.Hooper, D.C. and Walfson, J.S. Fluoroquinolone antimicrobial agents. *N. Engl. J. Med*. **324**: 384, (1991).

145.Rubin, R.H. and Swartz, M.N. Trimethopri-sulfamethoxazole. *N. Engl. J. Med*. **30**(3): 426, (1980).

146.Fletcher, C. First clinical use of penicillin. *British. Med. J*. **289**: 1721- 1723,(1984).

147.لجنة من تدريسي قسم علوم الحياة وعلم الاحياء المجهرية ، صفحة 90 ، جامعة بغداد / كلية العلوم (1991)

148.Sykes, R.B. and Mathew, M. The Beta-Lactamases of gram-negative bacteria and their role in resistance to Beta-Lactam antibiotics. *J. Antimicrob. Chemother*. **2**: 115-117, (1976).

149.Preston, K.E., Kacica, M.A., Limberger, R.T., Archinal, W.A. and Venezia, R.A. The Resistance and Intgrase Genes of PACMI, A conjugative Multiple-Resistance plasmid from *Klebsiella* Oxytoca. Plasmid, **37**: 105-108 , (1997).

150.Lauderdale, T.L., McDonald, C.L., Shiau, Y.R.; Chen, P.C., Wang, H.Y., Lai, J.F., Ho, M., TSAR participating hospital. The status of antimicrobial resistance in Taiwan among Gram-negative pathogens: the Taiwan surveillance of Antimicrobial Resistance (TSAR) program (2000). *Diagn. Microbial. Infect. Dis.* Mar. **48**(3): 211-219, (2004).

151.Ueda, Y and Sunagawa, M. *in vitro* and *in vivo* activity of novel 2-(thiazol-2-ylthio)-1-beta-methylcarbapenemes with potent Activity against multi resistant gram-positive bacteria. *Antimicrob. Agenets chemother.* Aug. **47**(8):2471-2478, (2003).

152.Gori, A., Espinasse, F.; Deplano, A., Nottof, C., Nicolas, M.H., and strulentes, M. J. Comparison of Pulsed- Field Gel Electrophoresis and Randomly Ampliphied DNA Polymorpholism analysis *J. Clin. Microbiology*. **34**: 2448-2453, (1996).

153.Lee, W. Rjoberts, S.M. and Labbe, *R.F.,Clin.Chem*.,**43**(1),154-157,(1997).

154.Sackheim, G.I. and Lehman, D.D. " Chemistry for the Health Science" 5th ed., (1985).

155.Atlas, R.. "Laboratory manual of experimental microbiology". 1st ed. (1995), Mosby, Inc. Missouri.

156.National Committee For Clinical Laboratory Standards (1988). Performance Standard For antibiotics Susceptibility Testing NCCLS. Villanova P.A.

157.Inobo, H.I., and Obanibi, H.B.T., Antimicrobial isolates to commonly used antibiotics. *African J. of Biotech*. **5** (5):487-489, (2005).

158.Baron, E.J. and Finegold, S.M. "Diagnostic Microbiology". 8th ed. (1990). The C.V. Mosby Co. Battimore.

159.Brooks, G.F, Butel, J. S., and Morse, S.A., Jawetz, Melnick and Adelbergs Medical Microbiology. 21st ed. (1989), Appleton and Lange California, pp:216-220.

160.Alexander,S. K. and Strete, D. , (2001) "Microbiology : A photographic Atlas For The Laboratory" Benjamin Cumming, Wesley Longman, Inc., pp.:35-110.

161.Maniatis, T., Fritch, E.F. and Sambrok, J. (1982). "Molecular cloning a laboratory manual. Gold spring Harbor Laboratory, New York.

162.Cruiclsshank, K., Duguid, J., marimon, B., Swain, R. "Medical Microbiology " , 12th ed. (1976), Churchill Livingstone. London.

163.Al-Shweely, Jameela, R.E., M.Sc., Thesis, Baghdad University, (2006)

164. Baron, E.J. and Finegold, S. M. (1994). Microorganisms Encountered in Urinary Tract in Baily and Scotts Diagnostic Microbiology. 9th ed. Mosby Company U.S.A.

165. Bauer, A.W., Kirby, W.M. M, Sherris, J.S. and Truk, M., Antibiotics susceptibility testing by standardized single disk method, *J. Clin. Path.*, **45**:.493, (1996).

166.Vandepitte, J, Engbaek, K., Piot, P. and Heuck, C.C. "Basic lab. Procedures in Clinical Bacteriology", W. H. O. - Geneva, (1991).

167.Kanneth, J., Ryan, C. and George, R., " Medical Microbiology" , 8th ed. (2003), P.: 193, U.S.A.

168.Najim, H. n, Kazal, K. F and Jalil, H. A. Antibiotics residues in milk of Baghdad area. The Iraqi J. Vet. Med., **12**: 32, (1986).

169.Al-Shammary, F. G. H.,. The Mixture effect of oily external from block cumin and alconolic bacteria, M.Sc. Thesis in Microbiology, College of Education, Al-Qaddisiah University, P.41, (2005).

170.Svehla, G."Vogel's qualitative inorganic analysis", 7th ed. (2001).Longman, India.

171.Tyagi, B.S, Srivastava, A.K. and Jain, P.C. " A manual of practical chemistry". Vol. I., 1st ed. (1978). Atman Ram and Sons, Delhi. India.

172.Al-Shammary, K.M., Biochemical and histopathological study in placentae of pregnant women with pre- eclampsia and eclampsia, M.Sc., Thesis, in Biochemisry, College of Science, Al-Nahrain University, PP.:33-37,(2002).

173.Fawcett,J.K. and Scott,J.E., J.Clin, Path., **13**,156-159, (1960).

174.Barham,D. and Trinder, P. Analyst,**97**:124, (1972).

175.Oliver, I.T., Biochem. J., **61**: 116, (1955).

176.Morrison, R.T. and Boyd, R.N., " Organic Chemistry " 4th ed. (1983).PP.:756-923, U.S.A.

177.Socrates, G., " Infrared Characteristic of Group Frequency ", (1980) John Wiley & Sons Inc.

178.Silverstein, R.M., Bassler, G.C. and Morrill, T.C. " Spectrometric Identification of Organic Compounds" 4th ed. (1980), Wiley, New York.

179.Pretsch, E., Scibel, J., Simon, W. and Clere, T. " Tables of Spectral Data for Structure Determination of Organic Compounds" , (1983), Springer- Verlag , Berlin.

180.Nakanishi, K., " Infrared Absorption Spectroscopy, 2nd. ed. (1980), Holden-Day, San. Francisco, Calif.

181.Sykes, P., " A Guide book to Mechanism in Organic Chemistry " 5th ed. (1981)., Longman, New York.

182.Shriner, R.L., Fuson, R.C., Curtin, D.Y. and Morrill, T.C., " The Systematic Identification of Organic Compounds", 6th. ed. (1980)., Wiley, New York.

183.Keller, R.J., " The Sigma Library of F.T-Infrared Spectra ",1st ed. (1986)., By Nicolet Instruments. St. Louis.

184.Keizur, J.J., Lavin, B. and Leidich, R.B. Latrogenic U.T.I. with *Pseudomonas cepacia* after transerectal ultrasound guided needle biopsy of the prostate. *J. Urology*, **149**: 523-552, (1993).

185.Shama, S., Sambasirarao, R., and Ravi, V. *Proteus* infection with reference to protection typing. *Indian, J. Med. Res*. **80**: 54-58,(1984).

186.Orrett, F. A. Prevalence of *Proteus* spp. In U.T.I in a regional hospital in trinided. *Chung – Hua - I- Hsuch - Isa- Chin –Taipei*.**62**:438-442 (Abstract), (1999).

187.Mehr, S.S., Powell, C.V. and Cuurti, N. Cephalosporin resistant urinary tract infections in young children. *J. Paediatr. Child. Health*. Jan-feb **40**(1-2):48-52, (2004).

188.Warren, J. W., Tenney, J. H., Hoops, J. M. Munciey, H. L. and Antony, W.C. A prospective microbiologic study of bacteria in patients with chronic is dwelling urethral catheters. *J. Infect. Disc.* **146**:719-723 (1982).

189.Khurana, S., Taneja, N. and Sharma, M. Extended spectrum beta-lactamase mediated resistance in urinary tract isolates of family Enterobacteriaceae. *Indian. J. Med. Res*. Oct. **116**:145-149 (2002).

190.Naylor, G.R.E. A 16 months analysis of U.T.I. in children, *J. Med. Microbial.* **17**:31, (1984).

191. Egorov, N.S.,"Antibiotics, A scientific approach", 1st ed. (1985), ,Mir Publishers, p.11.

192. Ling, J. M., Lam, A. W., Chan,E. W. and Chengy, A.F., What have we learn from community-aquired infections in Hong Kong?. *J. Antimicrobal. Chemother.* Apr. **51** (4): 895-904, (2003).

193. Gur, D., Kanar, G., Ceyhan, M., Secmeer, J. and Kanra, B., Kaymakoglu, epidemiology and antibiotics resistance of gram-negative urinary pathogens in pediatric patients. *Tuk. J. Pediater. Jan*-Mar, **41** (1):37-42, (1999).

194. Kahlmeter, G. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO-SENS project. *J. Antimicrobial. Chemother.* **51**:69-76, (2003).

195. zhanel, G., Karloessky, J.A., Hardring, G. K. M., Carrie, A., MAzzulli, T., Low, D. E., kibsey, P., Roscoe, D.L., Gibb, A.P., Rennie, R., Bolandeau, J., Dubois, J. ,Loo, V.,laverdire, M., and Hoban, D. J. A Canadian national surveillance study of urinary tract isolates from outpatients: comparison of the activities of trimethoprime- sulfamethoxazole ampicillin, mecillinam, nitrofuranatoin and ciprofloxacin. *Antimicrobial agents and Chemotherapy*. Apr., **44**:1089-1092, (2000).

196. Obi,C.L., Tarupiwa, A. and Simango, C. Scope of urinary pathogens isolated in the public health bacteriology laboratory, Harare: antibiotics susceptibility patterns of isolated and incidence of hemolytic bacteria. Cent. *Afr. J. Med.* Aug., **42**(80):244-249 (1996).

197. Ali, R.Y.M., Abdullah, F.M. and Ahmed, R.A., U.T.I in Mosul City, a bacteriological study. *J. Fac. Med*. **29**:391-396 (1987).

198. Almarjani, M.F. The effect of *Proteus mirabilis* isolated from human urinary tract infection on the morphology and physiology of rats kidney. M.Sc. thesis in Microbiology, College of Science, Al-Mustansiriya University. PP.:45- 60, (2000).

199.Lima, M.B.C., Deolivera, G.F.A. and Galvac, L.F., Trends in bacterial resistance and implication for treatment of infection. *J. Inter. Med. Res.* **18**(4):3-5, (1990).

200.Bradford, P.A. Extended spectrum Beta-lactamases in the 21st century characterization, epidemiology, and detection of this important resistance threat. *Clin. Microbial. Rev*. **14**: 933-951, (2001).

201.Murray, B.E, Vancomycin – resistant Enterococcal infection N. *Engle. J. Med*. **342**: 710-721, (2000).

202.Martinez- Pineiro, J. A., Deiriarte, E.G. and Armero, A.H., The problem of reoccurrences and infection after surgical removal of staghorn calculi. *Eur., Urol*., **8**(2): 94-101, (1982).

203.Hess, B., Metzger, R.M., Ackerman, D., Montaandon, A. and Jaeger, P., Infection-induced stone formation in a renal allograft. *Am. J. Kidney dis.* Nov., **24**(5): 868-872, (1994).

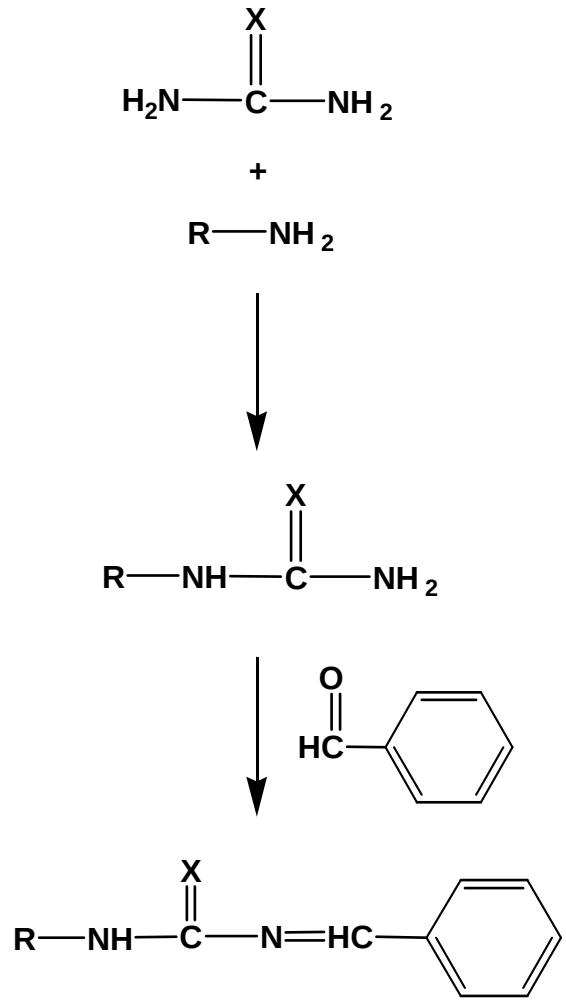
204.Schrier, R.W. & Gottschalk, C.W., " Diseases of the Kidney "5th ed., Vol. I, (1993)., Little , Brown & Company . Boston / Tornato / London. PP.: 579-1120.

205.Erley, C.M.M., Hirschbery , R.R., Hoefer, W., and Schaefer, K., Acute renal failure due to uric acid nephropathy in a patient with renal hyperuricemia . *Klin. Wocheaschr*. **67**: 308, (1989).

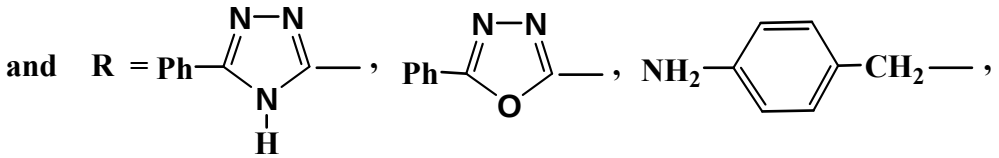
206.Schrier, R.W. & Gottschalk, C.W., " Diseases of the Kidney "5th ed., Vol. III , (1993).PP.: 2473-2680, Little , Brown & Company . Boston / Tornato / London.

207.Katzung , B.G. " Basic and Clinical Pharmacology " 4th ed., (1989).PP.: 280-295. Appleton & Norwallk, Los Altos, California.

208.Anderson, S.C. and Cockayne , S., " Clinical Chemistry Concepts & Application " (1993), PP.: 225-227.



Where X = O or S

and R = 

Scheme showed the overall steps of reactions to prepared compounds in this work

Case sheet

No.:				
Name:		**Age:**		**Sex:**
Jop. :				
Hypertension history:		**Yes**		**NO**
Diabetic:		**Yes**		**NO**
Chronic infection :		**Yes**		**NO**
Kidney stone:		**Yes**		**NO**

- **G.U.E.**
  Reaction:
  R.B.Cs.:
  Crystals:
  Albumin:
  Others:

- **Biochemical test results (Blood):**
  - **B.U.:**
  - **S.Cr.:**
  - **Uric acid:**
  - **R.B.S.:**
  - **Ca^{+2} :**

- **Culture results:**

  ☐ **Gram + ve** ☐ **Gram -ve**

- **Results of antibiotics sensitivity test *in vitro*:**

- **Results of prepared compounds activity *in vitro*:**

Summary

Novel series of 14 (urea and thiourea) derivative compounds were prepared from reaction of urea and thiourea with aromatic amine compounds (homocyclic and heterocyclic), in addition to preparation of 6 Schiff bases (C=N) compounds that were prepared from reaction of some derived compounds with benzyldehyde in this study. All the 20 prepared compounds were characterized according to F.T.I.R. spectra, purities were examined by T.L.C., and the melting points were measured to identify each of them.

From November 2005 through March 2006, a sample of (urine and sera) from 200 patients suffering U.T.I. and urinary stone attending (Diwaniya Teaching Hospital, Pediatrics and Gynecology Teaching Hospital, Health Centers and Private Clinical Laboratories) in Diwaniya, another sample was collected from 100 healthy persons as a control group.

The inhibition activity was measured for each of 9 commercially used antibiotics, and for the all prepared compounds *in vitro* against *Proteus* bacteria isolated from patients under this study and identified by valid laboratory methods (Api-20E method).

From (renal and bladder) 13 stones were collected from patients to detect the type of each stone by qualitative chemical analysis. Also the level of blood urea, serum uric acid, random blood sugar, serum creatinine and serum calcium, was measured for all samples in this study.

This study shows the following results:

- Bacterial growth for *Proteus* and other kind of bacteria were found in 138 patients out of the 200 (69 %), while *Proteus* spp. was isolated from 23 representing by 16.66 % of *Proteus* spp. out of the

138 patients of U.T.I.. Results of biochemical tests showed that *Proteus mirabilis* was found in 21 isolates with 91.3 %, and *Proteus vulgaris* in 2 isolates with 8.7 %.

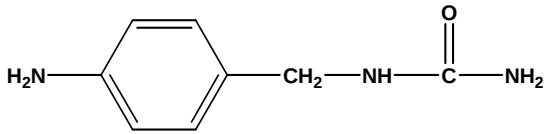
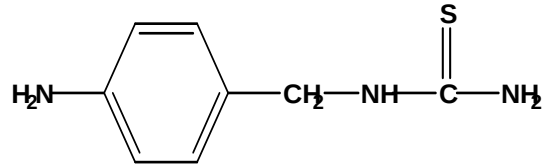
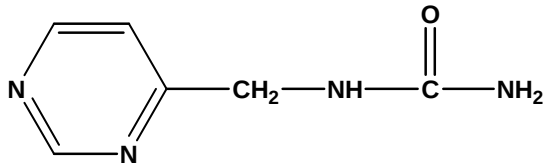
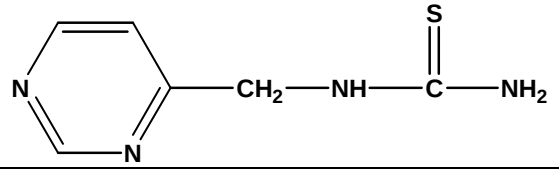
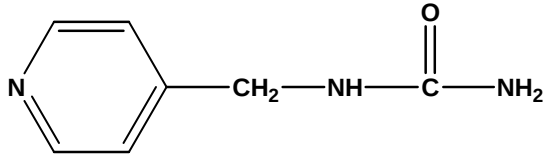
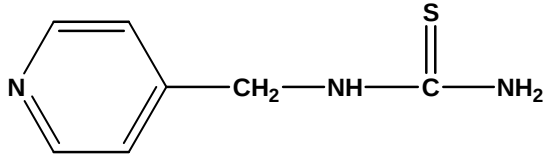
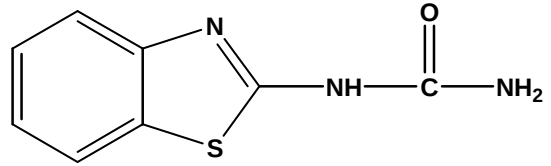
- Erythromycin was found to have the lowest inhibitory activity against *Proteus* spp. isolates with a percentage of resistance 86.9%, while Gentamycin have the highest inhibitory activity with a percentage of resistance 4.3 %. In bacterial sensitivity test among the 9 antibiotics was commonly used in this study.
- The prepared compounds showed inhibitory activity against growth of isolated *Proteus* spp. in (19 out of 20) compounds, [N-(5-phenyl-1,3,4-thiadiazole)-2-ylthiourae] shows the highest inhibition activity with a diameter of inhibition zone 30 mm., while the [N-pyridine-4-ylurea] shows no such activity in the same concentration, as compared with Gentamycin antibiotics which shows 18 mm diameter of inhibition zone.
- Struvite stones were commonest type of stones in patients with 61.5 %, followed by apatite carbonate stones with 30.7 % then mixed stones with 7.85 %.
- Statistical analysis shows a significant difference ($P < 0.05$) of the levels of blood urea, uric acid, serum creatinine and serum calcium for the patients when compared to that of control group. While no significant difference ($P > 0.05$) is observed or the R.B.S. level between the same groups.

Symbols and Abbreviations

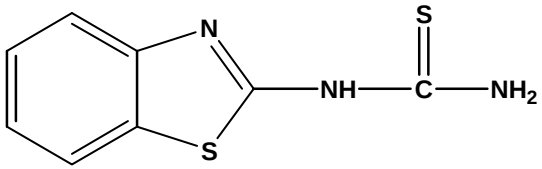
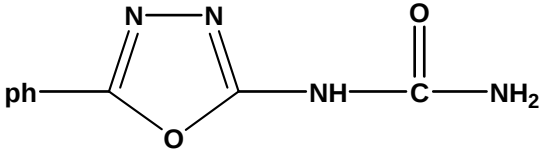
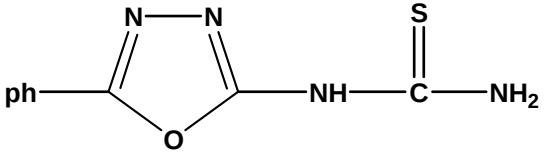
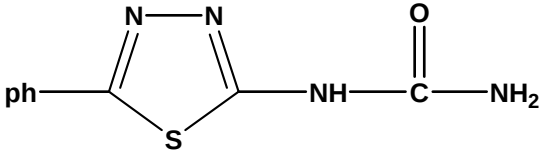
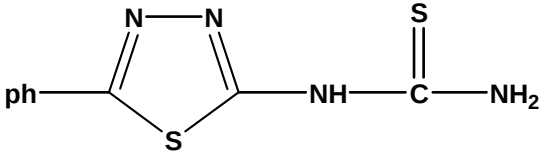
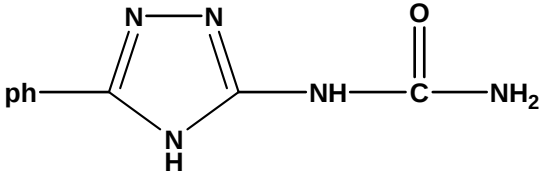
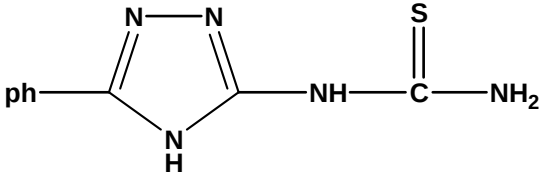
Abs.	Absorbance
µg	Microgram
ADH	Arginine dihydrolase
Aliph.	Aliphatic
Arom.	Aromatic
ATP	Adenosine Triphosphae
B.U.	Blood Urea
CIT	Citrate utilization
Conc.	concentration
D.W.	Distilled Water
dL	Deciliter
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
E.C.	Enzyme Commission
F.T.I.R.	Fourier Transform Infra Red
G.F.R.	Glomerular Filtration Rate
G.U.E.	General Urine Exam
G-6-P-DH	Glucose-6-Posphate-dehydrogenase
gm	gram
H.K.	Hexokinase
hr.	hour
I	Intermediate
Ibs.	Libras(pound/ $Inch^2$)
L	Liter
LDC	Lysine decarboxylase
LPS	Lipopolysaccharide
M	Molarity
M.H.A.	Mueller Hinton agar
Max.	Maximum
mg	milligram
min.	minutes
Min.	Minimum
mL	milliliter
mm.	millimeter

mRNA	messenger ribonucleic acid
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogenase
No.	number
ODC	Orinthine decarboxylase
ONPG	Orthonitrophenol galactoside
P	probability
P.	*Proteus*
PABA	Para-Amino Benzoic Acid
PMX	Polymyxine
ppt.	precipitate
p-sub.	Para-substitution
R	Resistant
R.B.S.	Random Blood Sugar
r.p.m.	round per minute
RBC	Red Blood Cell
RNA	Ribonucleic acid
S	Sensitivity
S.Cr.	Serum Creatinine
S.D.	Standard Deviation
sol.	Solution
spp.	Species
ß	Beta
T.L.C.	Thin Layer Chromatography
TDA	Tryptophane deaminase
tRNA	transfer ribonucleic acid
U.A.	Uric Acid
U.T.I.	Urinary Tract Infection
V	Volume
W.H.O.	World Health Organization

Table (3-2): Characteristic infrared absorption bands of (urea and thiourea) derivative compounds

No.	Structures	υ ($-NH_2$) cm^{-1}	υ (C=C) Arom. cm^{-1}	υ (C=O) cm^{-1}	υ (C=S) cm^{-1}	δ (*o*-sub.) cm^{-1}	δ (*m*-sub.) cm^{-1}	δ (*p*-sub.) cm^{-1}	υ (N-H) cm^{-1}	Others bands cm^{-1}
1		3350-3310	1548	1610	_	_	_	810	_	(C-H) Arom. 3030.6
2		_	_	_	1076.2	_	_	_	_	(C-H) Aliph. 2927.7
3		_	1560	1610.71	_	752.19	_	_	3359.62	(C-N) Arom. 1369.37
4		3546-3463	1571	_	1116.71	_	_	_	_	(C=N) Arom. 1626.45
5		_	1565.81	1623.24	_	_	_	_	3371.05	(C-H) Arom. 3058.89
6		3360-3340	1554.38	_	1135.99	_	_	_	3360.32	(C-H) Aliph. 2781.16
7		3407-3340	1498.59	1577.66	_	744.47	_	_	3340.20	(C-H) Arom. 3060.55

....Continued

No.	Structures	υ (-NH_2) cm^{-1}	υ (C =C) Arom. cm^{-1}	υ (C =O) cm^{-1}	υ (C =S) cm^{-1}	δ (*o*-sub.) cm^{-1}	δ (*m*-sub.) cm^{-1}	δ (*p*-sub.) cm^{-1}	υ (N-H) cm^{-1}	Others bands cm^{-1}
8		–	1531.37	–	1137.92	–	–	–	3340.6	(C-S) Arom. 927.70
9		3460-3313	1554.5	1643.2	–	–	–	–	3313.5	(C-O) Arom. 983.6
10		3421-3320	–	–	1210	–	–	–	–	(C-H) Arom. 3051.2
11		3438-3330	1571.88	1616.24	–	–	–	–	–	(C-S-C) Arom. 875.62
12		3402-3361	1546.80	–	1095.44	–	–	–	–	(C=N) Arom. 1612.38
13		–	1566.09	1610.45	–	–	–	–	3305.1	(C-N) Arom. 1143.71
14		3407-3350	–	–	1079.95	–	–	–	–	(C-H) Arom. 3076.25

الخلاصة

حضرت سلسلة جديدة شملت 14 مركباً من مشتقات اليوريا او الثايو يوريا من تفاعل اليوريا او الثايوريا مع أمينات حلقية (متجانسة او غير متجانسة)، بالإضافة إلى تحضير6 مركبات من قواعد شف (C=N) والمحضرة من تفاعل مشتقات اليوريا او الثايوريا مع الـ (benzyldehyde) ، بعد ذلك تم تشخيص جميع المركبات الـ 20 المحضرة بواسطة طيف الأشعة تحت الحمراء (.F.T.I.R)، و تم التأكد أيضا من نقاوة تلك المركبات بواسطة كروموتوغرافيا الطبقة الرقيقة (.T.L.C) وقيست لها درجة الانصهار (.m.p).

جمعت 200 عينه (أدرار ومصول) وذلك من تاريخ تشرين الثاني 2005 و لغاية آذار 2006 من المرضى الذين يعانون من خمج المسالك البولية وحصى الجهاز البولي والذين راجعوا (مستشفى الديوانيه التعليمي ، النسائية و الأطفال التعليمي ، المراكز الصحية و المختبرات الأهلية) في محافظة الديوانيه ، فضلاً عن ذلك أخذت 100 عينة من أشخاص أصحاء كمجموعة سيطرة.

تم قياس الفعالية التثبيطية لمضادات الحياة الشائعه وعددها 9 مستخدمة في هذه الدراسه وكذلك للمركبات المحضرة مختبرياً ضد بكتيريا المتقلبات المعزولة من المرضى قيد الدراسة، والمشخصة مختبرياً بأ ستخدام الطرق الروتينيه وخصوصاً طريقة الـ Api 20E الحديثه .

جمعت ايضاً 13 حصى بوليه من (كلية و مثانة) المرضى المذكورين لمعرفة نوع الحصى بطريقة التحليل الكيميائي النوعي لتلك الحصى . كذلك تم قياس مستوى الـ Urea، الـ Uric Acid، الـ Random Blood Sugar ، الـ Creatinine، والـ Calcium في أمصال مجموعة المرضى و مجموعة السيطرة .

أظهرت الدراسة النتائج الآتية:

- أن النمو الجرثومي الكلي لبكتريا المتقلبات والبكتريا الاخرى في العينات هي 138 حالة موجبة من بين 200 عينة اي بنسبة 69 % ، بينما كان عزل وتشخيص بكتريا المتقلبات 23 عزلة من بين 138 عينة اي بنسبة 16.66 % . كذلك أظهرت الفحوصات التشخيصيه الدقيقه وجود 21 عزلة من بكتريا الـ *Proteus mirabilis* اي بنسبة 91.3 % ، بينما كانت العزلات الـ 2 المتبقية من بكتريا الـ *Proteus vulgaris* اي بنسبة 8.7 % .

- لوحظ أن الـ Erythromycin اقل فعالية تثبيطية ضد نمو بكتريا المتقلبات وبنسبة مقاومة 86.9 % ، و بينما أعطى الـ Gentamycin أعلى فعالية تثبيطية و بنسبة مقاومة 4.3 % من بين 9 مضادات الحياة المستخدمة في الدراسة.
- أظهرت المركبات المحضرة مختبرياً فعالية تثبيطية ضد نمو بكتريا المتقلبات المعزولة ،حيث يمتلك المركب [N-(5-phenyl-1,3,4-thiadaizol)]-2-ylthiourea أعلى فعالية تثبيطية وبقطر .30 mm مقارنةً ببقية المركبات المحضرة،لكن لم يظهرالمركب N-pyridine-4-ylurea اي فعالية تثبيطية لنفس التركيز، مقارنةً بأختبار مضاد السيطرة الـ Gentamycin الذي أعطى قطر تثبيطي 18 mm .
- أن حصى الستروفيت هي الاكثر حدوثاً في المرضى من بقية أنواع الحصى وبنسبة 61.5 %، تليها حصى كربونات الابيتايت وبنسبة 30.7 % ثم حصى الخليط وبنسبة 7.85 %.
- اظهر التحليل الإحصائي لمستوى الـ Urea و الـ Uric Acid والـ Creatinine والـ Calcium في أمصال المرضى مقارنةً مع مجموعة السيطرة ان هناك فروقات معنوية واضحة ($P<0.05$)، بينما لم يظهر مستوى الـ Random Blood Sugar فرق معنوي ($P>0.05$) لأمصال نفس المجموعتين.