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Corn Silk Extracts as Urease Inhibitors of *Klebsiella* Bacteria in Iraqi Patients with kidney Stones

A Thesis

Submitted to the College of Science/Al-Nahrain University as a Partial Fulfillment of the Requirements for the Degree of M.Sc. in Bio Chemistry

By

Rafah Hilal Ismail

B.Sc. Chemistry /College of Science for women/Baghdad University(2014)

Supervised by

Assist. Prof. Dr. Salman A. Ahmed

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بسم الله الرحمن الرحيم

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Signature:

Name : **Assist. Prof. Dr. Salman A. Ahmed**

Scientific degree:

Date: / /2018

In view of the available recommendation, I forward this thesis for debate by the Examining Committee.

Signature:

Name: **Prof. Dr. Emad A. Yousif**

Scientific degree: Assistant Professor

Head of Department Chemistry

College of science

Al Nahrain university

Date: / /2018

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We, the examining committee certify that we have read this thesis entitled "**Corn Silk Extracts as Urease Inhibitors of Klebsiella Bacteria in Iraqi Patients with kidney stones** "and examined the student "**Rafah Hilal Ismail** "in its contents and that in our opinion, it is accepted for the Degree of Master of Science in Chemistry.

Signature:
Name: **Assist. Prof. Dr. Shatha A. Abd Al-Rahman**
Baghdad University
Date: / /2018
(Chairman)

Signature:
Name: **Assist. Prof. Dr. Ziyad H. Jawad**
AL-Mustansirya University
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(Member)

Signature:
Name: **Assist.Prof.Dr.Firas A. Hasan**
AL-Nahrain University
Date: / /2018
(Member)

Signature:
Name: **Assist.Prof.Dr.Salman A. Ahmed**
AL-Nahrain University
Date: / /2018
(Member/ Supervisor)

I, hereby certify upon the decision of the examining committee.

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Dedication

To the spring that never stops giving, to a strong and gentle soul who, taught me to trust in Allah….. to my mother

To Who still love me forever , the Moon and the Sun of my life …. my father

To whose love flows in my veins and my heart ,always remembers them, to my brother and sister.

To who spends his life caring for me ,respecting me ,and supporting me…my best friend my husband Alaa M, Zeadan

To my second family that supported and helped me a lot…. My uncle Moayed Z. Kalf and my aunt Ban I. Ali

To my life , my soul , my heart ,and most of all, the reason for my existence and struggle …. my son Yousif

To myself that suffered a lot.

Rafah H. Ismaill

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Summery

This study aimed to extract the crude from corn silk using (99.9% ethanol .80% ethanol and water) solvents then study the effect of those extract on inhibition urease enzyme which causes infection kidney stones

- This study included measurement of blood serum tests (uric acid ,glucose, Albumin ,total protein , Globulin) and kidney function tests among (100) patients (60 male and 40 female) of kidney stone in Baghdad city in Ghazi Al-Hariri Hospital for the period from December 2016 to February 2017. Also, (30) age-matched healthy people (15 male and 15 female) were enrolled as control group .The results of statistical analysis was showon that there is significant increase in urea and glucose. And no significant difference in, creatinine, uric acid, albumin ,total protein and globulin in comparison to that control group.

- In this study was diagnosis of struvite kidney stones by study urinalysis's results (pH, RBC. WBC, crystals) , urine culture , and stone analysis. 22% patients with renal stones were found to have infection kidney stones .

- Determination urease activity in urine of 22 patient with struvite kidney stone compare with 22 of control .The results of statistical analysis showed that there is highly significant difference ($P < 0.0001$) of urease activity in urine in comparison to that control group.

- Extraction of corn silk using (99.9% ethanol, 80% ethanol and water)solvents . Rutin, Kaepferol, and Quercetin were detected in those extracts by HPLC. The results were (0.012mg/L) of Quercetin in 99.9% ethanolic extract.(0.1398 mg/L),(0.15 mg/L), and (0.11 mg/L) of Rutin, Kaepferol, and Quercetin respectively in 80% ethanolic extract . (0.071 mg/L) and (0.091 mg/L) of Rutin and Kaepferol respectively in aqueous extract . It is noticed that only 80% ethanol has an ability to extract such three flavonoids.

- Studying the effect of corn silk extracts to inhibit urease enzyme from *Klebsiella Species* urease and pure urease enzyme. All such extracts revealed an inhibition of urease enzyme in the *Klebsiella Species* with (IC_{50} = 235.9 , 305.5 , and 247.3 mg/L) for 99.9% ethanol, 80% ethanol and aqueous extracts respectively, while standard urease inhibitor exhibits (IC_{50} =138.1mg/L).for thiourea .

  such extracts were utilized in the inhibition of pure enzyme and they exhibited an efficient inhibition of (IC50 = 133.9, 196.5, and 157.7 mg/L) for 99.9% ethanol, 80% ethanol and aqueous extracts respectively as compared with standard urease inhibitor of which gives(IC50 = 137.2 mg/L) in thiourea .

- Lastly, study kinetic parameter with and without inhibitors (80 % ethanolic extract) according to Linweaver- Burk plot . The results showed that the inhibition type is competitive inhibitor mode because the km is changed, while the V max remains unchanged

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Abbreviations

Symbol	Terms
UTI	Urinary Tract Infection
WBC	White blood cell
RBC	Red blood cell
CA	chemical analysis
CS	Corn silk
99.9%EE	99.9% ethanolic extract
80%EE	80 % ethanolic extract
AE	aqueous extract
IC50	half maximal inhibitory concentration
CT	computed tomography scan
AHA	Acetohydroxamic acid
EDTA	Ethylene diamine tetra acetic acid
HPLC	High-performance liquid chromatography
ELISA	enzyme-linked immunosorbent assay
TU	thiourea
V_{max}	maximum velocity of enzyme
k_m	Michaelis-Menten constant

CHAPTER ONE

Introduction & Literature Review

1.1 Nephrolithiasis

Renal stones are a common clinical problem with a subsequent burden for the health system [1]. It is one of the most common painful disease[2]. The symptoms related to the renal stones is highly dependent on the stones locations, the stones located within calyces are considered to be asymptomatic. While the initial symptoms of stone existing in the ureter is a cute onset of severe flank pain probably associated with nausea, vomiting and hematuria. The stone's movement in the ureter can cause obstruction of urine outflow from the kidney and as a developed case can cause post-renal acute kidney injury with rapid deterioration in renal function and infection which can be developed to pyelonephritis and-or sepsis [3].

Kidney stones develop when urine contain high concentration of soluble compounds containing calcium, oxalate (CaOx), and phosphate (CaP), resulting from dehydration or a genetic predisposition to over-excrete these ions in the urine[4].

1.1.1 Risks factors of kidney stone formation

- Low fluid intake [5]
- Diet of high protein, high sodium, and low calcium increase the risks of having some types of kidney stones evidence for many other dietary factors is mixed [5]
- Obesity increases the urinary excretion of promoters of crystallization and urine acidity and contributes to an increase of calcium oxalate stone formation [6].

- Kidney stones tend to recur approximately 50% people who form one stone form another within 10 years. The risk of recurrence ranges from 30-50% at 5 years in observational studies [7].
- Diabetes mellitus: increases the risk of nephrolithiasis by decreasing urinary pH which leads to uric acid stones, also high level of insulin associated with type 2 diabetes mellitus increase calcium salts saturation in urine [8].
- Gender : risk of kidney stones males are higher than females [9].
- Genetic Causes of kidney stones: the characterization of numerous rare single gene defects associated with nephrolithiasis and/or nephrocalcinosis has advanced the understanding of molecular pathways leading to stone formation [10].

1.1.2 Kidney stones types

There are four main types of kidney stones; calcium stones, uric acid stones, struvite stones,and cysteine stones [12].

1.1.2.1 Calcium based stones ; which fall into two types calcium oxalate (CaOx) which considered as a predominant component of kidney stones with rate of (75-80%) followed by calcium phosphate (5-10%) [13].The major cause of calcium stones is elevated urinary calcium level which caused by increased bone resorption, hyper-absorption of calcium from the intestine or impaired renal re-absorption of calcium [14]. Other causes are:

- An elevated urinary oxalate from diet or increase intestine oxalate absorption.
- decrease urinary citrate level (inhibitor of stone formation).

- Elevation of uric acid in the urine from excessive intake of animal protein [15].

1.1.2.2 Uric Acid stones ; its proportion within stone formers varying from (10%) to (20%) [16]. They primarily occur in acidic urine. However patients of type II diabetes mellitus (insulin resistance) in whom production and excretion of ammonia is low [17]. Some diseases are associated with uric acid stone formation consequently to the presence of very high levels of uric acid urinary excretion, which can be consequent to inborn defects of the metabolism of uric acid or to alterations that cause a high cell turnover (psoriasis, myeloproliferative disorders and other malignancies receiving chemotherapy). In other cases, uric acid stone formation can be consequent to diseases that are associated with lower urinary pH values as in patients with chronic diarrhea (inflammatory bowel disease or ileostomy) who have an increased risk of forming uric acid kidney stones by the consequent hypovolemia, which increases the supersaturation of urine, and by chronic gastrointestinal loss of bicarbonate leading to more acidic urine [18].

uric acid stones tend to be more frequent among patients with gout. In a matched study, pure uric acid stones were found in 50% of patients with gout and 20% of those without gout [11].

1.1.2.3 Struvite kidney stones ; or another name "infection stones" which have a proportion about (10-15%) of all kidney stones. are known to occur more frequently in women than in men (at a 2:1 ratio) a finding that might be attributable to the higher incidence of urinary tract infection (UTI) in women [19]. struvite calculi are important clinically because they can lead to sepsis and renal failure[20]. In fact these stones are mainly composed of magnesium ammonium phosphate $MgNH_4PO_4.6H_2O$ (struvite) and calcium

carbonate apatite $Ca_{10}(PO_4)_6.CO_3$ [21]. The urine composition does not seem to be a factor in the spontaneous formation of struvite crystals; therefore, stones containing struvite are considered associated with (UTI) [22]. They are primarily caused by urea splitting bacteria such as *proteus*, *pseudomonas*, *Klebsiella*, and *staphylococcus* [23].

These bacteria are responsible of urea hydrolyses (converting urea to ammonia) caused by urease enzyme produced by those bacteria which lead to increase the ammonia concentration in the urine making the urine alkaline in which struvite and calcium carbonate precipitate (normally these ions are soluble in normal urine pH) [24]. as shown in equation (1-1) and (1-2) [22].

$$\text{Urea} \xrightarrow{\text{Urease Enzyme}} 2.NH_3 + CO_2 \quad \text{Equation (1-1).}$$

$$NH_3 + H_2O \rightarrow {NH_4}^{+1} + OH^{-1} \quad \text{Equation (1-2)}$$

Increasing urinary pH leads to the elevation of the concentration of NH_4, CO_2 and PO_4 ions. These ions together with the ions of magnesium Mg^{2+} present in the urine lead to the crystallization of struvite, according to the following reaction Equation (1-3)

$$Mg^{2+} + NH_4 + PO_4 + 6H_2O \longrightarrow MgNH_4PO_4 \cdot 6H2O \quad \text{Equation (1-3)}$$

Struvite formation is usually associated with carbonate apatite, $Ca_{10}(PO_4)6CO_3$, precipitation because ions of calcium Ca^{2+} are present in urine.

Struvite kidney stones can grow rapidly over a period of weeks and can lead to obstruction, hydronephrosis, recurrent pyelonephritis, and decreased kidney function [22].

Crystal formation and growth in urine depend on the degree of crystalloid supersaturation and the concentration of promoters and inhibitors [25].

Infection stones do not always present with typical renal colic, as seen with many other stone types; nearly 70% present with flank or abdominal pain, 26% present with fever, 18% with gross haematuria, 8% are asymptomatic, 1% present with sepsis, and others can present with recurrent UTI [19].

The three key principles of treating struvite stones are: removal of all stone fragments, the use of antibiotics to treat the infection, and prevention of recurrence [19]. Several methods to remove stone fragments have been described in the literature, including acidification and urease inhibitors or citrate administration, should be adopted to minimize its effect on urinary saturation with respect to struvite [26].

Patients with infected struvite calculi, who do not receive any treatment, have about 50% chance of losing the kidneys [23,24]. however about 41% of the patients have 5-year survival rate with untreated unilateral struvite stones. Therefore, it is of prime importance to study the in *vitro* growth inhibition of struvite [28].

1.1.2.4 Cysteine stones; generally it constitutes (1-2%) of kidney stones. And it occurs as a sequelae of defect in amino acid re-absorption (cysteine, lysine, ornithine, and arginine) from proximal tubule which is an inherited disorder[27].Cysteine crystallization occur due to its insolubility property in urine[29]. Cystinuria is a genetic disease that clinically manifests as cystine renal stone formation. More than 80 % of people with cystinuria will form their first kidney stone in the first three decades of life [30]

In both humans and animals, stones in the urinary tract can obstruct the urine outflow, with subsequent extreme pain, hydronephrosis, and (possibly) rupture of the urinary bladder. In addition, disruption of kidney function can lead to metabolic imbalances, such as uremia, seizures, depression, anorexia, dehydration, even coma and death [20] .

1.1.3 Diagnosis of stones

1.1.3.1 Urine analysis; The aim of the routine study in clinical laboratories of urine samples is to identify and monitor renal and urinary tract illnesses. The first step of this study is based on different chemical reactions, which constitute the test strips methodology. To complete the study, the identification and count of the different cells and other particles present in urine samples in microscopy [31].

1.1.3.1.1 Dipstick ;

- **Assess pH** : the normal pH of urine is (4.5-7) [32]. However If (pH > 7) increases the possibility of struvite stones ,low urine pH increases the possibility of uric acid stones[31].
  - **Leukocyte esterase:** leukocyte esterase reagent strips are a rapid, cheap, and sensitive tool to identify inflammatory, it could be used as a screening tool in primary care practice. A positive result may indicate an inflammatory process [33]. it indicates WBC presence in urine [34].
  - **Nitrate test :** positive result indicates the presence of bacteria in a significant number [34] .

These two tests (Leukocyte esterase and Nitrate test) are useful clinical laboratory indicators existed that might predict the presence or absence of a

positive culture. Because of a seemingly low rate of positive cultures, would be helpful in deciding which urines should be cultured [35]

1.1.3.1.2 Microscopy

- **Crystal identification**.

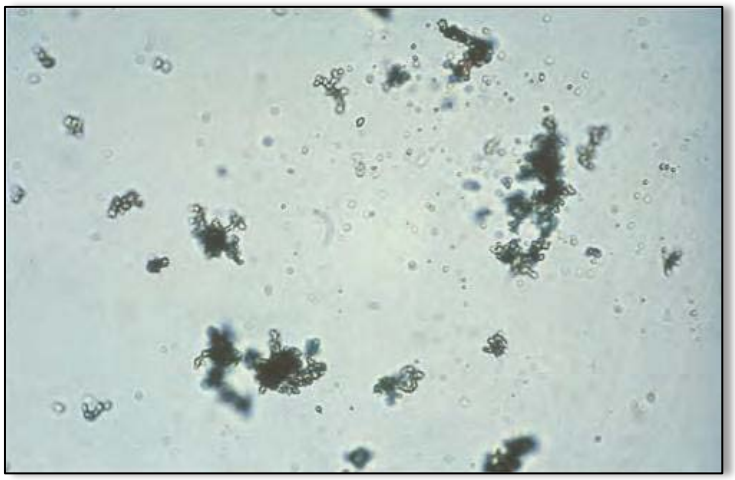
A variety of crystals may appear in the urine. They can be identified by their specific appearance and solubility characteristics. Crystals in the urine may present no symptoms, or they may be associated with the formation of urinary tract calculi and give rise to clinical manifestations associated with partial

or complete obstruction of urine flow [23] .

1. Amorphous urates crystal: appear microscopically as yellow-brown granules (Figure 1–1). Amorphous urates are found in acidic urine with a pH greater than 5.5 , whereas uric acid crystals can appear when the pH is lower [36].
2. Uric acid crystal: appear microscopically as yellow-brown (rosettes, wedges) (Figure 1–2). [36]. uric acid are found in acid pH the shape of crystals Polymorphous, whetstones, rosettes or prisms, rhombohedral prisms, hexagonal plate and are seen in uric acid stones [23]
3. Calcium oxalate crystal : appear microscopically as colorless octahedral dumbbells (Figure 1–3)[36]. Large amounts in fresh urine may indicate severe chronic renal disease, liver disease, ethylene glycol poisoning, diabetes mellitus, large doses of vitamin C [23].
4. Amorphous phosphates crystal : are appear microscopically as white colorless , granular in appearance (Figure 1–4), similar to amorphous urates but it is present in alkaline/ neutral pH [23].

5. Triple phosphate (Magnesium ammonium phosphate) crystal : are appear microscopically as colorless “coffinlids” three to six sided prisms with oblique ends (Figure 1–5).Can be seen in (UTI) urease splitting bacteria. more likely to form in alkaline urine. these crystals appear with struvite stones [37].
6. Ammonium bi-urate crystals: are appear microscopically as yellow-brown

have “Thorn apple” spheres, dumbbells, sheaves of needles shape (Figure 1–6) , more likely to form in alkaline urine [38].



Figure(1-1) Amorphous urates crystal [36].

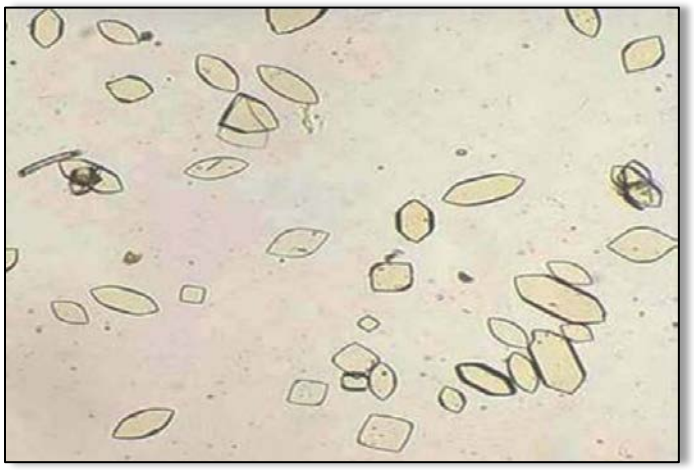


Figure (1-2) Uric acid crystal [37].

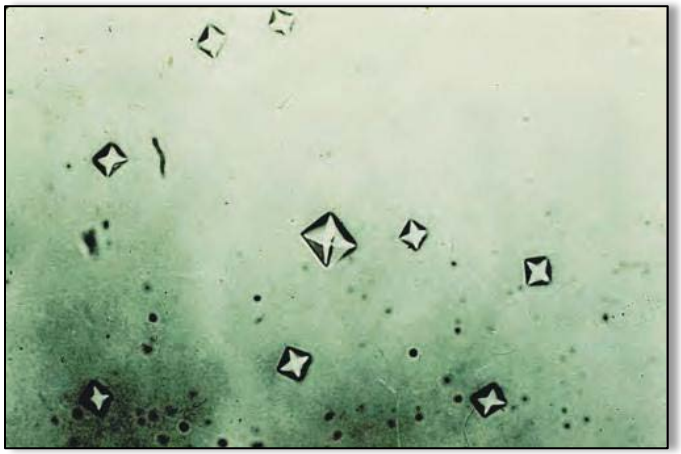


Figure (1-3) Calcium oxalate crystal [36].

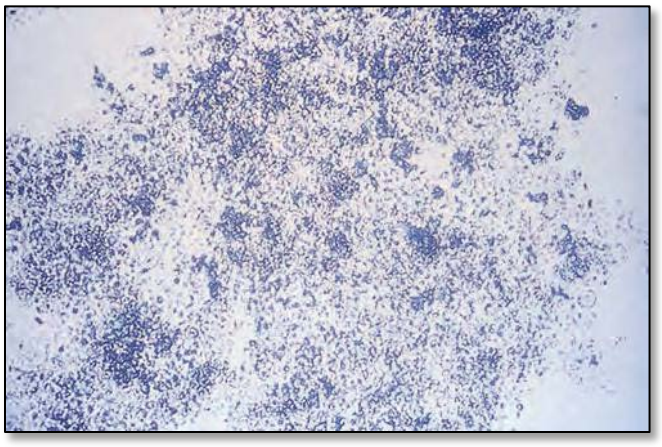


Figure (1-4) Amorphous phosphates crystal [36].

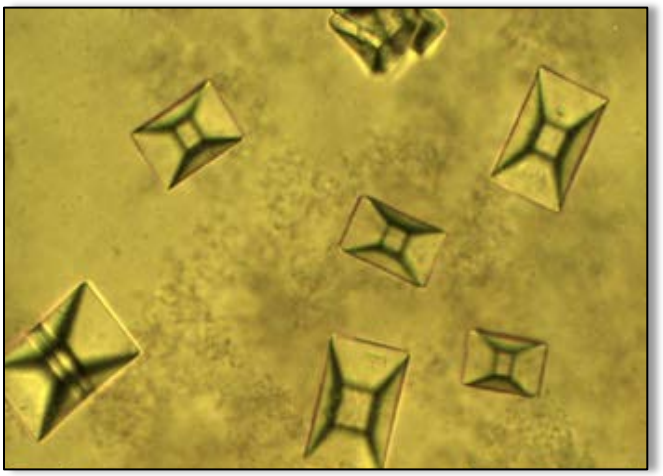


Figure (1-5) Triple phosphate (Magnesium ammonium phosphate) crystal [37].

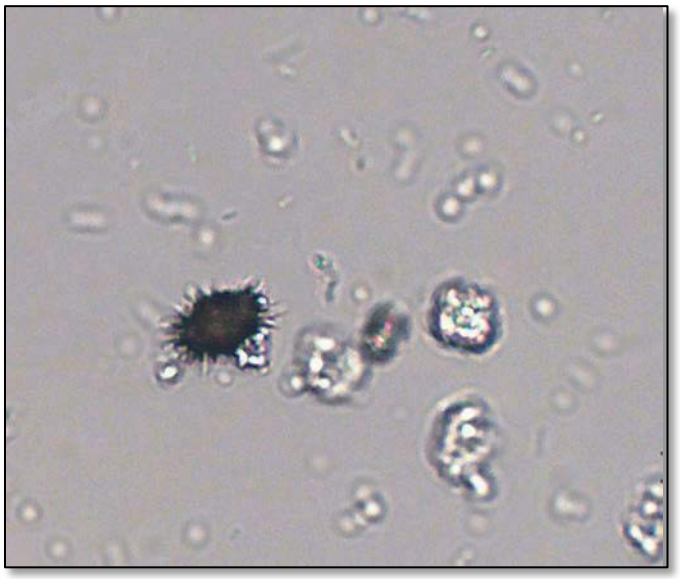


Figure (1-6) Ammonium bi-urate crystal [36].

Red Blood Cell ; In the urine, RBCs appear as smooth, non-nucleated, biconcave disks measuring approximately 7 mm in diameter (Fig. 1–7). They must be identified using high-power (40×) . RBCs are routinely reported as the average number seen in 10 hpfs. [36].The normal values (0-2) [32]. Moreover infection and kidney stones can cause microscopic haematurea [34]. Increased numbers of RBCs occur in several diseases including renal stones [23].

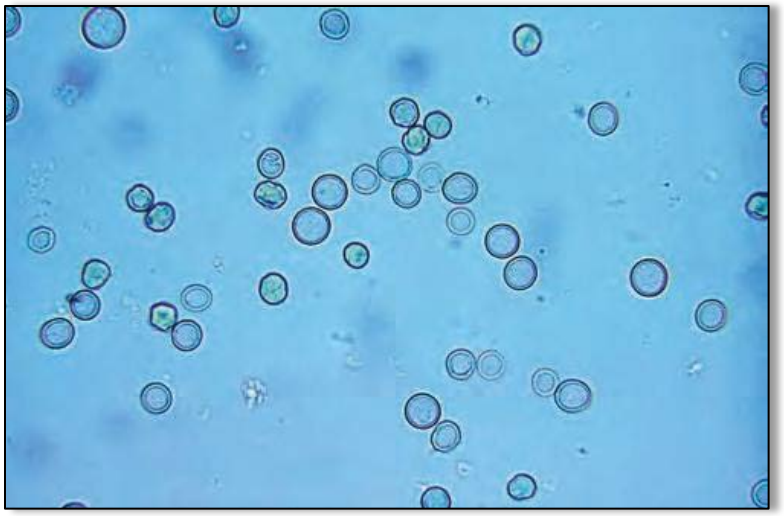


Figure (1-7)Red Blood cell in high-power (40×) [36].

White Blood Cell; WBCs are larger than RBCs, measuring an average of about 12 mm in diameter (Figure 1–8). The predominant WBC found in the urine sediment is the neutrophil. Neutrophils are much easier to identify than RBCs because they contain granules and multilobed nuclei. However, they are still identified using high-power microscopy and are also reported as the average number seen in 10 hpfs. Neutrophils lyse rapidly in dilute alkaline urine and begin to lose nuclear detail. The presence of urinary eosinophils is primarily associated with drug-induced interstitial nephritis. Small numbers of eosinophils may be seen with urinary tract infection (UTI) and renal transplant rejection. Mononuclear cells (Lymphocytes, monocytes, macrophages, and histiocytes) may be present in small numbers and are usually not identified in the wet preparation urine microscopic analysis. Because lymphocytes are the smallest WBCs, they may resemble RBCs. They may be seen in increased numbers in the early stages of renal transplant rejection. Monocytes, macrophages, and histiocytes are large cells and may appear vacuolated or contain inclusions. Specimens containing an

increased amount of mononuclear cells that cannot be identified as epithelial cells should be referred for cytodiagnostic urine testing [36].

Normally there should not be more than a few white blood cells in the urine (4–5 per high-power field). Infections or inflammations anywhere along the urinary tract cause an increase of WBCs in the urine [14].

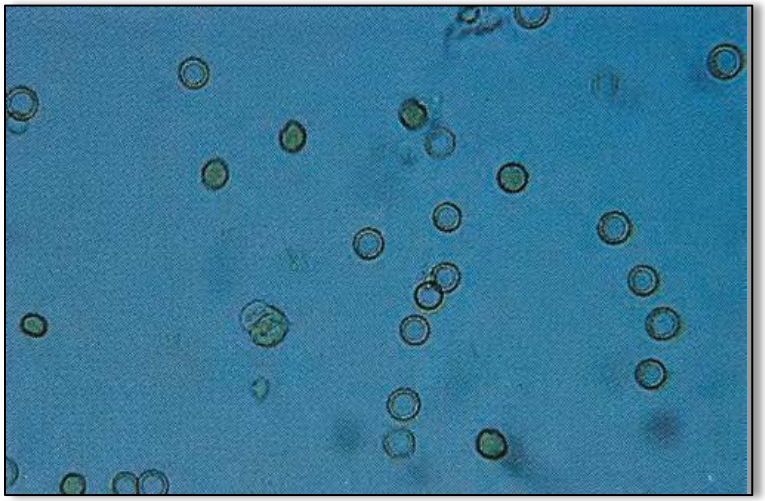


Figure (1-8) RBCs and one WBC (×400). Notice the larger size and granules in the WBC. [36].

Protein; normal value of protein in urine should be less than 30mg/dL

Urinary tract infection can cause mild proteinurea [40].

1.1.3.2 urine culture

The diagnosis bacterial urinary tract infection (kidneys, ureter, bladder, and urethra) can only be proven by culturing the urine [41]. The most common organism causing such infections is *E. coli.* Other gram negative rods, such as the *Proteus* or *Pseudomonas* groups, are occasionally present in UTI[39]. . urine culture should be carried out to all patients with signs of infection in urinalyses (if elevated urine WBCs are found.) [23].

1.1.3.3 Stone analysis

Analysis of spontaneously passed stone to collect the information about the chemical components of the stones, which helps guide the best treatment choice and preventing more likely stones from forming. However, while the use of chemical analysis for stone composition is declining ,it is still in use worldwide for the purpose of chemical analysis, the stone substance is dissolved and individual ions are identified, from which the original substances may be deduced using specific calculation scales. The results of chemical analysis appear as individual ion percentages (i.e. calcium, phosphate, etc.) as well as some deduced calculated mineral compositions. The use of chemical analysis is declining because analysis employing this method is very vulnerable to error [42].

1.1.3.5 Some clinical analysis tests

1.1.3.5.1 serum creatinine

Creatinine is a byproduct in the breakdown of muscle creatine phosphate resulting from energy metabolism [43]. It is produced at a constant rate depending on the muscle mass of the person and is removed from the body by the kidneys. Production of creatinine is constant as long as muscle mass remains constant. A disorder of kidney function reduces excretion of creatinine, resulting in increased blood creatinine levels. [23]. And excreted totally in urine, so it is used to estimate the function of kidney, when kidneys affected by diseases ,creatinine will be increased in the blood [44].

According to enzymatic method to measure creatinine level in the blood , its normal value range (0.6-1.2) mg/dL in male and (0.5-1.1) mg/dl in female [45]. The structure of creatinine shown in Figure (1-9) .

The amount of creatinine excreted is proportional to the total creatine phosphate content of the body, and thus can be used to estimate muscle mass. When muscle mass decreases for any reason (for example, from paralysis or muscular dystrophy), the creatinine content of the urine falls. In addition, any rise in blood creatinine is a sensitive indicator of kidney malfunction, because creatinine normally is rapidly removed from the blood and excreted [46]. The biosynthesis of creatine in humans accounts for 50% of the daily requirement and is a two-step process, as shown in Figure (1-10) [47].

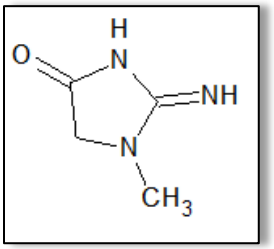


Figure (1-9): Chemical structure of creatinine

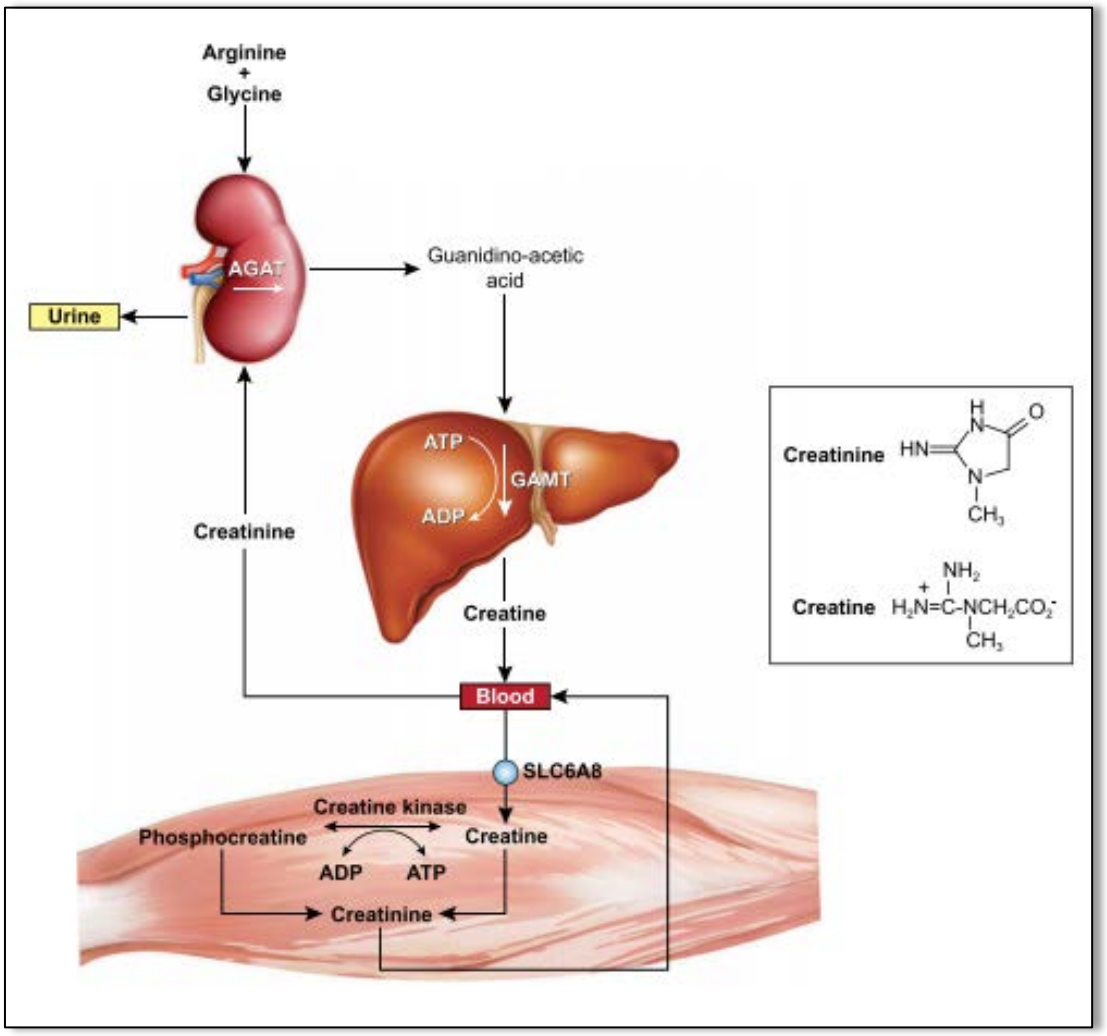


Figure (1-10) the biosynthesis of creatine and creatinine [47].

1.1.3.5.2 Blood urea nitrogen

Urea is the product of protein metabolism.it is produced in the liver. Diseases of the kidney that affect the excretion of urea result in high urea level in blood [44].Normal Serum concentrations of urea in the blood are between(8-25) mg/dl. [35]. Urea represents 45-50 % of non-protein nitrogen in blood. [44]. Its chemical structure is shown in Figure (1-11)

The urea cycle shown in Figure (1-12), first described in 1932 by Krebs and Henseleit [1932], is the principal method of nitrogen metabolism in urea-

producing organisms. Excess nitrogen, in the form of ammonia [48]. Urea accounts for about 90% of the nitrogen-containing components of urine. One nitrogen of the urea molecule is supplied by free ammonia and the other nitrogen by aspartate. The carbon and oxygen of urea are derived from CO_2 (as HCO^{3-}). Urea is produced by the liver and then is transported in the blood to the kidneys for excretion in the urine. However the mechanisms of transport of ammonia from the peripheral tissues to the liver for its ultimate conversion to urea by uses glutamine synthetase to combine ammonia with glutamate to form glutamine, a nontoxic transport form of ammonia. The glutamine is transported in the blood to the liver where it is cleaved by glutaminase to produce glutamate and free ammonia [46].

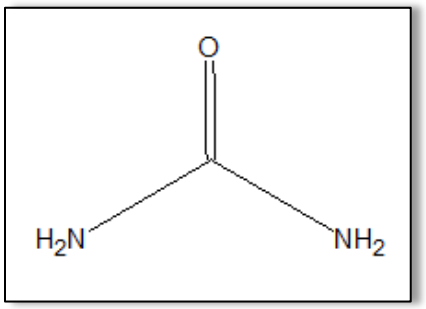


Figure (1-11): Chemical structure of urea

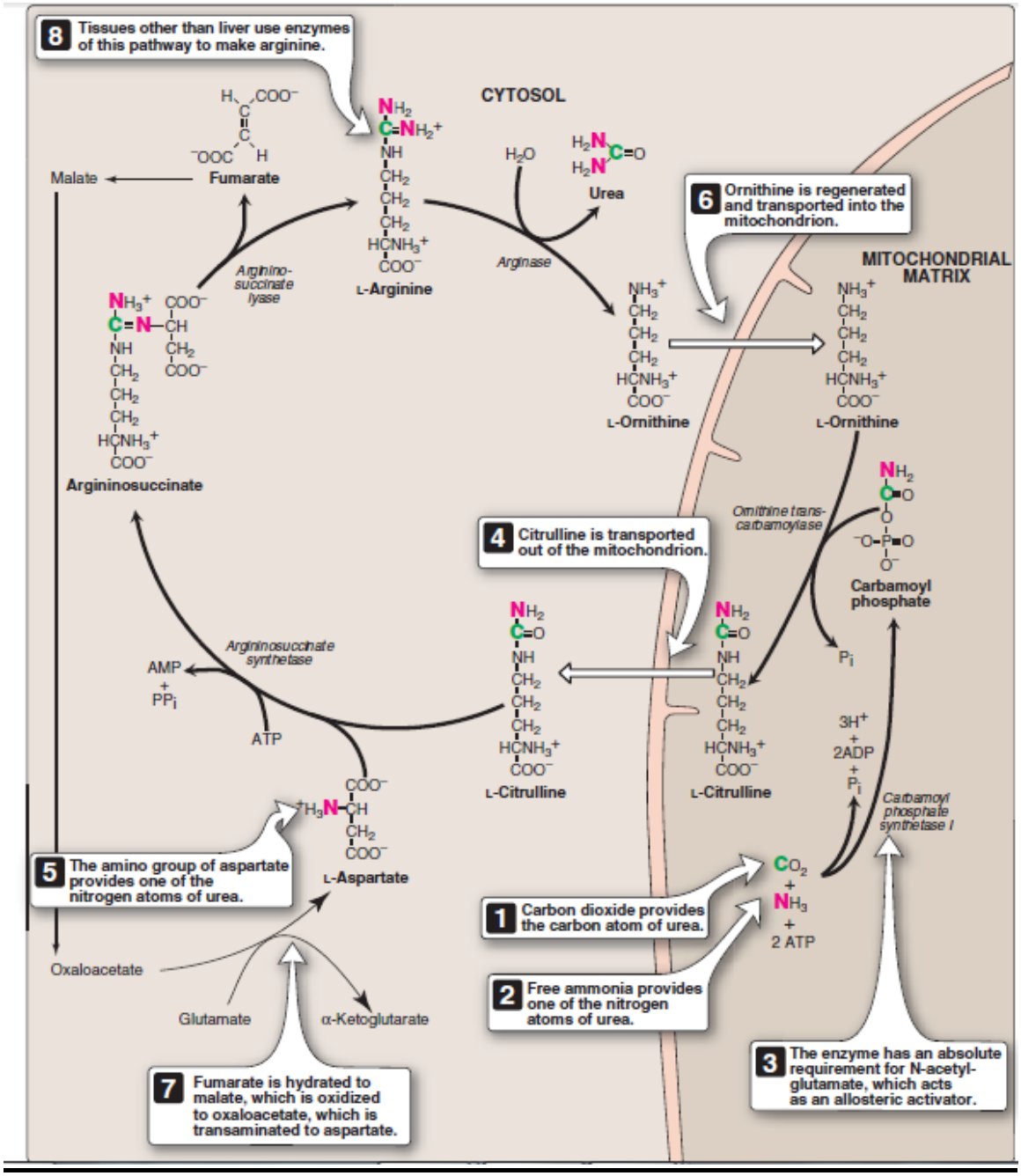


Figure (1-12) urea cycle [46].

1.1.3.5.3 Serum uric acid

It is the end product of purine metabolism, which is derived from cells death [49]. Uric acid is filtered by glomeruli then reabsorbed partially by the tubules and the rest is excreted in urine[44].Reduction in glomerular filtration rate due

to kidneys diseases lead to increase serum uric acid [50].The structure of uric acid shown in Figure (1-13). A summary of the steps in the production of uric acid are shown in Figure (1.14) [46].

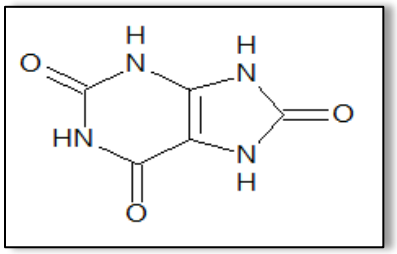


Figure (1-13): Chemical structure of uric acid

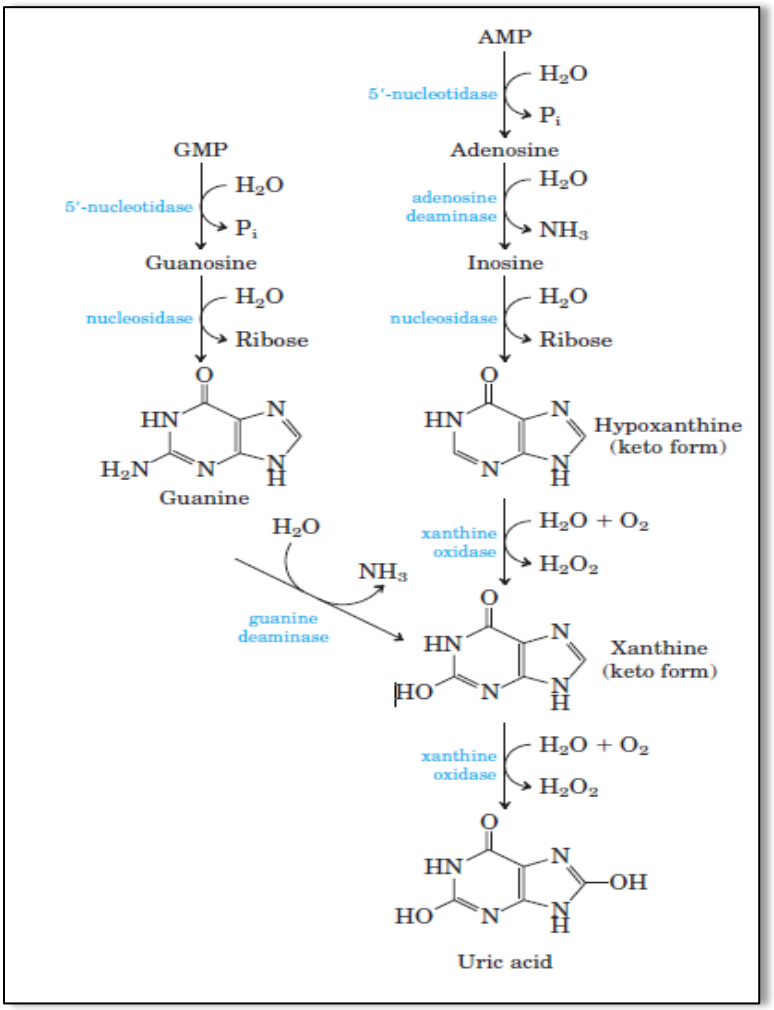


Figure (1-14) production of uric acid[51].

1.1.3.5.4 Total protein

Proteins are large biological molecules, made of one or more chains of amino acid. Proteins perform a vast array of functions within living organisms, including catalyzing metabolic reactions, replicating DNA, responding to stimuli, and transporting molecules from one location to another. Proteins differ from one another primarily in their sequence of amino acids, which is dictated by the nucleotide sequence of their genes, and which usually results in folding of the protein into a specific three-dimensional structure that determines its activity [52].

1.1.3.5.5 Albumin

Albumins are a family of globular proteins, the most common of which is serum albumin. The albumin family consists of all proteins that are water-soluble, are moderately soluble in concentrated salt solutions, and experience heat denaturation [52].

1.1.3.5.6 Globulin

The globulins are a family of globular proteins that have higher molecular weights than albumins and are insoluble in pure water but dissolve in dilute salt solutions. Some globulins are produced in the liver, while others are made by the immune system. Globulins, albumins, and fibrinogen are the major blood proteins. The normal concentration of globulins in human blood is about 2.6-4.6 g/dl [46].

1.2 Urease enzyme

(urea amidohydrolase EC 3.5.1.5) is an enzyme produced by certain types of bacteria , plant , and soil microorganism [24] Generally, bacterial ureases have three subunits in a trimer-of-trimers configuration (UreABC) , as epitomized by the proteins from *Klebsiella aerogenes* (Figure 1-15) [53].

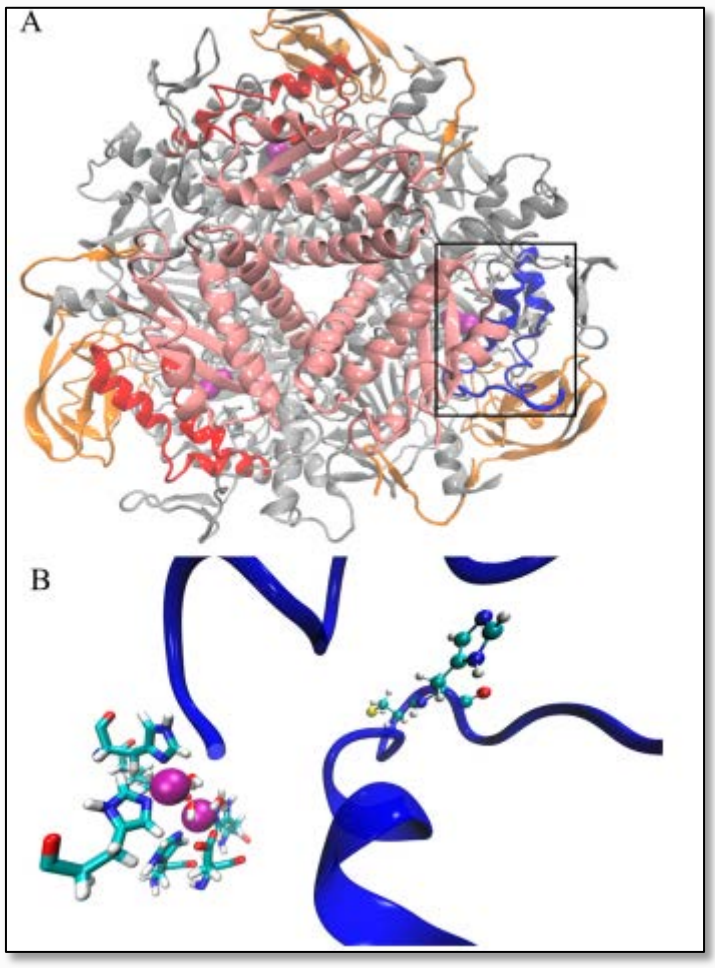
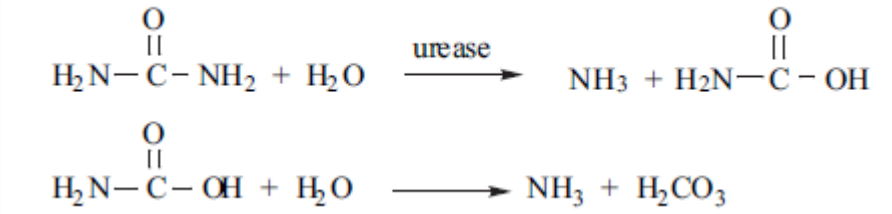


Figure (1-15).(A) The overall structure of K. 1 Figure 1.(A) The overall structure ofK. aerogenes urease is depicted in cartoon format. The three unique subunits are indicated by color; the trimer of alpha subunits (UreC) is depicted as gray the beta subunits (UreB) as orange, and the gamma subunits (UreA) as pink. Ni pairs are shown as magenta van der Waals spheres within the enzyme. The active site flaps, contained within the alpha subunits, are colored red,with the exception of the wide-open active site flap (boxed), which is colored in blue. (B) Expanded view of the wide-open active site flap and the nickel metallocenter (magenta spheres) with its coordinating ligands. The coordination sphere consists of two His per nickel, a terminal water molecule per nickel, a hydroxide bridging the nickels, a carbamylated Lys bridging the nickels, and a single Asp residue, all depicted in licorice representation and colored by atom type. The Cys319 and His320 residues of the active site flap are shown in CPK and colored by atom type. The active site flap is again represented in blue cartoon representation. [53]

Urease hydrolyze urea to form ammonia and carbonate ,carbonate hydrolysis to form ammonia (second molecule) and carbonic acid which responsible for pH increasing , as shown in equation below [54].



The major role of urease is to deliver a nitrogen source to the plants and microorganism from urea hydrolysis [24].

The urease enzyme isolated from microorganism differ from plant urease in structure ,but 50% of amino acid sequence are similar in both and both have Nickel ion at the active site [54]. However The knowledge on the urease active site was provided by the crystal structures resolved for bacterial ureases from *Klebsiella aerogenes* .The active site as shown in Figure (1-16) contain a binuclear nickel center in which the Ni-Ni distances were found close in value in both ureases, 3.7 and 3.5A in *Klebsiella aerogenes* enzyme. In the center the nickel(II) ions are bridged by a carbamylated lysine through its O-atoms, Ni(1) further coordinated by two histidines through their N-atoms, and Ni(2) by two histidines also through N-atoms and additionally by aspartic acid through its O atom. Besides, the Ni ions are bridged by a hydroxide ion (WB), which along with two terminal water molecules, W1 on Ni(1), W2 on Ni(2), and W3 located towards the opening of the active site, forms an H-bonded water tetrahedral cluster filling the active site cavity. It is this cluster that urea replaces when binding to the active site for the reaction [24].

Bacterial urease involved pathogenesis of many diseases in human and animals. Urease directly responsible for infection kidney stones , peptic ulcers caused by *H. pylori* bacteria [55].

In agriculture, urea fertilizer is used widely in the world urease activity on urea result in high amount of ammonia that evaporates ,this lead to plant damage firstly by depriving them from nutrients (Nitrogen) and secondary by increasing pH level in the soil [56].

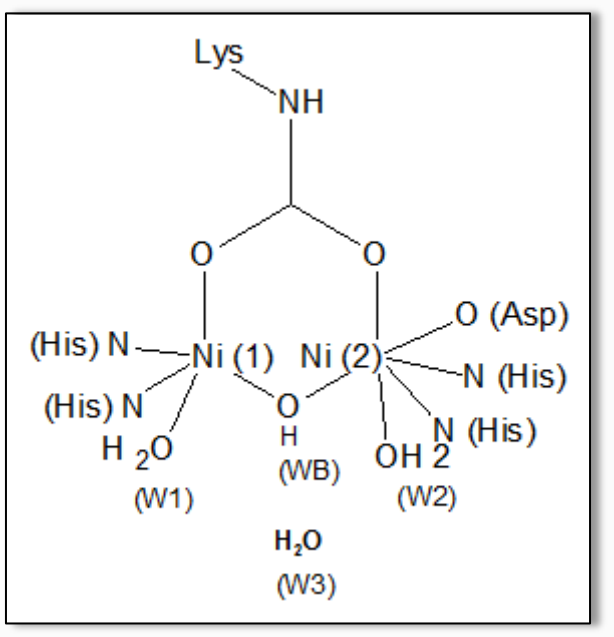


Figure (1-16): schematic structure of the active site of native[24].

1.2.1 Mechanism for urease action

A structure-based reaction mechanism for urease action is shown in (Figure 1-17). The mechanism entails a hydrated state of the enzyme (Figure 1-17A) that, upon entrance of urea when the flap is open, evolves to give an initial substrate-bound intermediate (Figure 1-17B). Urea binds to Ni(1) using the carbonyl oxygen and displaces the water molecules. This step is

corroborated by docking and density-functional quantum chemistry calculations, which also suggest that flap closure facilitates urea coordination to the second Ni(2) via its NH_2 group, stabilized by a specific H-bonding network (Figure 1-17C).

In this binding situation, the carbon atom of urea undergoes the nucleophilic attack by the Ni(2)-bridging hydroxide, whose bonds with the metal ions have been weakened by the binding of the substrate, leading to the tetrahedral intermediate (Figure 1-17D). The nickel-bridging OH group, now part of a diamino- (hydroxy) methanolate moiety and therefore very acidic, can transfer the hydrogen atom to the distal urea NH_2 group. This event could occur via the nearby oxygen atom of the Ni(2)-bound aspartate, which is able to undergo a dihedral rotation along the CR-Cβ bond, approaching alternatively the bridging hydroxide or the distal NH_2 group. The neutral imidazole side chain of the active site conserved histidine residue, moving nearer the active site upon closure of the flap, stabilizes the nascent C-NH_3 group (Figure 1-17E). The distal C-N bond is broken, ammonia is released, and the resulting carbamate decomposes into NH_4 and bicarbonate. The flap opening could facilitate the release of products and allow bulk water to rehydrate the active site to yield the native state of the enzyme (Figure 1-17A) [57].

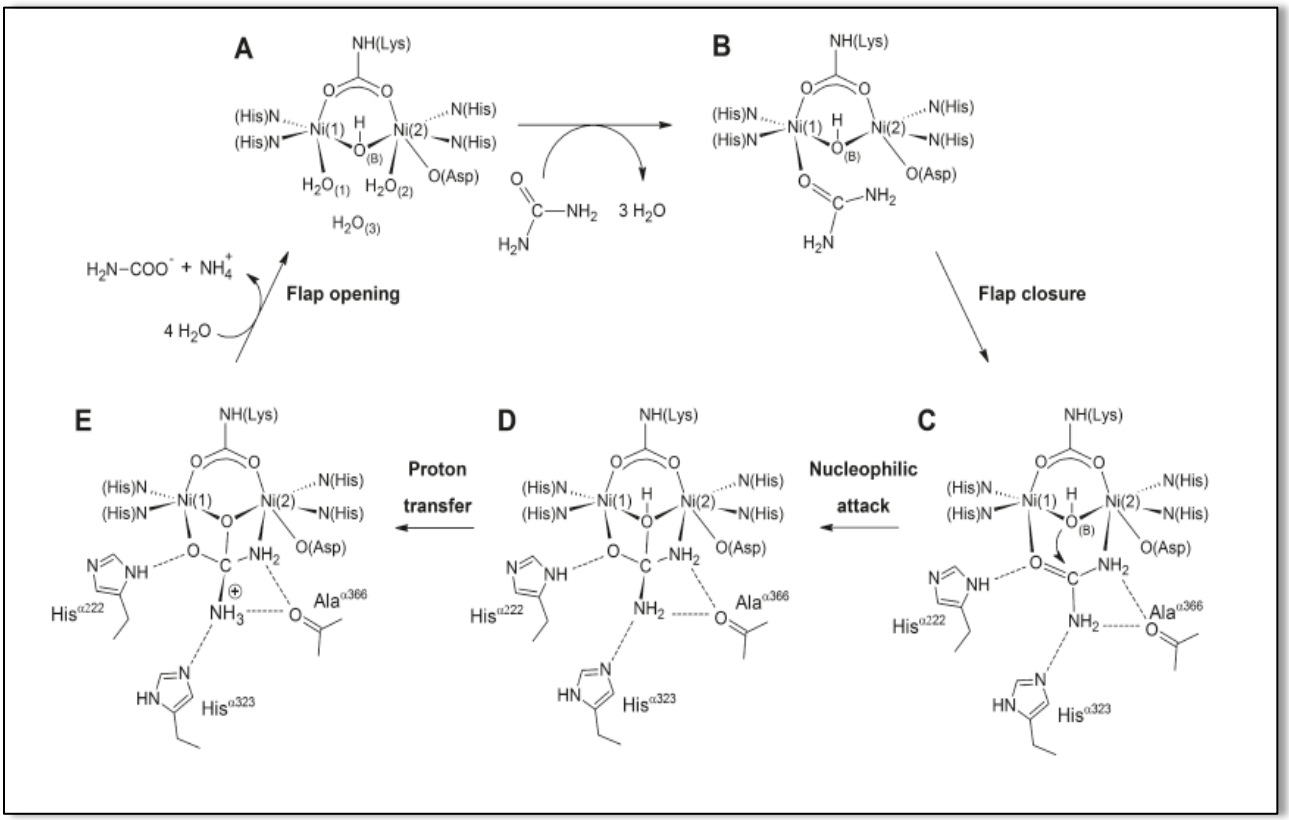


Figure (1-17) mechanism of urease action [57].

1.2.2 Urease inhibitors

Urease inhibition is an important field of research because of their practicable uses :

1. Urease inhibition used against urease produced by bacteria responsible for human diseases such as peptic ulcer caused by *H. pylori* and infection stones caused by urease producing organism such as *proteus* and *Klebsiella* [58].

2. Urease inhibitor combine with urea fertilizer to slow hydrolysis of urea by urease produced from soil microorganism and plants so, less

amount of nitrogen will be lost and soil so, less amount of nitrogen will be lost and soil will be protected from pH elevation[56].

Even though so many researches were studied about inhibition of urease only few of them giving a good result in controlling nitrogen loss from the soil .

1.2.3 Mechanism of urease inhibitors:

urease inhibitor can be divided into two categories [24].

1. substrate like inhibitor as can be seen in hydroxy urea and thiourea ,which compete with urea for binding with urease's active site .

2. Mechanism based inhibitors , the structure of these inhibitors are not similar to substrate's structure but they interfere with enzyme's action and inactivate it .

1.2.4 Some inhibitor for urease enzyme

1.2.4.1 hydroxamic acids; are important class of inhibitors of urease and which was found to be effective against a wide range of bacterial ureases .Hydroxamic acids are good metal chelates and their mechanism of inhibition involves binding to the metal ions of the active site of enzyme. The best studied hydroxamate of inhibitors is acetohydroxamic acid [59]. In medical application only Acetohydroxamic acid is used as urease inhibitor but it have many side effect such as :anaemia , rash ,nausea and ,headache [45]. The crystal structure of urease with acetohydroxamic (Fig.1-18). Acetohydroxamic binds to urease by displacing all four water/hydroxide molecules in the active site. The inhibitor o-atom bridges the Ni ions in

place of WB, reducing the Ni-Ni distance to 3.1Å, while its OH group coordinates to Ni(1) in place of W1. This results in that both Ni ions in the active site are penta-coordinated [24].

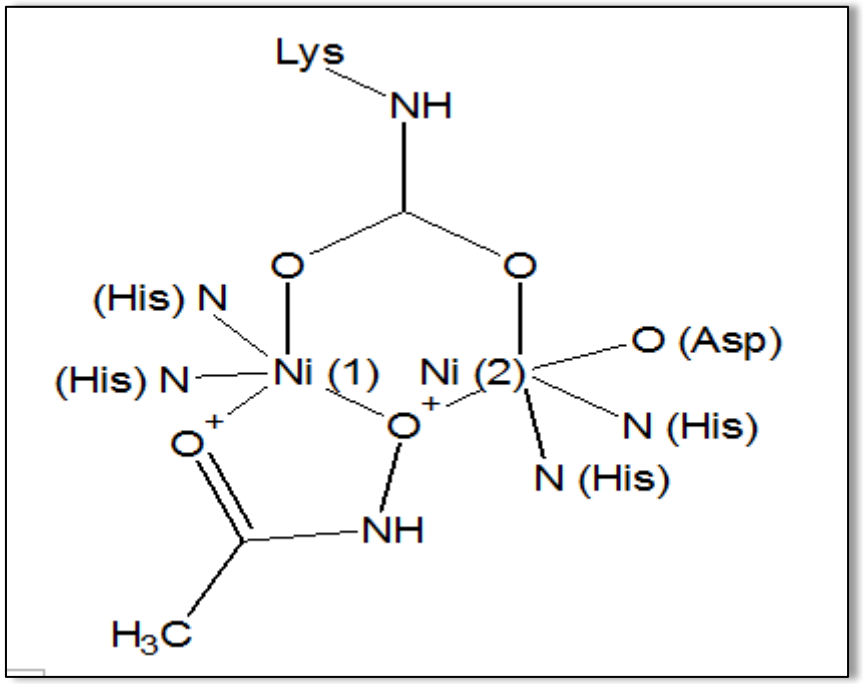


Figure (1-18): schematic structure of the active site of urease inhibited by Acetohydroxamic acid[24].

1.2.4.2 Phosphate-urease inhibitor

Phosphate used as urease inhibitor at neutral pHs had its inhibitory strength shown to be pH-dependent. This strength decreases with an increase in pH to cease at pH 7.0–7.5 .The inhibitory action of the phosphate was ascribed to $H_2PO_4^-$ ion a point verified by the crystal structure of urease–phosphate complex at pH 6.3 The mode of phosphate binding is shown in (Figure 1-19) In the structure, the $H_2PO_4^-$ molecule nearly perfectly replaces the cluster of four water molecules seen in the native enzyme. One oxygen of $H_2PO_4^-$ replaces WB and as an OH group bridges the Ni ions, retaining them at a distance of 3.8 Å.

The other oxygen and one OH replace W1 and W2 and bind to Ni(1) and Ni(2), respectively, while the other OH in $H_2PO_4^-$ is directed towards the opening of the active site [24].

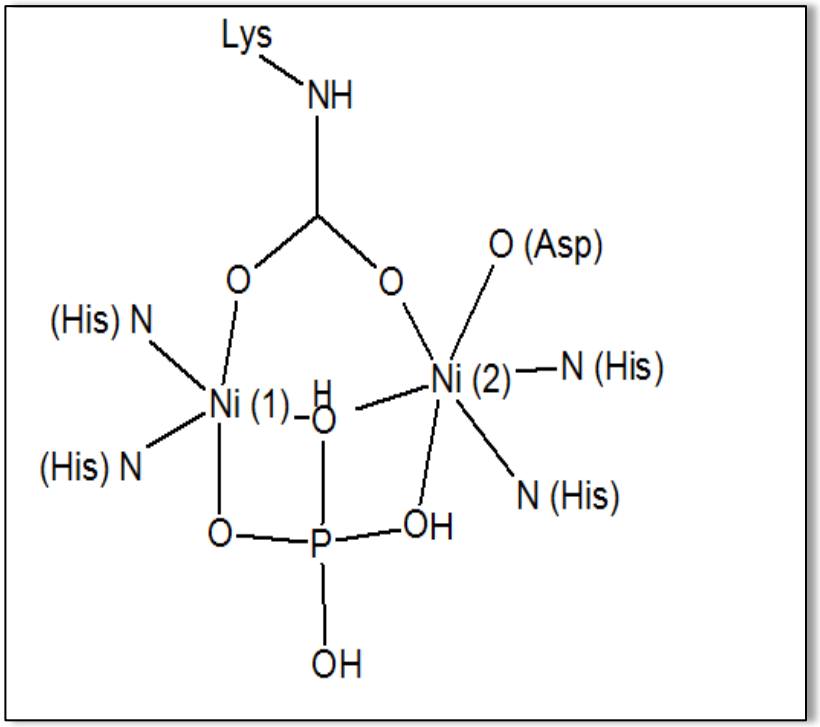


Figure (1-19): schematic structure of the active site of urease inhibited by phosphate[24].

1.2.4.3 Boric acid; is rapidly binding urease inhibitors, comparatively weak. suggestive of its action in the molecular form $B(OH)_3$. The crystal structure of boric acid-inhibited urease ,revealed that $B(OH)_3$ replaces in the active site W1–W3 water molecules, leaving in place the bridging WB. The inhibitor binds to the Ni ions with its two oxygen atoms, whereas its third oxygen points towards the cavity opening, the Ni-Ni distance being 3.6 A. Interestingly, a recent study of boric acid-urease complex did not exclude a possibility of a strong covalent bond formation between the bridging oxygen and boron [24]. as shown in (Figure 1-20) .

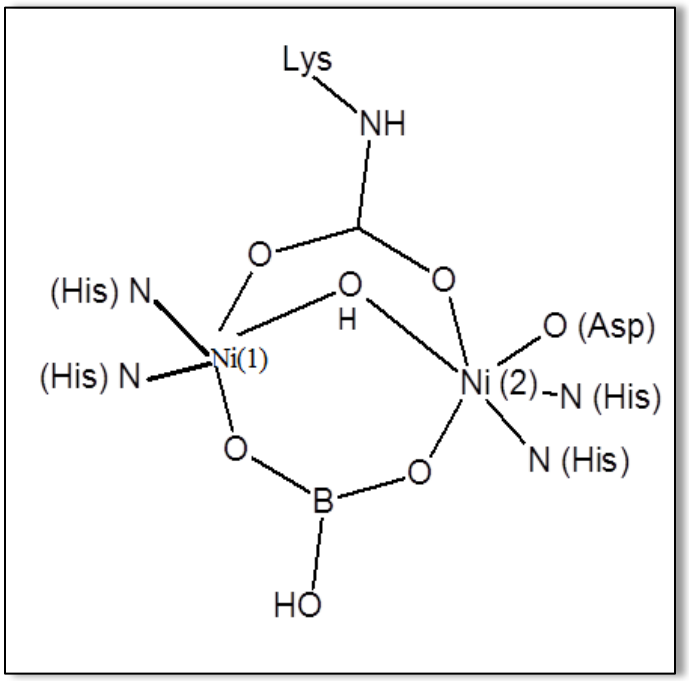


Figure (1-20): schematic structure of the active site of urease inhibited by boric acid[24].

1.3 Corn silk

Today, researchers have focused on the drug discovery from medicinal plants. Medicinal plants are regarded as an acceptable, cheap, easily available and safe source of active compounds for pharmaceutical use.The therapeutic effects of medicinal plants on kidney and urinary tract disorders have been variously studied and their efficacy has been demonstrated [60].

Corn silk is a long, weak, and shiny fibers at the top of corn's ear as shown in Figure (1-21) [61]. Traditionally, it is used for making tea as a healthy and medical drink in Asian communities especially in China [62]. However, corn silk becomes very important in drugs development, because of its bioactive constituents which include oxidant prevention agent limits, anti-diabetic activity, anti-proliferative effects, diuretic activity, anticoagulant activity, antifungal, anti-fatigue, and treating obesity [63]. In

addition, corn silk has been used as a treatment of many diseases like hyperglycemia, hypertension, cystitis, tumor, hepatitis, gout, diabetes, kidney stones, prostatitis, and nephritis [64]. Moreover, it is used medicinally as a mellow stimulant, diuretic, and demulcent. It is helpful in intense and incessant cystitis and in the bladder aggravation of uric corrosive, phosphatic grave, and employed in Gonorrhea [65]. It is also observed that corn silk have a positive effect on the kidneys, prevent kidney stone formation and reduce some of the symptoms of existing stones [66].

1.3.1 phytochemical

According to the phytochemical studies on corn silk, it revealed that corn silk contains a number of components like protein, vitamin, some minerals (Ca, K, Mg, Mn, and Zn), flavonoids ,alkaloids, tannins , saponins, carbohydrate , sugars and volatile components [64, 67].

Figure (1-21): corn silk

1.3.1.1Alkaloids

Alkaloids usually produced by variety of plants showing a great potential to be purified in relatively large quantities using new advanced techniques in the area of plant biotechnology. Alkaloids are chemical compounds that are characterized by having nitrogen atom in their structures. They are large group of chemicals divided into several categories, such as true alkaloids,

protoalkaloid, polyamine alkaloids, peptide and cyclopeptide alkaloids, and pseudoalkaloids. [67].

1.3.1.2 Steroids

A steroid is an organic compound with four rings arranged in a specific molecular configuration. [69]. Steroids are one type of components in living organisms and the importance of these as a part of a healthy diet has been emphasized in the recent dietary recommendations. steroid from have been studied including inhibiting tumor growth, cellular immune stimulating, anti-inflammatory, antioxidant and anti-diabetic properties . However, sterols could be used on the prevention and treatment of the cardiovascular and cerebrovascular diseases (CCD) through higher intake of phytosterols in a natural diet to low levels of total cholesterol and LDL (low density lipoprotein)-cholesterol in the serum [70]. The structure of Steroids show in (Figure 1-22)

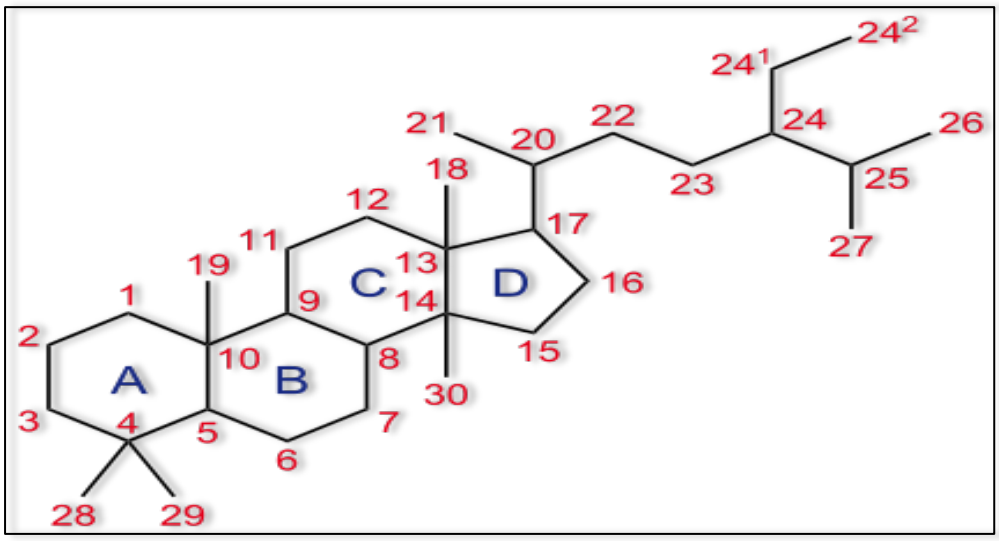


Figure (1-22) the structure of Steroids [73].

1.3.1.3 tannins

Tannins are defined as phenolic compounds . Condensed tannins consist of flavanoid units (flavan-3-ol) linked by carbon-carbon bonds [71]. that have

high molecular weight ranging from 500 to more than 3000 which they found in plants leaves, bark, wood and bound to proteins that form insoluble or soluble tannin-protein complexes [72].

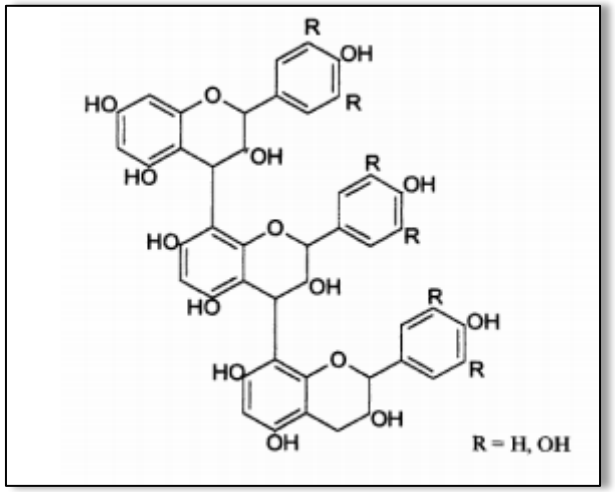


Figure (1-23) the structure of tannin

1.3.1.4 Saponins

are triterpene or steroid glycosides found in a wide variety of plants and certain marine organisms possessing various biological and pharmacological activities [74].

1.3.1.5 Flavonoids

Physiologically, Flavonoids (the original term is a Latin name called "flavus" word; which means yellow) play a vital role as the major former of blue, red, and purple pigments of plants tissues [75]. Phenol was considered as a dominant component in flavonoid formation, the three carbon atoms were formatted for connecting two rings of phenol to form a heterocyclic ring by joining them with an oxygen atom as shown in Figure (1-24) [76]. Flavonoids are the essential group of polyphenolic. They exhibit important effects on radicals' scavengers and health strengthen properties. Polyphenols have abilities to inhibit free radicals, to decrease the cardiovascular problems, and

to produce a strong anti-inflammatory and anti-cancer activity [77]. Flavonoids effectively used to treat the obesity, hypertension, and dyslipidemia in both humans and animals [78]. Flavonoids fall into several types like flavones, flavanones, anthocyanins isoflavones, and flavonols [79].

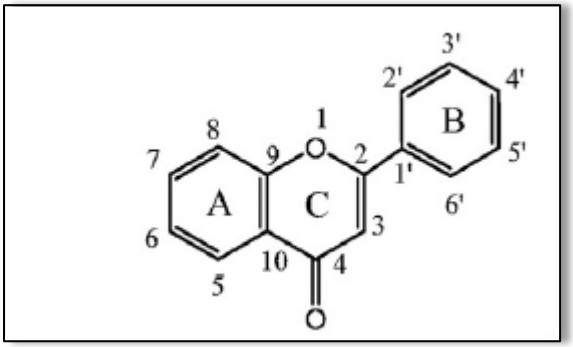


Figure (1-24); basic flavonoid structure

1.3.1.5.1 Quercetin

Quercetin is a plant polyphenol belongs to flavonol of flavonoids. Mostly, It's available in many human foods especially fruits, leaves, grains, and vegetables [80]. It characterizes by a phenyl benzo (c) pyrone derived structure as represented by its chemical structure [81]. as shown in figure (1-25) .

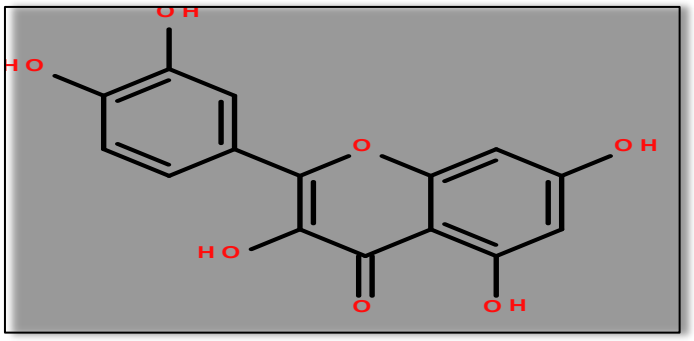


Figure (1-25): The chemical structure of Quercetin

1.3.1.5.2 Rutin

It is the glycoside which combine the disaccharide rutinose (α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranose) and the flavonol quercetin [78]. Lots of researches proved the significant effects on preventing several types of diseases [82]. It has several pharmacological characteristics like anti-inflammatory, antioxidant, anti-carcinogenic, antiviral, antiallergic, and stronger scavenger of superoxide radicals [83]. The chemical structure of rutin is exhibited in Figure (1-26).

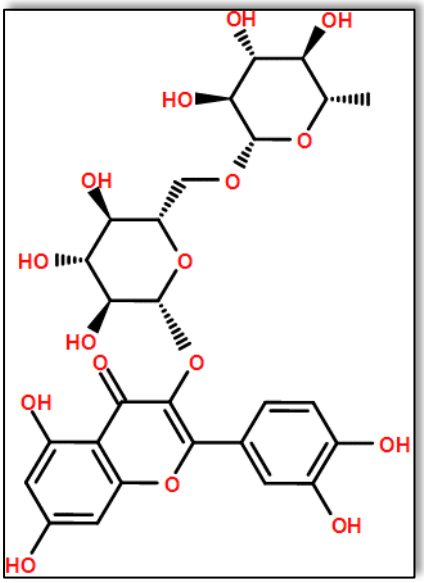


Figure (1-26): The chemical structure of Rutin.

1.3.1.5.3 Kaepferol

It is known as a natural flavonol (one of the flavonoids type). It is one of the most common food flavonoids; initially, it found in tea propolis, and grape fruit [84]. It is popular due to its antioxidant activity and its efficient utilization in cytoprotection agents. Previous experiments revealed that Kaepferol produces anti-proliferation activity and contains apoptosis in a

variety of human cancer cell lines in vitro, for examples, leukemia, and cancers related to non-small cell lung, prostate, esophageal, cell lung, colon and oral cavity [83]. Figure (1-27) presents the chemical structure of Kaepferol.

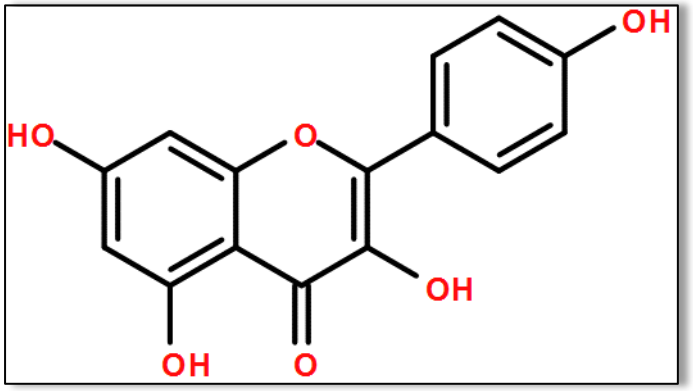


Figure (1-27): Chemical structure of Kaepferol

1.3.2 Toxicity of corn silk

The interest in using herbal medicines has increased over the years. Being natural and traditionally used make users think herbal medicines are safe and harmless. Thus, it is important to carry out toxicity studies and determine the safety of herbal products. A recent study using male and female Wistar rats confirmed that CS is non-toxic in nature There were no histopathological and adverse effects observed at a CS concentration of 8.0% (w/w) consumed for 90 days. This content corresponds to a mean daily CS intake of approximately 9.354 and 10.308 g/day/kg body wt. for males and females, respectively. As such, the intake of CS has no adverse effects and this supports the safety of CS for human consumption [85].

Aims of the study:

This study aimed to :-

1. Determination of urease activity in urine of patient with kidney stone
2. Diagnosis of infection kidney stones by Study urinalysis's results (PH, RBC. WBC, crystals) , urine culture , and stone analysis among patients with renal stones.
3. Study the relation between age ,gender with renal stones .
4. Extraction of corn silk with different ratios of (ethanol; water) solvents and Detection of Rutin, Kaepferol, and Quercetin in this extracts by HPLC .
5. Study the effect of corn silk extracts in inhibition of urease enzyme , and prevent formation of infection kidney stones .
6. study Kinetic parameter with and without inhibitors according to Linweaver- Burk. plot.

CHAPTER TWO

Materials and methods

2.1 Patients

The present study comprised of 100 individuals patient with kidney stones (group1) (60 male and 40 female).The patients aged from 20 to 75 years old , were attending to the Urologic Department in Ghazi Al-Hariri Hospital for the period from December 2016 to February 2017.Patients were diagnosed by urologist in the hospital. Patients didn't Suffers from any other disease.The study included 30 healthy individuals as control (group 2) (15 males , 15 females)

2.2 Samples

Samples, blood and urine were collected . The collected blood (5 ml) was left for around 15 minute at room temperature, centrifuged immediately at (1500 xg) for 10 minutes to separate the serum from the cells to determine some clinical analysis (blood urea nitrogen, creatinine , serum uric acid, glucose, albumin and serum total protein).The urine samples were collected to done general urine examination and urine cultures .

2.3 Instrumentals and chemicals

2.3.1 Instrumentals

Table (2-1): The instrumental with the supplied company used in this study

NO.	Instrument	Company
1	Autoclave	FANEM.
2	Balance	TP Series
3	Centrifuge	KHT-400
4	ELISA reader	Human reader
5	Incubator	Memmert

6	Lyophilize freeze-dryer	CHRIST
7	Microscope	N-200M
8	Spectrophotometer	APEL
9	Water bath	DFS
10	Rotary evaporator	Heidolph
11	Ultrasonic	Thermoline
12	vortex mixer	vm-300

2.3.2 Chemicals

Table (2-2) chemicals with the supplied company used in this study

No.	Chemicals	Company
1	Albumen Kit	Biomaghreb
2	Brain heart infusion	Oxoide
3	Creatinine Kit	Randax
4	Ethanol	SIGMA-ALDRICH
5	Glucose Kit	Spinreact
6	Kovac”s Reagent	Kimadia
7	MacConKeyAgar	HIMEDIA
8	methanol	SIGMA-ALDRICH
9	Phenol	SIGMA-ALDRICH
10	phosphate-Buffered saline	HIMEDIA
11	sodium hydroxide	SIGMA-ALDRICH

12	sodium hypochlorite	HIMEDIA
13	standard flavonoids	Central Drag House CDH
14	Total protein Kit	Biosystim
15	Urea	Central Drag House CDH
16	Urea broth	HIMEDIA
17	Urea Kit	Randox
18	urease enzyme	Sigma-Aldrich
19	Uric acid Kit	Spinreact

2.4 Determination of some biochemical parameters

2.4.1Serum Urea:

- principle

The analysis was made according to Bethelot method [86]. The procedure is based on the reaction below :

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

ammonium ions can react with the reagent that contain salicylate and hypochlorite to form a green complex (2-2di- carboxylindophenol).

- Reagent composition ; the reagents shown in Table (2-3)

Table (2-3) Reagents of urea Kit

Reagent composition	Concentration
R1a enzyme (urease)	≥ 5000 Unit/l
R1b phosphate buffer pH 7.0	120mmol/l,
Sodium salicylate	63.4mmol/l
Sodium nitroprusside	5.00 mmol/l
EDTA	1.5 mmol/l

R2 Sodium hypochlorite	18 mmol/l
Sodium hydroxide	750 mmol/l
Standard CAL	8.33 mmol/l (50mg/dl)

- Procedure

1. Two tubs were labelled as standard , sample .
2. Ten ml of standard 8.33 mmol/l were added to standard tube .
3. Ten ml of serum were added to sample tube .
4. To each tubes 1ml of working reagent (R1) was added .
5. All tubs were shacked , incubated for at least 3 min at 37 C.
6. Two hundred µl of sodium hypochlorite (R2) (18 mmol/l) were added to each tube .
7. Tubes were shacked . incubated for 5 min . the mixture was read at 600nm against blank.

- Calculations**:**

Conc. of Urea (mg/dl) = OD sample / OD standard × conc. Of standard.

2.4.2 Serum Creatinine:

- principle

In alkaline solution creatinine react with picric acid and form a red colour complex . The creatinine concentration directly proportional to The amount of the complex formed [87].

Reagent composition; shown in Table (2-4)

Table (2-4) Reagents of creatinine Kit

Contents	Initial concentrations of solution
standard CAL	177 µmol/l (2mg/dl)
R1a picric acid	35mmol/l

R1b NaOH	0.32mol/l

- Procedure

1. Two tubes labelled as standard , sample.
2. 0.2 ml of standard (177 µmol/l (2mg/dl)) was added to standard tube.
3. 0.2 ml of serum was added to sample tube.
4. To each tubes , 2ml of working reagent (R1a + R1b) was added.
5. All tubs were shacked , the absorption was read after 30 seconds A1 of the standard and sample. Exactly 2 minutes, the absorbance was A2 of sample and standard at wavelength 492 nm.

- calculations:

$A_2 - A_1 = \Delta$ A standard or Δ A samples.

creatinine concentration in serum = Δ A sample /ΔA standard × conc. of standard.

2.4.3 Serum Uric Acid :

- principle

uricase can oxidized Uric acid to allantoine and ($2H_2O_2$), which under the effect of POD, 2,4 Dichlorophenol sulfonate (DCPS) and 4-aminophenazone (4-AP) to form a red compound (quinoneimine).

$$2H_2O + \text{Uric acid} + O_2 \xrightarrow{\text{uricase}} CO_2 + \text{Allantoine} + 2H_2O_2$$

$$2H_2O_2 + \text{4-AP} + \text{DCPS} \xrightarrow{\text{POD}} 4H_2O + \text{Quinoneimine}$$

The uric acid concentration in the sample is proportional to of red color formed [88].

- Reagents: Reagents of uric acid shown in Table (2-5)

Table (2-5) Reagents of uric acid Kit

Contents		Initial concentrations of solution
R1	phosphate	50mmol/l
Buffer pH 7.4	2-4 (DCPS)	4 mmol/l
R2	Uricase ENZYM	60 Unit/l
Enzymes	POD	660 Unit/l
	Ascorbate oxidase	200Unit/l
	4-AP	1mmol/l
Uric acid standard	aqueous uric acid	6 mg/dl

- Procedure

1. Two tubes were labelled as standard , sample .
2. One ml of standard (6 mg/dl) were added to standard tube.
3. One ml of serum were added to sample tube .
4. To each tubes, 2ml of working reagent (R1 + R2) was added.
5. All tubs were shacked ,incubate at 37 C for 5 min and read (A) of samples and standards against the blank at wavelength 520 nm.

- Calculations:

Conc. of uric acid mg/dl = A Sample /A standard × standard conc.

2.4.4 Serum total protein

- principle

The analysis was made according to Biuret method [89]. In alkaline medium the protein in the sample will reacts with copper (II) ion and form a color complex that measured by spectrophotometry .

- Reagent composition: shown in Table (2-6) .

Table (2-6); Reagent composition of total protein kit

	Contents of	Initial concentrations of solution
Reagent 1	Copper (II) acetate	6 m/L,
	KI	12 m/L,
	NaOH	1.15 m/L,
Standard Protein	Bovine serum albumin.	69.8 g/l

- Procedure

1. Two tubes were labelled as standard , sample.
2. Twenty µL of standard (69.8 g/l) were added to standard tube .
3. Twenty µL of serum were added to sample tube .
4. To each tubes 1 ml of working reagent (R1) was added.
5. All tubes were shacked , let to stand for 10 minutes at room temperature. and read the absorbance (A) of samples and standards against the blank at wavelength 545 nm .
6. The color is stable for at least 2 hours .

• Calculations:

The concentration of protein in the sample is calculated by the equation

conc. of sample = A Sample /A standard × standard conc.

2.4.5 Serum Albumin

• principle

The method is depend on the specific binding of bromocresol green (BCG), an anionic dye, and the protein at acid pH resulting shift in the absorption wavelength of the complex .The intensity of the color formed is proportional to the concentration of the albumin in the sample [90].

BCG +Albumin ⟶ BCG-albumin complex

Table (2-7) shows reagent composition of albumin kit

Table (2-7) show Reagent composition of albumin kit

	Contents of	concentrations
Reagent (1)	BCG	0,14 gram/l
	Succinate buffer	75 mm/l
	Brij	7 ml /l
Standard of Protein	Bovine serum albumin.	50 gram / l 724 µm/l

- Procedure

1. Two tubes labelled with standard , sample .
2. Ten µL of standard (50 gram / l) were added to standard tube .
3. Ten µL of serum was added to sample tube .
4. To each tubes , 2 ml of working reagent (R1) was added .
5. All tubs were shacked , let to stand for 5 minutes at room temperature. And the absorbance was read (A) of samples and standards against the blank at wavelength 628 nm .
6. The color is stable for at least. 30 minutes.

- Calculations

Conc. of sample = A Sample / A standard × standard Conc.

2.4.6 Serum Globulin

Globulin = Total protein – albumin .

2.4.7 Serum Glucose:

- Principle

Serum glucose is oxidized by glucose oxidase (GOD) to form gluconic acid. The formed (H_2O_2) is detected by peroxidase (POD) that contain a chromogenic oxygen acceptor, phenol- aminophenazone presence of peroxidase (POD) [91].

β-D-Glucose +H_2O +O_2 $\xrightarrow{GOD}$ H_2O_2 +Gluconic acid

phenol+ H_2O_2 +Aminophenazone $\xrightarrow{POD}$ H_2O + Quinone

glucose concentration in the samples is proportional to the color formed (pink).

- Reagents: composition of the reagents in glucose kit is shown in Table (2-8).

Table (2-8):Reagent composition of Glucose kit

	Contents of	concentrations
R1 Buffer	TRIS pH 7.4 Phenol	92 mm/l 0.3 mm/l
R2 Enzymes	GOD POD Ascorbate oxidase 4-AP	15000 Unit/l 1000 Unit/l 200 Unit/l 2.6 mm/l
Glucose standared	aqueous Glucose	100 mg/dl

- Procedure

1. Two tubes were labelled as standard , sample.
2. Ten µL of standard (100 mg/dl) was added to standard tube.
3. Ten µL of serum was added to sample tube .
4. To each tubes 1 ml of working reagent (R1+R2)) was added .

5. All tubes were shacked , incubate at 37 C for 10 min, and the absorbance was read (A) of samples and standards against the blank at wavelength 505 nm.

- Calculations:

Conc. glucose in the serum mg/dl = A Sample /A Standard × standard Conc. mg/dl

2.5 General urine examination for 100 patient with kidney stone (group 1)

2.5.1 Protein test (Sulphosalicylic acid test):

Three drops of (20%) sulphosalicylic acid was added to 1 ml of urine then the specimen was heated. If the cloudiness continue means positive result (presence of protein) .as show in Table (2-9) .

2.5.2 urine test strip

1. Assess pH .
2. Leukocyte esterase .
3. Nitrate test .
4. Ketone bodies .
5. Sugar .
6. Bilirubin .
7. Specific gravity .

2.5.3 Microscopic urinalysis examination for 100 patient with kidney stone (group 1)

1. Urine of well-mixed (usually 10-15 ml) is centrifuged in a test tube at relatively low speed (about 2-3,000 rpm) for 5-10 minutes until a moderately cohesive button is produced at the bottom of the tube.
2. The supernatant is decanted and a volume of 0.2 to 0.5 ml is left inside the tube.

3. The sediment is suspended in the remaining supernate by flicking the bottom of the tube several times.
4. A drop of suspended sediment is poured onto a glass slide and covered with slip .
5. By microscopy , the red blood cells, white blood cells, bacteria, Crystals (include calcium oxalate, triple phosphate and amorphous phosphates) and bacteria are identified .

2.6 Preparation of phenol reagent and alkali reagent

- **phenol reagent** : sodium nitroprusside (25 mg) and phenol (5g) was dissolved in deionized water 500 ml. This reagent was stored in a dark-colored bottle at 5°C [94].
- **buffered hypochloride reagent** 2.5 g NaOH in NaClO (4.2 ml) solution were dissolved .The deionized water was added to complete the final volume of 500 ml. This reagent was stored in a dark-colored bottle at room temperature[94].

2.7 Urease activity of bacteria in urine from 22 patient with struvite kidney stone compared with 22 control

- principle

The urease activity test in urine had been run spectrophotometrically in 96-well plate. The activity of urease was estimated by the rate of release of ammonia, that change absorbance (A) .The net reaction volume was 200 µL.

- procedure

1. Twenty five µ L of urine incubated with Urea (55 µL; 100 mM) at 30 °C for 10 minutes.
2. After incubation, 70 µL of alkali reagents and 45µL of phenol were added.

3. The plate was incubated for 50 minutes at 30 °C.
4. the absorbance (A) was read at wave length 360 nm on ELISA reader plate [92].

- Calculation:

$$\text{Activity (unit/ml)} = \frac{\text{absorbancy 420nm}}{\text{slope} \times \text{enzyme volume} \times \text{incubation time}}$$

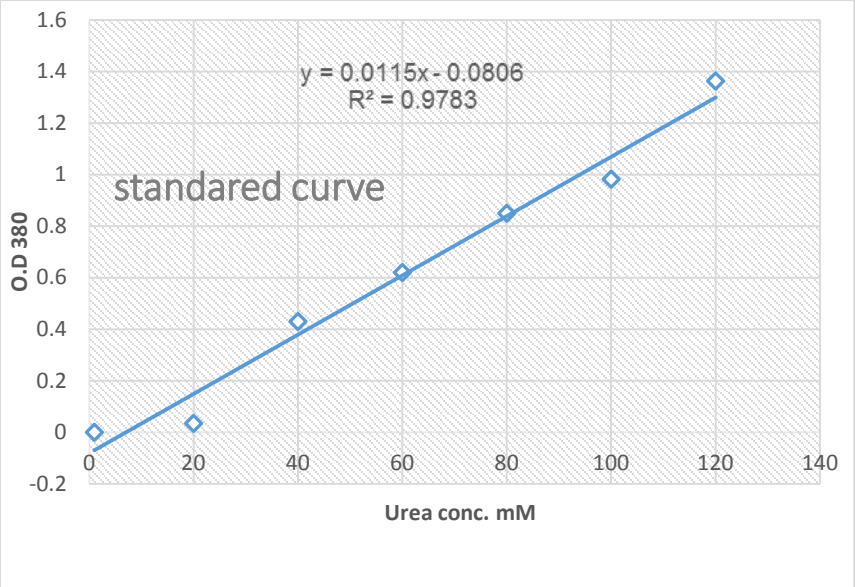


Figure (2-1): Standard Curve of urea

2.8 The Bacteriology

2.8.1 culture of urine from 100 patient with kidney stone (group 1)

Performed on MacConkey agars, inoculating 0.001 mL of urine and streaking the surface to quantitative colony counts .The plates were aerobically incubated for 24 hr. at 35°C.

2.8.2. Identification of bacteria in urine of 100 patient with kidney stone (group 1)

Identification of suspected isolates was done according to the colony morphology and biochemical tests .

2.8.2.1 Colony morphology All isolates were primarily identified according to the general culture characteristic (color, shape, texture and size) of the colony onto MaCconky agar and eosin methylene blue EMB agar after incubation overnight at 37ºC. Other characteristics were observed like lactose fermentation.

2.8.2.2 Biochemical tests to identify bacteria in urine of patients with kidney stone

Following tests were used to identify bacteria .

2.8.2.2.1 Citrate utilization test

Simmon citrate agar slant was stabbed with fresh bacterial isolates and incubated at 37 ºC for 24 hrs. Changing the color from green to blue is indicating positive result. This test used to detect the bacterial ability to utilize sodium citrate as a carbon sourc .

2.8.2.2.2 Oxidase test reagent

A filter paper was moistened with several drops of freshly prepared 1% oxidase reagent , and then a small portion of the tested colony was picked up by a wooden stick and placed on moistened filter paper. The color conversion to blue or purple color within 30 second indicate a positive reaction.

2.8.2.2.3 Motility test

Tube containing motility media was stabbed once in the center of media with an inoculating needle, then incubated at 37°C for 24hrs. The motile bacteria spread out from the injected line of inoculation.

2.8.2.2.4 Urease test

Urea agar slant was inoculated heavily over the entire slant surface and incubated at 37°C for 24hrs. Urease test is positive if the indicator was changed to purple-pink color, while keeping the media its yellow-orange color indicates a negative result. This test used to detect bacterial capacity to produce urease enzyme which hydrolyzes urea to ammonia and carbon dioxide.

2.8.2.2.5 Indole test

Peptone broth was inoculated with a new culture of each suspected isolate and incubated at 37°C for 24hrs.Avolume of 0.5 ml from Kovac's reagent , was added directly to the bacterial culture tube and if the culture produces tryptophanse which hydrolyze tryptophan to indole, pyruvic acid and ammonia, red ring will appear at the top of the broth and this indicated a positive result.

2.8.3 storage of bacteria

The bacteria storage in Brain heart infusion .

2.9 Extraction of corn silk part

2.9.1 Plant materials

The samples of corn plant were collected at harvesting time where their materials are fully maturated and developed. Firstly, the corn silk flowers were gathered from corn fields of the faculty of Agriculture's farm of Baghdad University in February 2017. Secondly, they were dried in a shaded well-ventilated place. Thirdly, Cuts (0.4 mm) them using a knife mills then keeping them stored in glass containers at room temperature for further processing [93].

2.9.2. Preparation of the crude extract [93].

1. Three Erlenmeyer flask labelled with 99.9% ethanol extract , 80% v/v ethanol/water extract and water extract contain 1L of 99% ethanol, 80% v/v ethanol/water and water respectively
2. one hundred g of chopped corn silk were added to each flask and exposed to a hot continuous extraction in a Ultrasonic at steady temperature of (50 ± 1.0 °C) for 5 hours (cycles 1)
3. decanted the solvents from each flask , added 1L of each solvents and exposed to a hot continuous extraction in a Ultrasonic at steady temperature of (50 ± 1.0 °C) for 5 hours (cycles2)
4. Repeat step 3 (cycles3)
5. Each of three previous extracts was filtered through Whatman No. 1 filter paper to remove the debris.
6. Then, each filtered sample was condensed by a rotary flash evaporator under vacuum at 50°C.
7. lyophilizing each condensed samples in a freeze-dryer to obtain a crude 99.9% ethanol Extract (99.9%EE), 80% ethanolic extract (80%EE), and water extract (AE).
8. Lastly, all extracts were stored at 4 °C for subsequent analysis

- The same experiment was returned to the one hundred g of the corn silk powder Which was crushed by the electric mill with 80% ethanol.

- Calculation:

The below equation used to determine the yield as percentage of the quantity of the initial material of (100g).

$$\text{Yield } \% = \frac{\text{yield} * 100}{100 \text{ g}}$$

2.9.3 Detection some Flavonoids by HPLC [76].

- **Procedure**

1. From each crude 0.1 g was dissolved in methanol of volume 100 ml .
2. Twenty µL of each dissolved was filtered through 0.45 µm membrane
3. Then diluting with methanol-water (80 to 100%) and methanol with 1% Orthophosphoric acid.
4. Then analyzing them for detecting the compounds (Rutin, Kaepferol, and Quercetin) using reversed phase HPLC, column= (250×4.6mm Id) 5 mm particle size. The UV-Vis detector was set at 280 nm.
5. The place of flavonoids in the HPLC results was defined through comparing the peak retention time between extracts and standard flavonoids solution; (retention time of compound Rutin, Kaepferol , and Quercetin were 2.85, 3.08, and 7.31 min; respectively).

- calculation

  The content of flavonoids was calculated through regression equation between peak area and flavonoids content

2.10 urease inhibition part

2.10.1 Preparation concentration

1. The stock solutions of organic solutions were prepared by dissolving dried organic extracts and dried aqueous extract by phosphate-Buffered(pH=7).

2. Prepared from it various concentration (1000, 500, 250, 125, 62.5) mg\L diluted with phosphate-Buffered(pH=7) for organic extract and aqueous extract.

3. Then filter it with micro filter 0.45µm and used or stored at 4C° until further use.

2.10.2 *Klebsiella* Species urease inhibition assay

2.10.2.1 Activation of microorganisms

The specimen of the colonies were taken by a loop that contains 5 ml of sterilized Brain heart agar. The loop has been shaken well and incubated in the incubator for 24 hours at 37 °C. The loop was sterilized via flame before using it to ensure that the planted bacteria are not contaminated

- **Principle of urease inhibition assay**

  The inhibition of urease examination was performed spectrophotometrically in 96-well Microplate , Urease activity was continuously measured with the rate of ammonia generation .

- **Procedure of urease inhibition assay**

1. Dissolve 38.71 g of urea broth powder in 1000 ml distilled water.
2. Then, thoroughly mixed to dissolve the medium completely then sterilized the products by Autoclave.
3. After that, 40% urea was sterilized by filtration and added to the medium.
4. After activation, under a sterile tube and aseptic ambience, the desired colony was taken by a loop to the test tube that contains 5 ml of the sterilized urea broth
5. The solution: (100µl) of bacteria diluted of Klebsiella species was incubated with 100µl of extracts (99.9% EE), (80% EE), and (AE) dissolved in phosphate puffer in concentrations of (1000, 500, 250, 125, 62.5, and 31.25 mg/L) at 30 °C for 24 hour.
6. Change in absorbance (optical density) was measured at 630 nm on ELISA plate reader in comparison with standard urease inhibitor i.e. thiourea (TU) [92].

- **Calculation**

The percentage of inhibition was calculated by using the formula given below.

$$\% \text{ Inhibition} = 100 - \left\{\frac{\text{Absorbance of Test Compound}}{\text{Absorbance of Control}} \times 100\right\}$$

Measuring the effects of different concentrations of inhibitors on production of ammonia was used to evaluate the IC50 of the active compounds are calculated by plotting the relation between % Inhibition and concetration of inhibitors.The IC50 values were determined using Graphpad Prism7 software.

2.10.3 Urease inhibition assay in pure enzyme

- Principle of urease inhibition assay

This test aimed to determine urease activity in monitoring of the products of enzyme catalyzed hydrolysis of urea into carbon dioxide and ammonia. We used the indophenol assay, in which ammonium ions reacts with NaClO and monochloramine is formed. Addition of the phenolic solution to monochloramine results in quinonechlorimine. The imine interacts with the phenol and indophenol occurs. In acidic solution indophenol is of yellow color, but after alkalization of the solution the blue product is formed – Berthelot reaction [94].

The urease inhibition assay has been run spectrophotometrically in 96-well plate. The net reaction volume was 200 µL

1. Twenty five µL of urease enzyme incubated with 5 µL of Extracts (99.9%EE), (80%EE), and (AE) dissolved in phosphate buffer in concentration (1000, 500, 250, 125, 62.5, and 31.25 mg/L) at 30 °C for 15 minutes.

2. Urea (55 µL; 100 mM) was added and the plate was incubated again at 30 °C for 10 minutes.
3. After incubation, added to each well 70 µL of alkali reagents and 45µL of phenol reagents The plate was again incubated at 30 °C for 50 minutes.
4. The activity of urease was continuously estimated with the rate of release of ammonia, and the absorbance changing (optical density) was observed at 360 nm on ELISA plate reader. Thiourea used as a standard compound [92].

- **calculation**

The percentage of inhibition was calculated by using the formula given below.

$$\% \textbf{ Inhibition} = 100 - \left\{\frac{\text{Absorbance of Test Compound}}{\text{Absorbance of Control}} \times 100\right\}$$

Measuring the effects of different concentrations of inhibitors on production of ammonia was used to evaluate the IC50 of the active compounds are calculated by plotting the relation between % Inhibition and concetration of inhibitors. The IC50 values were determined using Graphpad Prism7 software.

2.10.4. Determination of Kinetic Constants of urease:

1. Twenty five µL of urease enzyme incubation with 5 µL (96.8 mg/L) of Extracts (80%EE) (with inhibitor) and 5 µL phosphate buffer (without inhibitor) , at 30 °C for 15 minutes.
2. 55 µL of various concentrations of urea solution graduated from (10 - 120 mM) are prepared in 5 µL phosphate buffer (pH 7) and incubated at 37^{o}C for 10 minutes.

3. After incubation, added to each well 70 µL of alkali reagents and 45µL of phenol reagents ,The plate was again incubated at 30 °C for 50 minutes.
4. Then , absorbance changing (optical density) was observed at 360 nm on ELISA plate reader. Thiourea used as a standard compound [92].

- This experience was repeat on urease enzyme in patient urine and control urine

- **calculation**

The enzyme activity was measured and the initial rate of interaction was estimated. The values of Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) are calculated by plotting the relation between rate of the reaction V and substrate concentration [S] using Linweaver- Burk. plot [90].

$$\frac{1}{V} = \frac{K_m + [S]}{V_{\max}[S]} = \frac{K_m}{V_{\max}}\frac{1}{[S]} + \frac{1}{V_{\max}}$$

2.11 Statistical analysis

Performed statistical analysis by statisticians with the graphpad prism 7.00 and also Excel 2007. Independent sample t-test was used for tables with means and standard deviation for data analysis . P value of ≤ 0.05 was used as the level of significance.

CHAPTER THREE

Results And Discussion

3.1 Clinical analysis

3.1.1 Biochemical parameters in serum of patients with kidney stone and control

Urinary stones have plagued humans since the earliest records of civilization, the etiology of stones remains speculative. The renal stones are very common in our community and one of the most painful diseases.

So, many Biochemical species in serum have been determined to know the effect of these species in stones formation. And for the purpose of comparsion it seems logical to use normal values obtained as much as possible from normal controls.The results of urea ,creatinine ,uric acid and glucose are shown in table (3-1)

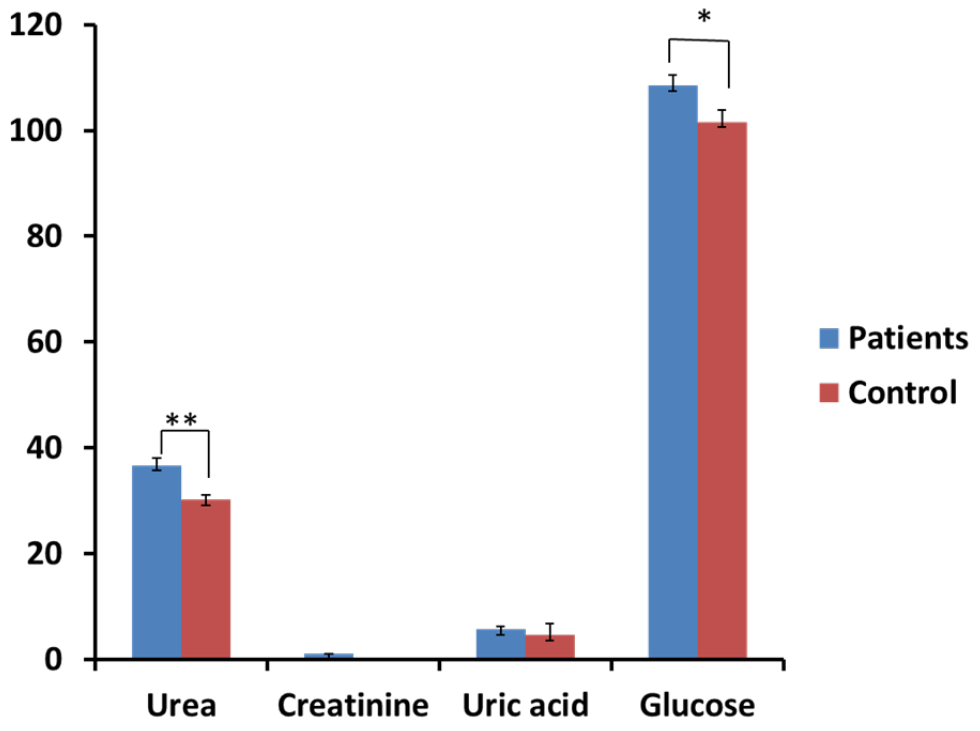


Figure (3-1) urea ,creatinine ,uric acid and glucose in serum of patients with kidney stone Compare with control group

P** < 0.001 high significant , p*< 0.01 =significant , p < 0.05 = no significant

Serum urea is measured as a screening test in the chemical evaluation of renal function.The results of statistical analysis show that there were high significant differences ($P < 0.001$) in urea level .

Serum creatinine is measured as a screening test in the chemical evaluation of renal function.Serum creatinine does not show a significant biological rhythm and somewhat independent of nutritional status. But there was a higher level of serum creatinine in males and that is due firstly, to the greater muscle bulk in the males compared with the age, weight matched females and secondly, to the greater physical activity in the males.

Serum uric acid is measured as a screening test in the chemical evaluation of renal function. The results of statistical analysis show that there were no significant differences in Serum uric acid level when it was compared between control group and patients of kidney stones .

In this study glucose was determined to know if there is any relation between formation of renal stones and glucose.The results showed significant difference when it was compared between control group and patients of kidney stones. which mean that the high level of glucose effects the formation of stone in the kidney. However, Diabetes mellitus increases the risk of nephrolithiasis by decreasing urinary pH which leads to uric acid stones, also high level of insulin associated with type 2 diabetes mellitus increase calcium salts saturation in urine [8].

In this study , albumin ,total protein and globulin were determined to know if there is any relation between formation of renal stones and albumin ,total protein and globulin

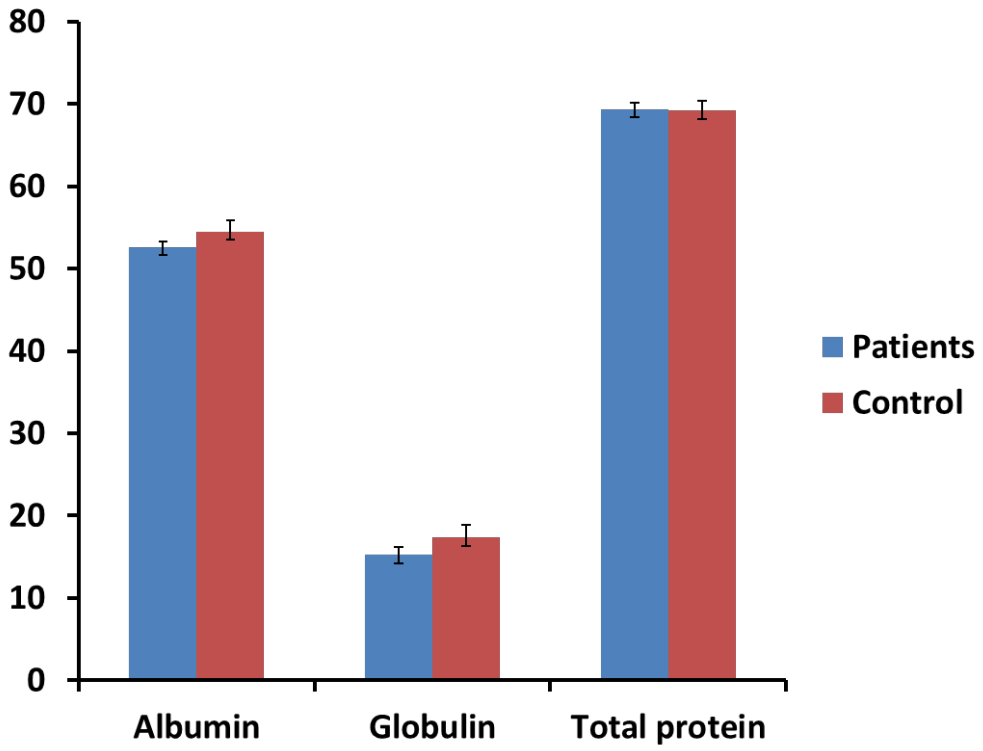


Figure (3-2) ; Total protein , albumin and globulin in serum for patient with kidney stone and control group

P** < 0.001 high significant , p*< 0.01 =significant , p < 0.05 = no significant

The results showed no significant difference in albumin ,total protein and globulin as show in table (3-2). However , not necessarily that kidney stones cause significant differences in renal function because they do not cause severe damage in kidney that leads to a significant increase in these biochemical species.

3.1.2 General urine examination

General urine examination was done for 100 patients with kidney stone, the result showed in Table (3 – 1):

Table (3 – 1): General urine examination for 100 patients with kidney stone

Color	Brown	yellow	Bloody
percent	17 %	66%	17%

Appearance	Clear	Turbid
Percent	29%	71%

Reaction (PH)	Acidic	Alkaline
Percent	82%	18%

Protein	nil	1-10	10-20	20-30
percent	71	17%	6%	6%

Microscope examination

RBC.s	1-10	10-20	20-30	30-40
percent	31%	1%	12%	56%

pus	Nil	1-10	10-20	20-30
percent	16	60%	12%	12%

crystals	Ca-oxalate	Amorphase-urate	Amorphase-phosphat	Uric acid
Percent	24%	55%	18%	3%

3.1.3 stone type

Kidney stone type was realize by CT scan or stone analysis kit. the result was shown in Table(3-2) . The results showed that 22% of the patients have struvite stones and (10 % ,33% ,19% ,16%) have (Ca. Oxalate stones ,uric acid stones Ca. phosphate stones and cysteine stones) respectively.

These results approximately dis agree with those reported by Aggarwal et al [1] who mention that 75% of stones are primarily calcium oxalate, but up to

50% of these include calcium hydroxyl phosphate ,10–20% are composed of struvite, 5% are composed of uric acid and 1-2% are composed of cysteine.

Table (3 – 2): kidney stone type from (100) patients with kidney stone

Stone type	Ca. Oxalate stones	Uric acid stones	Struvite stones	Ca.phosphate stones	Cysteine stones
Percent	10%	33%	22%	19%	16%

3.1.4 Urease activity test in urine

The urease activity was measured in urine of 22 patient with struvite kidney stones compare with 22 of control the result shown in Figure (3-3) The results of statistical analysis show that there is very highly significant difference ($P < 0.0001$) of urease activity in urine

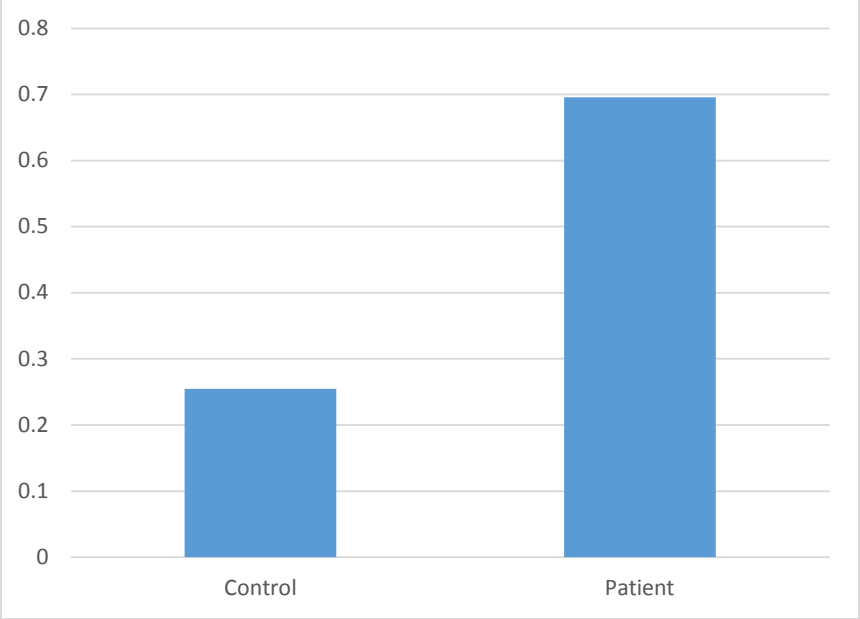


Figure (3-3) Comparison of Urease activity in urine patient with struvite kidney and control

3.2 Bacteriology part

3.2.1 Result of urine culture

The urine culture was performed for 100 patients with kidney stones, making sure that the patients did not take any anti - bacterial medication. The results showed that 71% of the patients have no growth in their urine culture and 29% have positive urine culture(13% ,10%, 5% and 1% of *Klebsiella* ,*E-coli*, *Proteus*, and *Pseudomonas*) respectively. All results of urine culture is shown in Table (3-3). Ureolytic infection- induced stones are estimated to constitute 15–20% of all urinary stones. *Proteus*, *Pseudomonas*, and *Klebsiella* are the most common bacterium responsible for struvite stone[24].*Escherichia coli* causes the majority of asymptomatic bacteriuria, cystitis, pyelonephritis, and catheter-associated urinary tract infection (UTI)[95].

Table (3 - 3): Bacteria types and Percentage in urine culture

bacteria types	Percentage
Klebsiella	**13%**
Proteus	**5%**
E-coli	**10%**
Pseudomonas	**1%**
No growth	**71%**

3.2.1.1 Diagnosis of bacteria

Primary bacteria identification with nonspecific media MacConkey agar show in Table (3-4), is aselective and differential culture medium for bacteria designed to selectively

isolate Gram-negative. *Klebsiella, Proteus, E-coli, and Pseudomonas* they have growth on MacConkey agar.MacConkey agar distinguishes those Gram-negative bacteria that can ferment the sugar lactose (Lac+) from those that cannot (Lac-) fermentation. *Klebsiella* and *E-coli* are lactose fermenting .The bile salts precipitate in the immediate neighbourhood of the colony, causing the medium surrounding the colony to become hazy. *Proteus* and *Pseudomonas* are Non-lactose fermenting. Table (3 - 5): shows bacteria identification in Citrate utilization test ,indole test , Oxidase test, motility test and Urease test.

Table (3 - 4): The Primary bacteria identification with MacConkey agar

bacteria species	MacConKey Agar	ferment the sugar lactosue
Klebsiella	**Positive(+)**	**Lac+**
Proteus	**Positive(+)**	**Lac-**
E-coli	**positive(+)**	**Lac+**
Pseudomonas	**Positive(+)**	**Lac-**

Table (3 - 5): Bacteria identification with, Citrate utilization test indole test , Oxidase test, motility test and Urease test

	Citrate utilization test	indole test	Oxidase test	motility test	urease test

Klebsiella	Positive(+) (blue color)	Positive(+) or Negative(-)	Negative(-)	Negative(-)	Positive(+)
Protuse	Negative(-) (green color)	Positive(+) or Negative(-)	Negative(-)	Positive(+)	Positive(+)
E-coli	Negative(-) (green color)	positive(+)	Negative(-)	Positive(+)	Negative(-)
Pseudomonas	Positive(+) (blue color)	Negative(-)	Positive(+)	Negative(-)	Negative(-)

3.3 Plant extract

3.3.1 extraction of corn silk

Table (3-6) show yield of extracts of corn silk with respect to solvents. The percentage yields were calculated against 100g of corn silk material subjected to each extraction method .The percentage yield of aqueous extract (AE) (was high yield than others 9.1%. The next was **80%** ethanol extract (80%EE) with (2.4%). The percentage was (0.93) for 99.9% ethanol extract (99.9%EE) . These results disagree with those reported by Nurhanan, et al. [105] who mention that the yield of extracts are found to be in ethanol higher than water. However, more polar aglycones or flavonoid glycosides are extracted with pure alcohols or with water−alcohol mixtures, and for less polar flavonoids (isoflavones, flavanones, methylated flavones, and flavonols) [103]

According to the results shown in Table (3-6), it is noticed that the water solvent gives higher yield than others which can considered as factor in solvent cost reduction.

When we returned the experiment to the powder of the corn silk which was crushed by the electric mill with 80% ethanol the yield of extracts was only

0.84 g , however, the yield of extracts of powder of the corn silk less than yield of extracts of cut corn silk (0.4mm) because when the particle sizes are too small, unhomogeneous extractions can form and the analyte re-adsorption on the matrix surfaces, which hinders the extraction. In our study, because of the re-adsorption of the extracted solutes, a lower flavonoid yield was found when the particle size was smaller than 0.4mm. Hence, a particle size of 0.4 mm was selected for subsequent tests [97].

Table (3-6); Comparison analysis of extraction yield, In 100g of corn silk .fractions obtained using different solvents.

Type of solvents used in extraction	Yield(g)	Color of extract	yield%
99.9%ethanol	**0.93**	yellow	**0.93%**
80%ethanol	**2.4**	light brown	2.4%
Water	**9.13**	brown	**9.1%**

3.3.2Detection of some Flavonoids by HPLC

Corn silk is a rich source of phenolic compounds, especially flavonoids [67]. Four types of flavonoids in corn silk identified by HPLC . (Rutin, Kaepferol and, Quercetin) . The solubility of organic compounds in different solvents plays an important role in their separation and purification applications [97]. Solubility of flavonoids can be influenced by factors including an OH or OCH_3 substituent on ring B, the connection position of ring B, the bond between C_2 and C_3 of ring C (C_2=C_3 or C_2-C_3) and an OH substituent at 3-positionl. When ring C of the flavonoid contains a double bond (C_2-C_3),

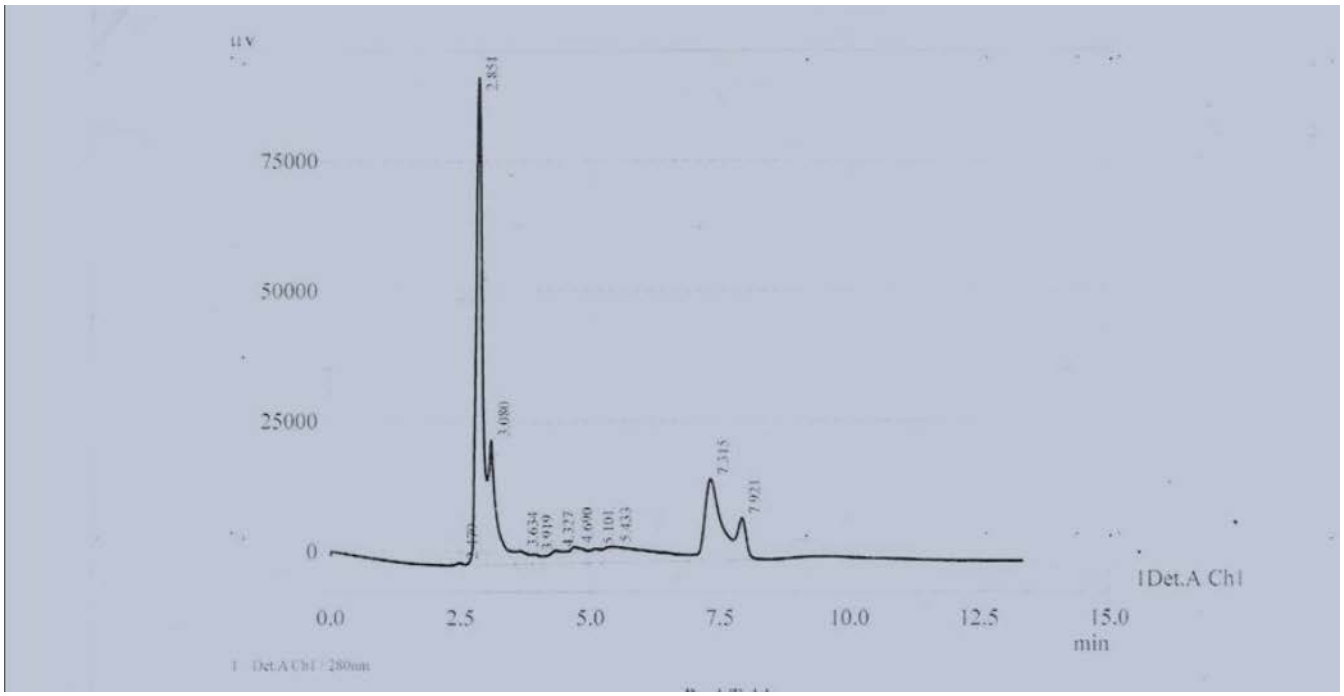
aqueous solubility increases with increasing numbers of OH substituent on ring B [98]. Figure (1-24);

At a constant temperature, the solubility of flavonoids in water + ethanol mixtures increase with increasing ethanol contents, and the highest solubility were obtained when a water + ethanol mixture was used as solvent. So in our results the concentration of Rutin and Kaepferol in 80% ethanolic extract higher than in aqueous extract [97].

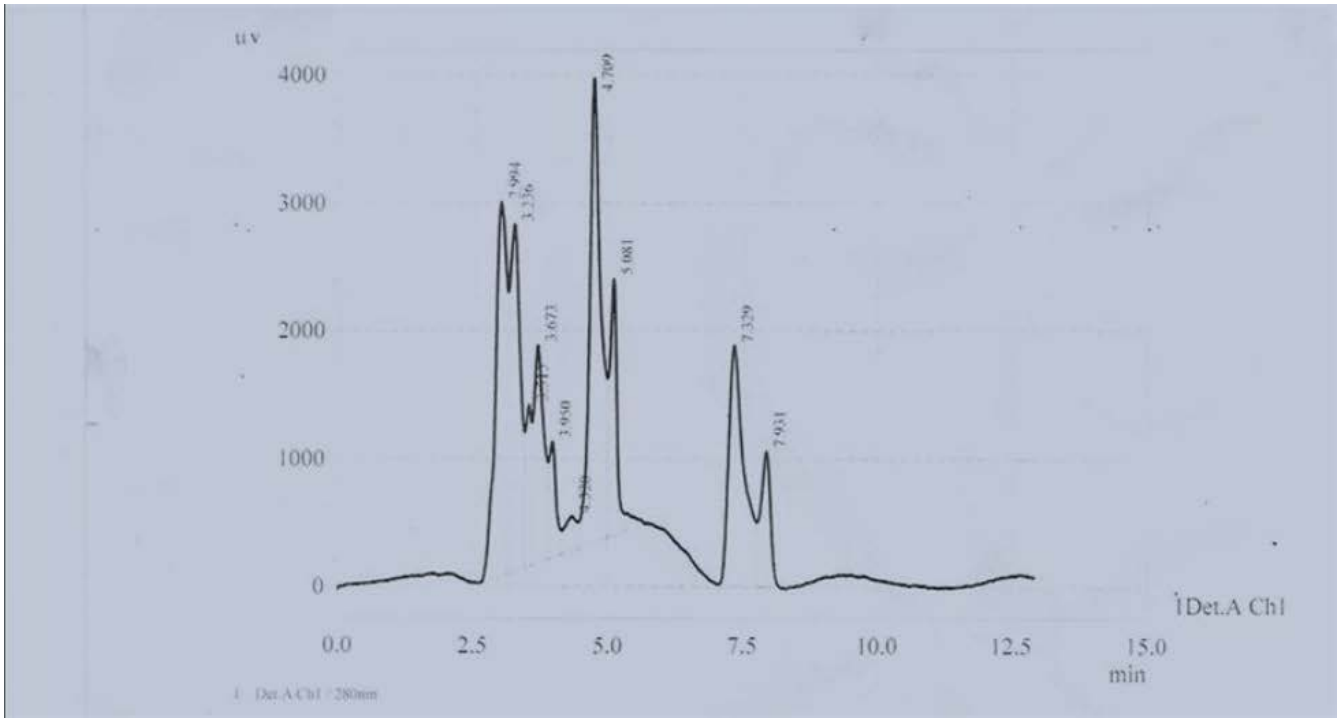
The results of four flavonoids detection using reversed phase HPLC are exhibited in the Table (3-7). The results of (Rutin, Kaempferol and, Quercetin) concerning flavonoids constitution of corn silk extracts were: (0.012 mg/L) of Quercetin in 99% ethanolic extract, (0.13, 0.15 and, 0.11 mg/L) of (Rutin, Kaempferol, and Quercetin) respectively in 80% ethanolic extract, and (0.071, 0.091 mg/L) of (Rutin, and Kaempferol) respectively in aqueous extract. It is seen that 80% ethanol gives better and higher concentrations than others and it is suitable for all three types of flavonoids.

Table(3-7); Concentrations of (Rutin, Kaepferol and, Quercetin) in (99.9%EE) (80%EE), and (AE).

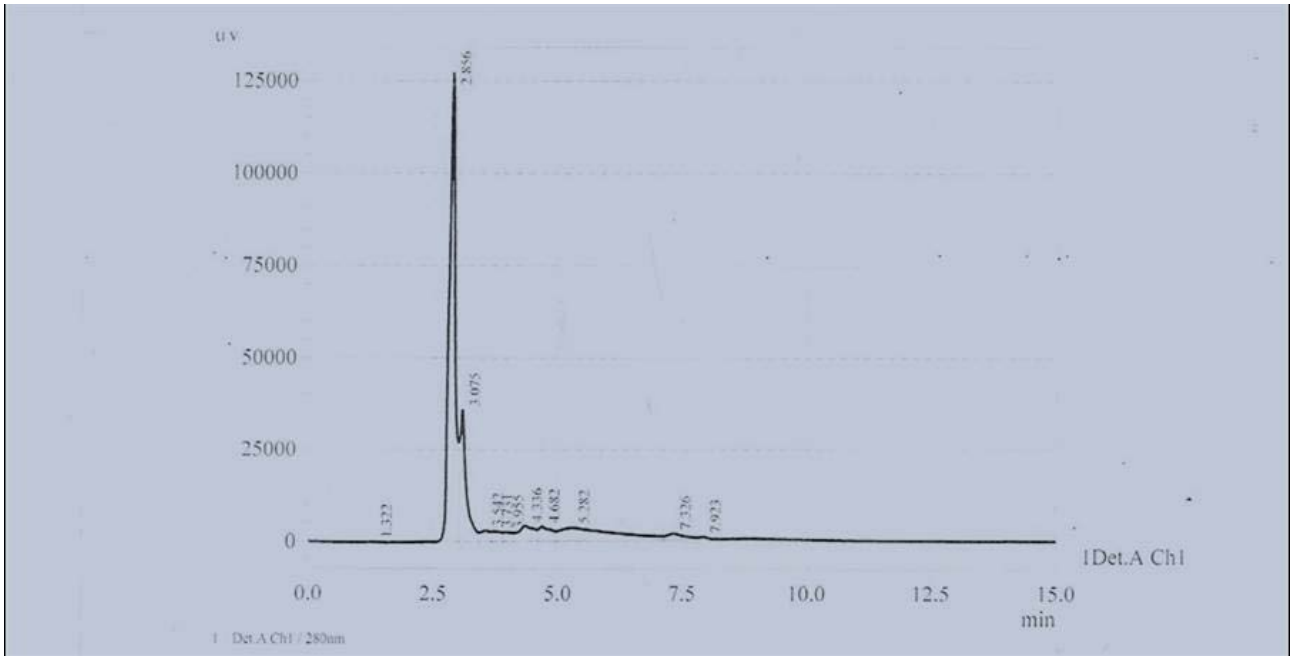
Type of flavonoides	R time(min.)	Conc.in 99.9% ethanol extract (mg/L)	Conc.in 80% ethanol extract (mg/L)	Conc.in aqueous extract (mg/L)
Rutin	**2.851**	-----	**0.1398**	**0.071**
Kaepferol	**3.080**	-----	**0.150**	**0.091**
Quercetin	**7.315**	**0.012**	**0.110**	------
Maysin	**4.209**	------	-------	------



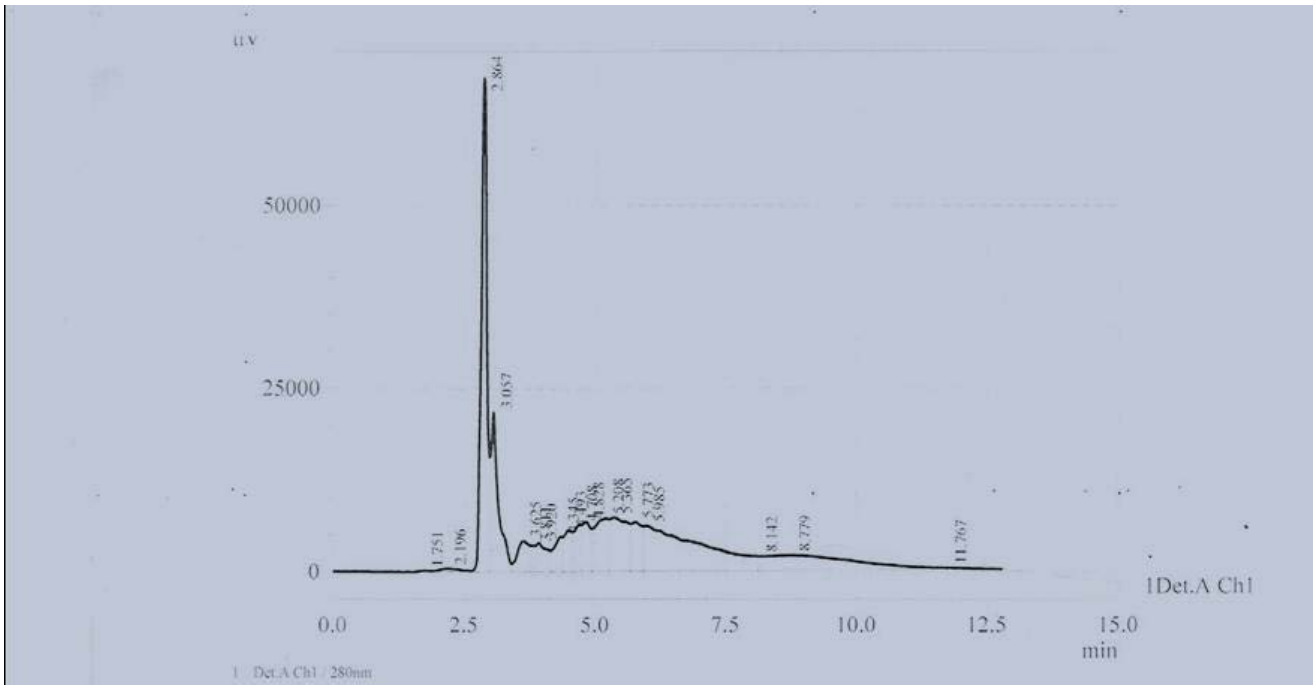
Figure(3-4); HPLC of standard flavonoids (retention time of Rutin ,kaepferol , and Quarcetin, were 2.85, 3.08, and 7.31 min, respectively).



Figure(3-5) HPLC of 99.9% ethanolic extract



Figure(3-6) HPLC of 80% ethanolic extract



Figure(3-7) ; HPLC of aqueous extract

3.4 Study urease inhibition by corn silk extract.

Studies on enzyme inhibition is an important area of pharmaceutical science. These studies in the past have led to the discoveries of several successful drugs, useful against a variety of pathophysiological conditions. Natural products have played an important role in the development of new therapeutic agents against urease enzyme, such as flavonoids, that exhibited excellent urease inhibitory activity. Specific inhibitors interact with enzymes and block their activity towards their corresponding natural and synthetic substrates[93].

3.4.1 Urease inhibition in *Klebsiella species*

Urea Broth medium was developed by Rustigian and Stuart .This medium is especially recommended by Indian Pharmacopoeia .The pink color is given as a positive result of the bacteria produced urease Klebsiella aerogenes . Because Urea Broth Medium convert to alkaline as the use of urea by the organisms release ammonia through the incubation, showed by pink color [102] The inhibitory activity of Extracts (99.9%EE), (80%EE), and (AE) to Klebsiella Species are shown in the table (3-8). It is noticed that all three extracts (99.9%EE), (80%EE), and (AE) show a potent urease inhibitory activity and the IC_{50} values are shown in Figure (3-8), (3-9) ,(3-10). (IC_{50} = 235.9 , 305.5 , and 247.3 mg/L) respectively as compared with Thio urea as shown in Figure (3-11),which shows inhibitory of (IC_{50} =138.1mg/L). The extract

(99.9%EE) have less IC50 so it's the best urease inhibitor from the other extracts.

Table (3-8). Klebsiella species urease inhibitory activity of Extracts (99.9%EE) (80%EE), (AE) as compared with standard urease inhibitor thiourea .

Conc. / species	Inhibtion % of thiourea	Inhibtion % of 99.9E.E.	Inhibtion % of 80% E.E.	Inhibtion % of A.E.
31.25	29.78	23.72	9.927	28.81
62.5	34.62	29.53	23.97	34.62
125	38.25	33.89	31.23	37.77
250	39.70	38.98	32.62	39.22
500	45.76	41.64	36.25	40.67
1000	52.30	44.30	42.42	44.3099
IC_{50}	138.1	235.9	305.5	247.3

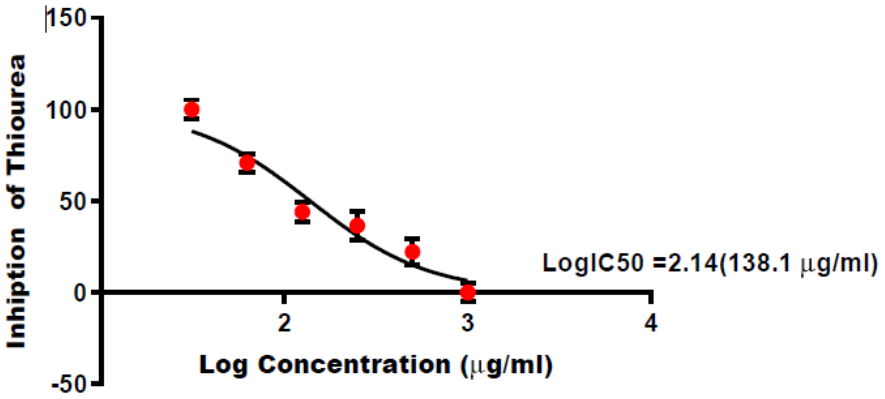


Figure. (3-8).The IC_{50} of Klebsiella species urease inhibition by thiourea

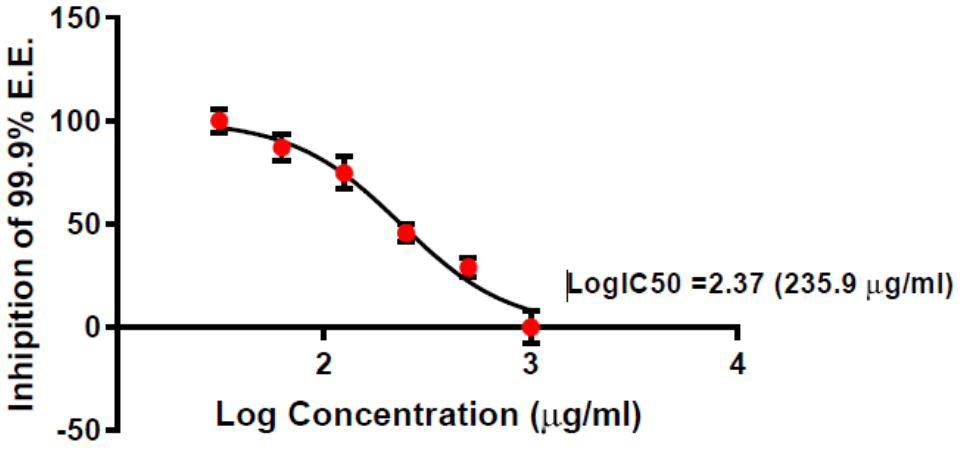


Figure (3-9).IC50 of Klebsiella species urease inhibition by Different concentrations of 99.9%ethanolic extract (99.9%EE)

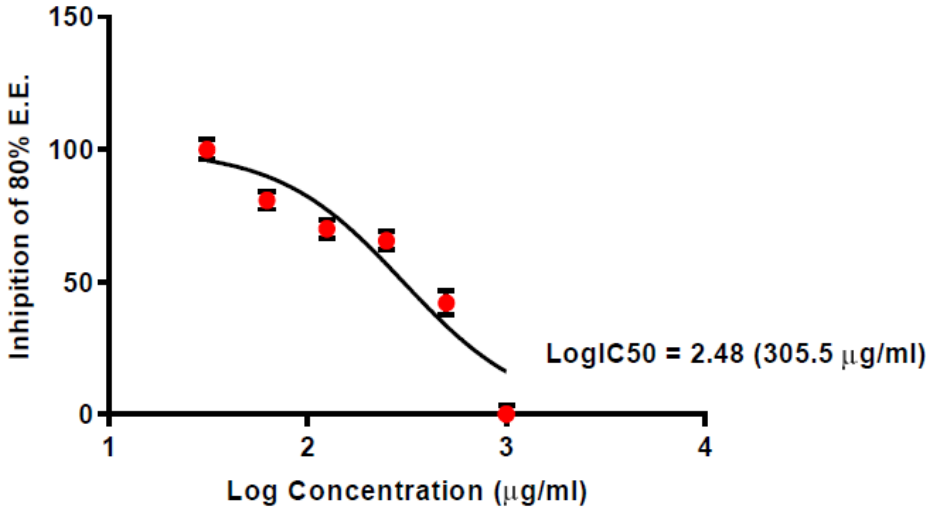


Figure (3-10).IC50 of Klebsiella species urease inhibition by different concentrations of 80%ethanolic extract (80%EE)

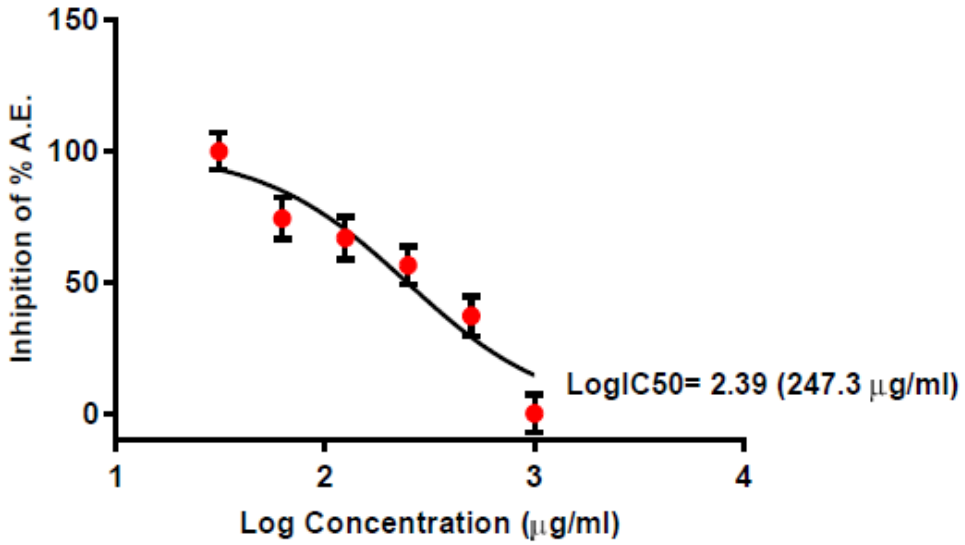


Figure (3-11).IC50 of Klebsiella species urease inhibition by different concentrations of aqueous extract (AE)

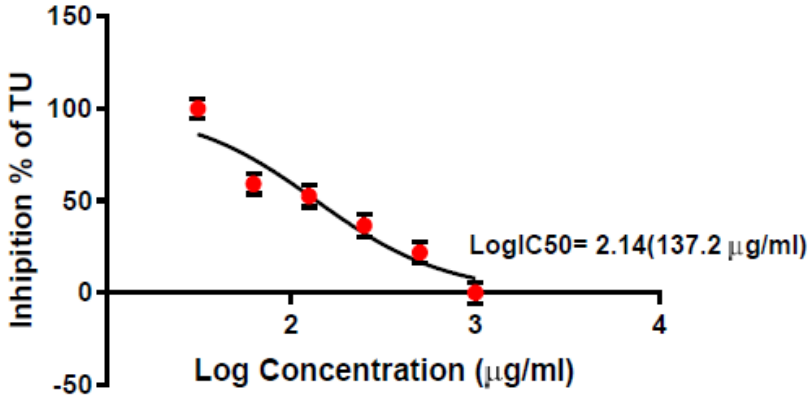
3.4.2 Pure Urease activity inhibition

This test aimed to determine urease activity in monitoring of the products of enzyme catalyzed hydrolysis of urea into carbon dioxide and ammonia [94]. Table (3-9) shows the pure inhibitory activity of the three extracts (99.9%EE), (80%EE), and (AE).

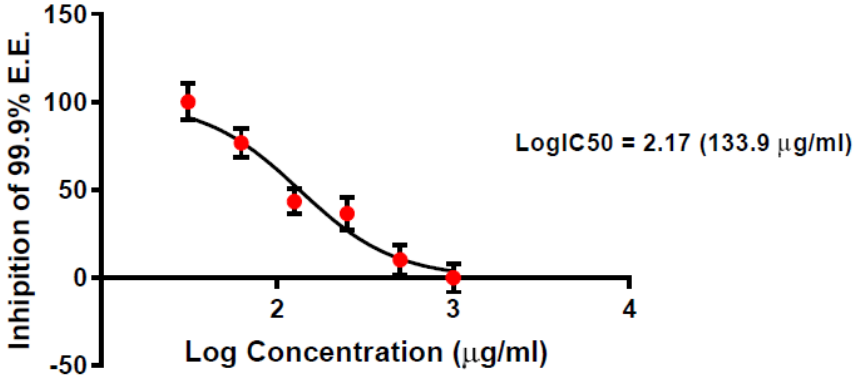
Table(3-9):pure urease inhibitory activity of extracts (99.9%EE) (80%EE), (AE) in Comparative to standard urease inhibitor i.e. thiourea

Conc. (mg/L)	Inhibition % of thiourea	Inhibition % of.(99.9%EE)	Inhibition% of(80%EE)	Inhibition% of (AE)
31.25	35.65	33.77	33.40	33.61
62.5	39.64	35.08	36.10	36.18
125	42.31	38.07	39.90	37.65

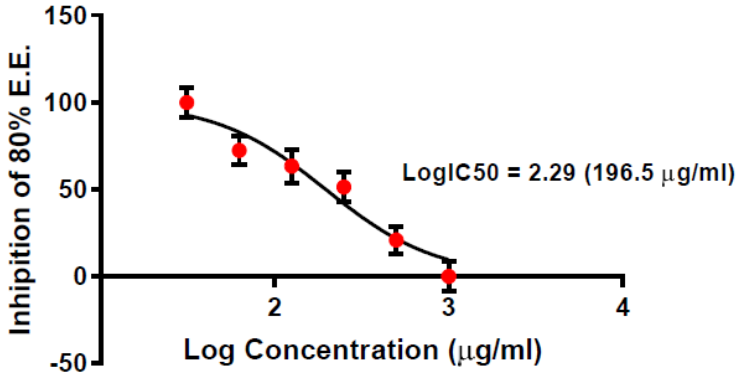
250	45.20	39.17	41.26	39.95
500	46.51	43.36	42.42	44.57
1000	53.99	46.30	45.98	46.82
IC50	137.2	133.9	196.5	157.7



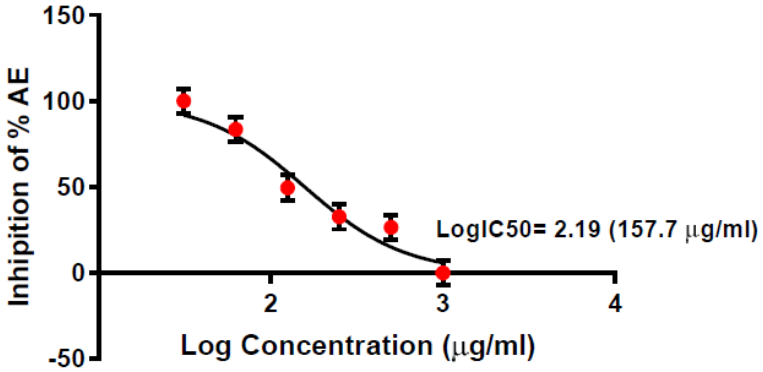
Figure(3-12).IC50 of pure urease inhibition by different concentrations of standard urease inhibitor



Figure(3-13).IC50 of pure urease inhibition by different concentrations of 99.9% ethanolic extract (99.9% EE)



Figure(3-14) IC50 of Pure urease inhibition by different concentrations 80% ethanolic extract (80%EE)



Figure(3-15) IC50 of Pure urease inhibition by different concentrations aqueous extract (AE)

It is noticed that all three extracts (99.9%EE), (80%EE), and (AE) show a potent urease inhibitory activity and the IC50 values are shown in Figure (3-12), (3-13) ,(3-14). (IC_{50} = 133.9, 196.5, and 157.7 mg/L) respectively as compared with Thiourea as shown in Figure (3-15), which exhibits (IC_{50} = 137.2 mg/L). The extract (99.9%EE) have less IC_{50} so it's the best urease inhibitor from the other extracts.

However, these results were compared to Acetohydroxamic acid which exhibits (IC50 =18.7 mg/L) reported by Kumar, et al [105] .

Acetohydroxamic acid is useful urease inhibitor , its used as drug (Lithostat) that is a potent and irreversible inhibitor of bacterial and plant urease usually used for urinary tract infections. Which it has two nickel ions bound inside the active site. But this compound have severe side effects, such as teratogenicity, psychoneurologic and musculo-integumentary symptoms[106]. So , researches nowdays are focusing on finding urease inhibitor as effective as Acetohydroxamic acid but without side effects

The urease enzyme has nickel ions in the active site, which is essential for its activity [100]. The large amounts of flavonoids present in corn silk [67] are responsible for inhibition of the enzyme because the Flavonoids have properties to form chelate complexes by interactions with metal ions (Ni) [101].

Structure-activity relationship (SAR) indicates that the presence of two consecutive OH groups enable the flavonoid to interact with the enzyme, most likely through hydrogen bonding with the active site residues of enzyme. They may also chelate with the ‘’Ni’’ ions at the active site. Additionally, OH groups are also capable of forming electrostatic interactions (i.e. hydrogen bonding) with charged active site residues, including histidine, arginine and aspartic acid[92].

3.5 Enzyme kinetic

To check the inhibition mechanism of these extracts, the kinetics studies of the most active extract (99.9% ethanolic extract) were performed. In the kinetics studies along with different concentration of the substrates(urea, 10 – 120 mM). This experiment was done on pure urease enzyme the results shown in Table 3-10 , Figure 3-16, urease enzyme in patient urine the results

shown in Table 3-11 , Figure 3-17, and urease enzyme in control urine the results shown in Table 3-12 , Figure 3-18,

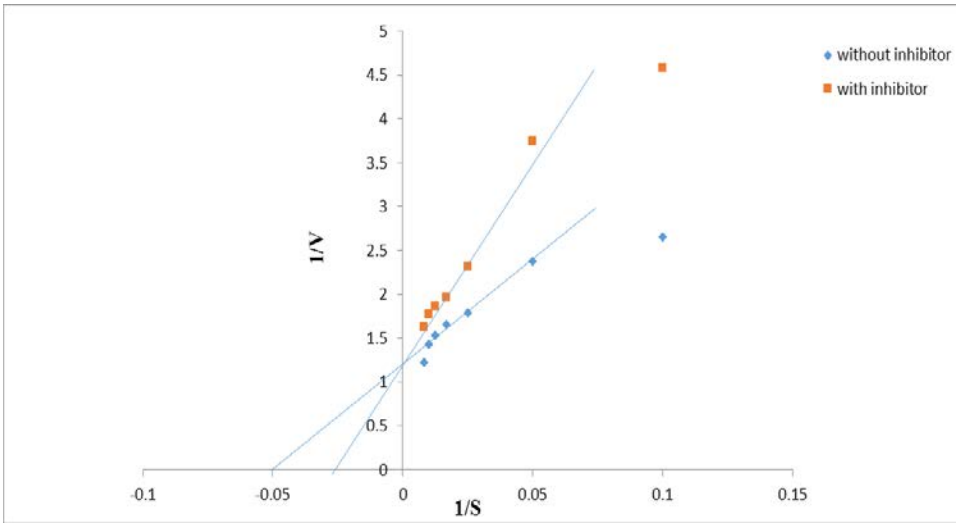


Figure (3-16): Kinetic constants of pure urease enzyme according to Linweaver- Burk.

Table (3-10): Kinetic constants of pure urease enzyme according to Linweaver- Burk.

Kinetic Constants	Km (mM)	V max (unit/ml)
Without inhibitor	20	0.76
With inhibitor	40	0.76

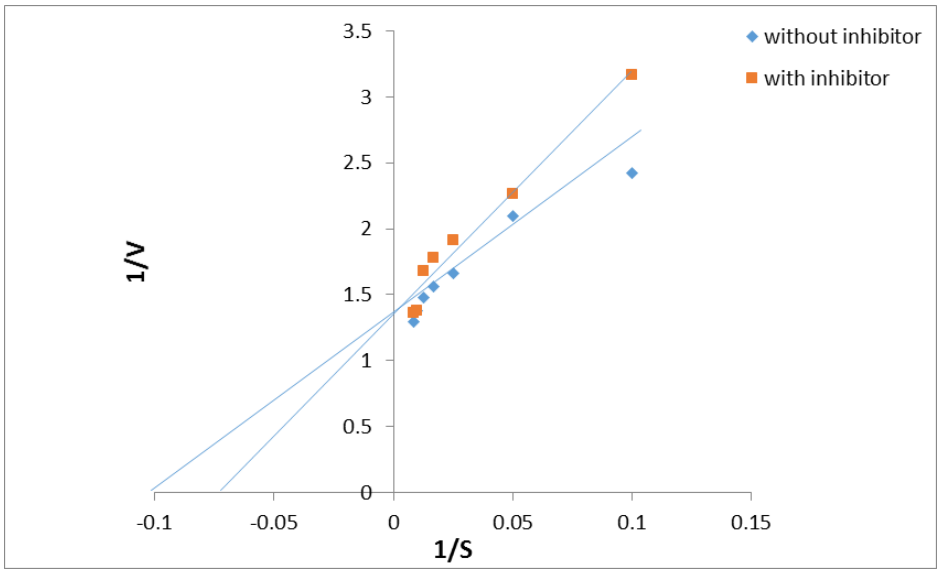


Figure (3-17): Kinetic constants of urease enzyme in patient urine according to Linweaver- Burk.

Table (3-11): Kinetic constants of urease enzyme in patient urine according to Linweaver- Burk.

Kinetic Constants	Km (mM)	V max (unit/ml)
Without inhibitor	10	0.71
With inhibitor	13	0.71

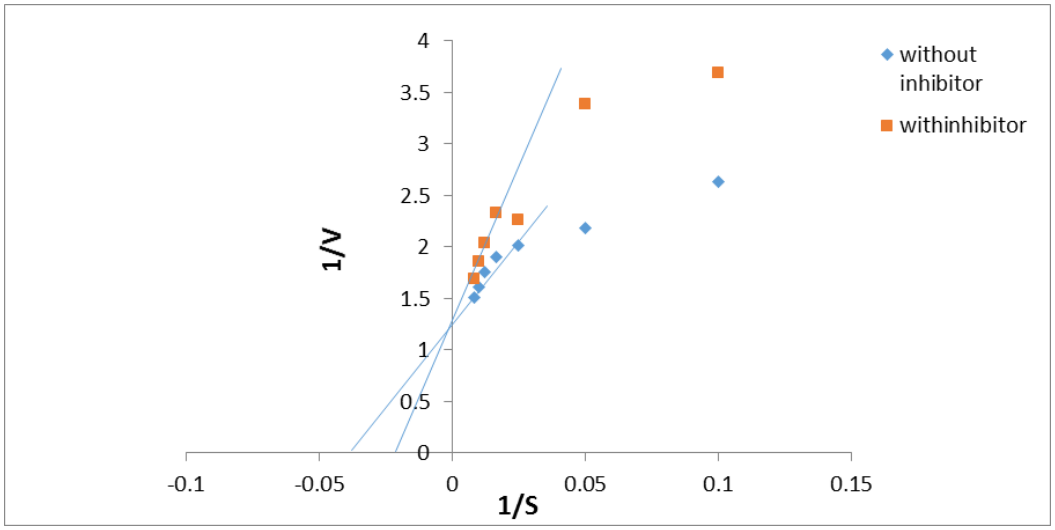


Figure (3-18): Kinetic constants of urease enzyme in control urine according to Linweaver- Burk.

Table (3-12): Kinetic constants of urease enzyme in control urine according to Linweaver- Burk.

Kinetic Constants	Km (mM)	V max (unit/ml)
Without inhibitor	25	0.8
With inhibitor	50	0.8

Michalis-Menten constant refers to the enzyme affinity towards substrate, whereas Vmax occurs when all available enzyme molecules present in the form of enzyme-substrate complex (ES).

It can be noticed that enhancing substrate concentration enhances the rate of the reaction because of increasing the collisions between substrate and enzyme molecules until reaching a certain concentration, after that further increasing in substrate concentration has no effect on the reaction rate because that enzyme becomes saturated and its active site is occupied with substrate.

The type of inhibition was determined by Lineweavere Burk plots, the reciprocal of the rate of the reaction were plotted against the reciprocal of substrate concentration to monitor the effect of inhibitor on both Km and Vmax. In the presence of 99.9% ethanolic extract, the Vmax of jack bean urease was not affected, while the Km increased which indicated a pure competitive type of inhibition. As proposed in SAR, these compounds interact either with the active site residues of the enzyme or they chelate with the Ni ions present at active site of the enzyme [92].

Conclusions:

We found in this study that :

1. males increased risk of developing stones more than females that could be related to high urinary citrate concentration in females and type of hormones in males and females and also females have low level of calcium than males .
2. The pH of urine is the most important factor in the stone formation for both calcium oxalate and uric acid stones alkaline pH form struvite stones while acidic pH form uric acid stones .
3. There is highly significant difference ($P < 0.0001$) of urease activity in urine patient with struvite kidney stones Compare with urine control .
4. Percentage of struvite stones was 22% that caused by different type of bacteria (Klebsiella and protuse) .
5. The water solvent gives higher yield than others which can considered as factor in solvent cost reduction.
6. Only 80% ethanol has an ability to extract such three flavonoids Rutin, Kaepferol, and Quercetin .
7. The extracts were utilized in the inhibition of urease enzyme and 80% ethanolic extract exhibited an highest efficient inhibition .
8. Lastly, It was study Kinetic Constants of pure urease enzyme and urease in urine According to Linweaver- Burk. .

Recommendations

1. To avoid stone formation , take more water because it helps to flush away the substances that is recommended to form stones in the kidney.
2. Patients should know exactly what kind of stone that they had because the therapy is depend on the type of stone that the patients have .

Suggestion of the future work:

1. For Patients with struvite stone Syntheses same nontoxic chemical compound that can make chelating complex with Ni atom in urease enzyme and inactive the active site .
2. Isolation and purification of each flavonoids from corn silk and study the possibility of using it as urease inhibitor .
3. Comparison of inhibition between purified flavonoids and crud extraction of corn silk .

References

References

1. Aggarwal K. P., Narula, S., Kakkar, M., & Tandon, C. (2013). Nephrolithiasis: molecular mechanism of renal stone formation and the critical role played by modulators. BioMed research international, 1-21.
2. Sekkoum, K., Cheriti, A., Taleb, S., & Belboukhari, N. (2016). FTIR spectroscopic study of human urinary stones from El Bayadh district (Algeria). Arabian Journal of Chemistry, 9(3), 330-334.
3. Perera, I. (2016). Renal stones in children: evaluation and medical management. Sri Lanka Journal of Child Health, 45(1), 3-10.
4. Han, H., Segal, A. M., Seifter, J. L., & Dwyer, J. T. (2015). Nutritional management of kidney stones (nephrolithiasis). Clinical nutrition research,4(3), 137-152.
5. Fink, H. A., Wilt, T. J., Eidman, K. E., Garimella, P. S., MacDonald, R., Rutks, I. R., ... & Monga, M. (2013). Medical management to prevent recurrent nephrolithiasis in adults: a systematic review for an American College of Physicians Clinical Guideline. Annals of internal medicine, 158(7), 535-543.
6. Domingos, F., & Serra, A. (2010). Nephrolithiasis is associated with an increased prevalence of cardiovascular disease. Nephrology Dialysis Transplantation, 26(3), 864-868.
7. Han, H., Segal, A. M., Seifter, J. L., & Dwyer, J. T. (2015). Nutritional management of kidney stones (nephrolithiasis). Clinical nutrition research, 4(3), 137-152.
8. Taylor, E. N., Stampfer, M. J., & Curhan, G. C. (2005). Diabetes mellitus and the risk of nephrolithiasis. Kidney international, 68(3), 1230-1235.
9. Rejnmark, L., Vestergaard, P., & Mosekilde, L. (2011). Nephrolithiasis and renal calcifications in primary hyperparathyroidism. The Journal of Clinical Endocrinology & Metabolism, 96(8), 2377-2385.
10. Palsson, R. (2014). Genetic causes of kidney stones. Urinary Stones: Medical and Surgical Management, 57-69.

References

11. Ferraro, P. M., & Curhan, G. C. (2017). Serum Uric Acid and Risk of Kidney Stones. American Journal of Kidney Diseases, 70(2), 158-159.
12. Wang, X. G., Fan, B., Wang, W., Jin, Y. Y., Jin, Y., Yang, Z. Q., & Li, X. C. (2017). The value of performing dual-source dual-energy computed tomography in analysis strategy for urinary mixed stone. Int J Clin Exp Pathol, 10(2), 1519-1528.
13. .Ou, Y., Xue, J. F., Tan, C. Y., Gui, B. S., Sun, X. Y., & Ouyang, J. M. (2015). Inhibition of urinary macromolecule heparin on aggregation of Nano-COM and Nano-COD crystals. Molecules, 20(1), 1626-1642.
14. Kelly, C. R., & Landman, J. (2012). The Netter Collection of Medical Illustrations-Urinary System e-Book (5). Elsevier Health Sciences.2nd Ed 176-177.
15. Goldfarb, D. S., MacDonald, P. A., Gunawardhana, L., Chefo, S., & McLean, L. (2013). Randomized controlled trial of febuxostat versus allopurinol or placebo in individuals with higher urinary uric acid excretion and calcium stones. Clinical Journal of the American Society of Nephrology, 8(11), 1960-1967.
16. Maalouf, N. M. (2014). Uric Acid Stones. Urinary Stones: Medical and Surgical Management, 26-35.
17. Coe, F. L., Evan, A., & Worcester, E. (2005). Kidney stone disease. Journal of Clinical Investigation, 115(10), 2598.
18. Trinchieri, A., & Montanari, E. (2017). Biochemical and dietary factors of uric acid stone formation. Urolithiasis, 1-6.
19. Flannigan, R., Choy, W. H., Chew, B., & Lange, D. (2014). Renal struvite stones [mdash] pathogenesis, microbiology, and management strategies. Nature reviews Urology, 11(6), 333-341.
20. Pang, J., Borjeson, T. M., Parry, N., & Fox, J. G. (2015). Struvite Urolithiasis in Long–Evans Rats. Comparative medicine, 65(6), 486-491.

References

21. Iqbal, M. W., Youssef, R. F., Neisius, A., Kuntz, N., Hanna, J., Ferrandino, M. N., ... & Lipkin, M. E. (2016). Contemporary management of struvite stones using combined endourologic and medical treatment: predictors of unfavorable clinical outcome. Journal of endourology, 30(7), 771-777.
22. Bazin, D., André, G., Weil, R., Matzen, G., Emmanuel, V., Carpentier, X., & Daudon, M. (2012). Absence of bacterial imprints on struvite-containing kidney stones: a structural investigation at the mesoscopic and atomic scale. Urology, 79(4), 786-790.
23. Macegoniuk, K. (2013). Inhibitors of bacterial and plants urease. Folia Biologica et Oecologica, 9(1), 9-16.
24. Krajewska, B. (2009). Ureases I. Functional, catalytic and kinetic properties: A review. Journal of Molecular Catalysis B: Enzymatic, 59(1), 9-21.
25. Sun, W. D., Wang, J. Y., Zhang, K. C., & Wang, X. L. (2010). Study on precipitation of struvite and struvite-K crystal in goats during onset of urolithiasis. Research in veterinary science, 88(3), 461-466.
26. Trinchieri, A. (2014). Urinary calculi and infection. Urologia, 81(2), 93.
27. Rimer, J. D., An, Z., Zhu, Z., Lee, M. H., Goldfarb, D. S., Wesson, J. A., & Ward, M. D. (2010). Crystal growth inhibitors for the prevention of L-cystine kidney stones through molecular design. Science, 330(6002), 337-341.
28. Chauhan, C. K., & Joshi, M. J. (2013). In vitro crystallization, characterization and growth-inhibition study of urinary type struvite crystals. Journal of Crystal Growth, 362, 330-337.
29. Ahmed, K., Dasgupta, P., & Khan, M. S. (2006). Cystine calculi: challenging group of stones. Postgraduate medical journal, 82(974), 799-801.
30. Lee, M. H., Sahota, A., Ward, M. D., & Goldfarb, D. S. (2015). Cystine growth inhibition through molecular mimicry: a new paradigm for the prevention of crystal diseases. Current rheumatology reports, 17(5), 33.

References

31. Mong A, M., Penniston, K. A., & Goldf Arb, D. (2015). Pocket Guide to Kidney Stone Prevention. Springer International Publishing, 2015th Edition.115-117
32. Simerville, J. A., Maxted, W. C., & Pahira, J. J. (2005). Urinalysis: a comprehensive review. Am Fam Physician, 71(6), 1153-62.
33. Rodríguez-Muguruza, S., Malumbres, S., Olive, A., Valero, O., Holgado, S., Mateo, L., & Martínez-Morillo, M. (2017). AB1055 Leukocyte esterase reagent strips for rapid diagnosis of inflammatory synovial fluid , 76(2) , 1424-1424.
34. Mundt, L. A., & Shanahan, K. (2010). Graff's textbook of routine urinalysis and body fluids. Lippincott Williams & Wilkins, 2nd Ed , 200-201.
35. Lindh, W. Q., Pooler, M., Tamparo, C. D., Dahl, B. M., & Morris, J. (2013).Delmar's comprehensive medical assisting: administrative and clinical competencies. Cengage Learning.5th Ed .1299-1307.
36. Strasinger, S. K., & Di Lorenzo, M. S. (2014). Urinalysis and body fluids. FA Davis 6th Ed , 72-138.
37. Sink, C. A., & Weinstein, N. M. (2012). Practical veterinary urinalysis. John Wiley & Sons 1st Ed , 55-59.
38. Prywer , J., Torzewska, A., & Płociński, T. (2012). Unique surface and internal structure of struvite crystals formed by Proteus mirabilis. Urological research, 40(6), 699-707.
39. Banks, A. (2013). Laboratory tests and diagnostic procedures with nursing diagnoses. Pearson , 8th Ed , 59-82 .
40. Simerville, J. A., Maxted, W. C., & Pahira, J. J. (2005). Urinalysis: a comprehensive review. Am Fam Physician, 71(6), 62-1153
41. Ghani, S., Karim, S. A., & Izhar, R. (2016). frequency of urine culture positivity in pregnant women with urinary symptoms and positive urine detail report, 22 (3), 13-18

References

42. Gilad, R., Williams, J. C., Usman, K. D., Holland, R., Golan, S., Tor, R., & Lifshitz, D. (2017). Interpreting the results of chemical stone analysis in the era of modern stone analysis techniques. Journal of nephrology, 30(1), 135-140.
43. Mathew G. , Lincy J. , & Ashna S, (2017). Evaluation of Clinical Outcome and Estimation of Effect of Serum Creatinine Levels in Parkinson's Patients with the Administration of Ropinirole. International Journal of Science and Research, (Vol. 6). 2319-7064
44. Maheshwari, N. (2008). Clinical biochemistry. Jaypee Brothers Publishers.
45. Finney, H., Newman, D. J., & Price, C. P. (2000). Adult reference ranges for serum cystatin C, creatinine and predicted creatinine clearance. Annals of clinical biochemistry, 37(1) ,49-59.
46. Harvey, R. A., & Ferrier, D. R. (2011). Biochemistry. Lippincott Williams & Wilkins.
47. Macomber, L., Minkara, M. S., Hausinger, R. P., & Merz Jr, K. M. (2015). Reduction of urease activity by interaction with the flap covering the active site. Journal of chemical information and modeling
48. Mitchell, S., Ellingson, C., Coyne, T., Hall, L., Neill, M., Christian, N., ... & Summar, M. (2009). Genetic variation in the urea cycle: a model resource for investigating key candidate genes for common diseases. Human mutation, 30(1), 56-60.
49. Alvarez-Lario, B., & Macarron-Vicente, J. (2011). Is there anything good in uric acid?. QJM: An International Journal of Medicine, 104(12), 1015-1024.
50. Johnson, R. J., Kang, D. H., Feig, D., Kivlighn, S., Kanellis, J., Watanabe, S., ... & Mazzali, M. (2003). Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal disease?. Hypertension, 41(6), 1183-1190.

References

51. Nelson, D. L., Lehninger, A. L., & Cox, M. M. (2013). Lehninger principles of biochemistry. Macmillan.6th Ed.
52. Mehdi, W. A., Eyada, Z. N., & Ismail, R. A. (2014). Study the Effect of Gases Rising From Electric Generators on Sera Protein, Iron, Transferrin and other Biochemical Parameters in Iraqi Workers Employed on the Operation of Diesel Generators. Australian Journal of Basic and Applied Sciences, 8, 521-526.
53. Macomber, L., Minkara, M. S., Hausinger, R. P., & Merz Jr, K. M. (2015). Reduction of urease activity by interaction with the flap covering the active site. Journal of chemical information and modeling, 55(2), 354-361
54. Amtul, Z., Siddiqui, R. A., & Choudhary, M. I. (2002). Chemistry and mechanism of urease inhibition. Current medicinal chemistry, 9(14), 1323-1348.
55. Follmer, C. (2010). Ureases as a target for the treatment of gastric and urinary infections. Journal of clinical pathology, 63(5), 424-430.
56. Junejo, N., Khanif, M. Y., Hanfi, M. M., Yunus, W. W., & Dharejo, K. A. (2011). Role of inhibitors and biodegradable material in mitigation of nitrogen losses from fertilized lands. African Journal of Biotechnology, 10(18), 3504-3514.
57. Zambelli, B., Musiani, F., Benini, S., & Ciurli, S. (2011). Chemistry of Ni2+ in urease: sensing, trafficking, and catalysis. Accounts of chemical research, 44(7), 520-530.
58. Strasinger, S. K., & Di Lorenzo, M. S. (2014). Urinalysis and body fluids. FA Davis ,6th Ed ,102-38 .
59. Fischbach, F. T., & Dunning, M. B. (2015). A manual of laboratory and diagnostic tests. Lippincott Williams & Wilkins ,8th Ed ,178-278.
60. Bahmani, M., Baharvand-Ahmadi, B., Tajeddini, P., Rafieian-Kopaei, M., & Naghdi, N. (2016). Identification of medicinal plants for the treatment of kidney and urinary stones. Journal of renal injury prevention, 5(3), 129.

References

61. Hu, Q. L., Zhang, L. J., Li, Y. N., Ding, Y. J., & Li, F. L. (2010). Purification and anti-fatigue activity of flavonoids from corn silk. International Journal of Physical Sciences, 5(4), 321-326.

62. Cuina, W., Tiehua, Z., Jun, L., Shuang, L., Cheng, Z., Erlei, W., & Jingbo, L. (2011). Subchronic toxicity study of corn silk with rats. J. Ethnopharmacol.

63. Chen, S., Chen, H., Tian, J., Wang, Y., Xing, L., & Wang, J. (2013). Chemical modification, antioxidant and α-amylase inhibitory activities of corn silk polysaccharides. Carbohydrate polymers, 98(1), 428-437.

64. Sarepoua, E., Tangwongchai, R., Suriharn, B., & Lertrat, K. (2013). Relationships between phytochemicals and antioxidant activity in corn silk.International Food Research Journal, 20(5), 2073-2079.

65. Nessa, F., Ismail, Z., & Mohamed, N. (2012). Antimicrobial activities of extracts and flavonoid glycosides of corn silk (Zea mays L). International Journal of Biotechnology for Wellness Industries, 1(2), 115-120.

66. Gul, H., Kinza, S., Shinwari, Z. K., & Hamayun, M. (2017).effect of selenium on the biochemistry of zea mays under salt stress.pakistan journal of botany, 49, 25-32.

67. Solihah, M. A., Wan Rosli, W. I., & Nurhanan, A. R. (2012). Phytochemicals screening and total phenolic content of Malaysian Zea mays hair extracts 19(4): 1533-1538.

68. Khalil, A. (2017). Role of Biotechnology in Alkaloids Production. In Catharanthus roseus .Springer International Publishing. 59-70.

69. Lednicer, D. (2011). Steroid chemistry at a glance , John Wiley & Sons , 10 .

70. . Zhang, H., Cao, X., Liu, Y., & Shang, F. (2017). Rapid recovery of high content phytosterols from corn silk. Chemistry Central Journal, 11(1), 108

References

71. Bianchi, S., Kroslakova, I., & Mayer, I. (2016). Determination of Molecular Structures of Condensed Tannins from Plant Tissues Using HPLC-UV Combined with Thiolysis and MALDI-TOF Mass Spectrometry ,6 (20) , 1-14 .

72. Hassanpour, S., MaheriSis, N., & Eshratkhah, B. (2011). Plants and secondary metabolites (Tannins): A Review , 1 (1) , 47-53.

73. Moss, G. P. (1989). Nomenclature of steroids (Recommendations 1989). Pure and Applied Chemistry, 61(10), 1783-1822.

74. Xu, Q. M., Shu, Z., He, W. J., Chen, L. Y., Yang, S. L., Yang, G., ... & Li, X. R. (2012). Antitumor activity of Pulsatilla chinensis (Bunge) Regel saponins in human liver tumor 7402 cells in vitro and in vivo. Phytomedicine, 19(3), 293-300.

75. Procházková, D., Boušová, I., & Wilhelmová, N. (2011). Antioxidant and prooxidant properties of flavonoids. Fitoterapia, 82(4), 513-523.

76. Ren, S. C., Qiao, Q. Q., & Ding, X. L. (2013). Antioxidative Activity of Five Flavones Glycosides from Corn Silk (Stigma maydis). Czech Journal of Food Science, 31(2), 148–155

77. Nurhanan, A. R., & Wan Rosli, W. I. (2013). Evaluation of Polyphenol Content and Antioxidant Activities of Some Selected Organic and Aqueous Extracts of Cornsilk (Zea mays Hairs). Journal of Medical and Bioengineering (JOMB), 1(1), 47-50.

78. Panchal, S. K., Poudyal, H., Arumugam, T. V., & Brown, L. (2011). Rutin attenuates metabolic changes, nonalcoholic steatohepatitis, and cardiovascular remodeling in high-carbohydrate, high-fat diet-fed rats. The Journal of nutrition, 141(6), 1062-1069.

References

79. Huang, W. W., Chiu, Y. J., Fan, M. J., Lu, H. F., Yeh, H. F., Li, K. H., ... & Yang, J. S. (2010). Kaempferol induced apoptosis via endoplasmic reticulum stress and mitochondria-dependent pathway in human osteosarcoma U-2 OS cells. Molecular nutrition & food research, 54(11), 1585-1595.

80. Nabavi, S. F., Russo, G. L., Daglia, M., & Nabavi, S. M. (2015). Role of quercetin as an alternative for obesity treatment: you are what you eat. Food chemistry, 179, 305-310.

.

References

81. Nabavi, S. M., Nabavi, S. F., Eslami, S., & Moghaddam, A. H. (2012). In vivo protective effects of quercetin against sodium fluoride-induced oxidative stress in the hepatic tissue. Food Chemistry, 132(2), 931-935

82. Zeng, H., Wang, Y., Kong, J., Nie, C., & Yuan, Y. (2010). Ionic liquid-based microwave-assisted extraction of rutin from Chinese medicinal plants.Talanta, 83(2), 582-590

83. Javed, H., Khan, M. M., Ahmad, A., Vaibhav, K., Ahmad, M. E., Khan, A., & Safhi, M. M. (2012). Rutin prevents cognitive impairments by ameliorating oxidative stress and neuroinflammation in rat model of sporadic dementia of Alzheimer type. Neuroscience, 210, 340-352.

84. Lee, K. M., Lee, K. W., Jung, S. K., Lee, E. J., Heo, Y. S., Bode, A. M., ... & Dong, Z. (2010). Kaempferol inhibits UVB-induced COX-2 expression by suppressing Src kinase activity. Biochemical pharmacology, 80(12), 2042-2049.

85. Hasanudin, K., Hashim, P., & Mustafa, S. (2012). Corn silk (Stigma maydis) in healthcare: a phytochemical and pharmacological review. Molecules, 17(8), 9697-9715.

86. Patton, C. J., Crouch, S. R., Anal. Chem., 49: 464 – 469; 1977.

87. Bartels, H., Bohmer, M., Clin. Chem. Acta 37: 193; 1972.

88. Youge, D. S. (2002). "**Effect of disease on clinical lab**", 4th ed.

89. Lubran, M. M. (1978). The measurement of total serum proteins by the Biuret method. Annals of Clinical & Laboratory Science, 8(2), 106-110.

References

90. Doumas B.T.,Watson W.A., and Biggas H.G., (1971) Albumin standards and the measurement of the serum albumin with bromocresol green ,Clinical Chemistry Acta.,31 (1) .87-96.

91. Burtis, C. A., & Ashwood, E. R. (1999). Tietz textbook of clinical chemistry.

92. AJ Awllia, J., Al-Ghamdi, M., Huwait, E., Javaid, S., Rasheed, S., & Iqbal Choudhary, M. (2016). Flavonoids as Natural Inhibitors of Jack Bean Urease Enzyme. Letters in Drug Design & Discovery, 13(3), 243-249

93. Liu, J., Wang, C., Wang, Z., Zhang, C., Lu, S., & Liu, J. (2011). The antioxidant and free-radical scavenging activities of extract and fractions from corn silk (Zea mays L.) and related flavone glycosides. Food Chemistry,126(1), 261-269

94. Hubalek, J., Hradecky, J., Adam, V., Krystofova, O., Huska, D., Masarik, M., ... & Zehnalek, J. (2007). Spectrometric and voltammetric analysis of urease–Nickel nanoelectrode as an electrochemical sensor. Sensors, 7(7), 1238-1255.

95. Foxman, B. (2014). Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. Infectious disease clinics of North America, 28(1), 1-13.

96. Ferreira, O., & Pinho, S. P. (2012). Solubility of flavonoids in pure solvents.Industrial & Engineering Chemistry Research, 51(18), 6586-6590.

97. Razmara, R. S., Daneshfar, A., & Sahraei, R. (2010). Solubility of quercetin in water+ methanol and water+ ethanol from (292.8 to 333.8) K. Journal of Chemical & Engineering Data, 55(9), 3934-3936.

References

98. Zhang, H., Wang, M., Chen, L., Liu, Y., Liu, H., Huo, H., ... & Qi, A. (2017). Structure-solubility relationships and thermodynamic aspects of solubility of some flavonoids in the solvents modeling biological media. Journal of Molecular Liquids, 225, 439-445.

99. Macegoniuk, K. (2013). Inhibitors of bacterial and plants urease. Folia Biologica et Oecologica, 9(1), 9-16.

100. Boer, J. L., Quiroz-Valenzuela, S., Anderson, K. L., & Hausinger, R. P. (2010). Mutagenesis of Klebsiella aerogenes UreG to probe nickel binding and interactions with other urease-related proteins. Biochemistry, 49(28), 5859-5869.

101. Symonowicz, M., & Kolanek, M. (2012). Flavonoids and their properties to form chelate complexes , Biotechnology and Food Sciences 76 (1), 35-41

102. Mac Faddin, J. F. (1976). Biochemical tests for identification of medical bacteria. Williams & Wilkins Co..

103. Liu, J., Lin, S., Wang, Z., Wang, C., Wang, E., Zhang, Y., & Liu, J. (2011). Supercritical fluid extraction of flavonoids from Maydis stigma and its nitrite-scavenging ability. Food and bioproducts processing, 89(4), 333-339.

104. Nurhanan, A. R., & WI, W. R. (2012). Evaluation of Polyphenol Content and Antioxidant Activities of Some Selected Organic and Aqueous Extracts of Cornsilk (Zea Mays Hairs). Journal of Medical and Bioengineering (JOMB) Vol, 1(1).

105. Kumar, S., & Kayastha, A. M. (2010). Acetohydroxamic acid: a competitive inhibitor of urease from soybean (Glycine max). Proteins Proteomics, 1, 3-8.

References

106. Kosikowska, P., & Berlicki, Ł. (2011). Urease inhibitors as potential drugs for gastric and urinary tract infections: a patent review. Expert opinion on therapeutic patents, 21(6), 945-957.

الخلاصة

هدفت هذة الدراسه الى استخلاص حرير الذره بأستخدام المذيبات (99.9 % ايثانول و80% ايثانول والماء) .ودراسة تأثير هذه المستخلصات لتثبيط انزيم اليوريز الذي يسبب حصى الكليه.

- تضمنت الدراسة قياس بعض المتغيرات (اليوريا , الكرياتنين , حمض اليوريك, السكر,الالبومين ,البروتين الكلي والكلوبيلين) في مصل 100 مصاب بحصى الكليه في مستشفى غازي الحريري مقارنه مع 30 من الأصحاء .فكانت نتائج التحليل الاحصائي انه يوجد زياده معنويه بالنسبه لليوريا والسكر مقارنه مع الأصحاء ولاتوجد فروق معنويه بالنسبه لباقي المتغيرات.
- دراسه تشخيص نوع الحصى ستريوفيت من خلال نتائج تحليل الادرار العام وزرع الادرار وكذلك تحليل مكونات الحصى . فكانت النتائج 22% من مرضى حصى الكليه يمتلكون نوع حصى ستريوفيت
- تقدير فعاليه انزيم اليوريز في أدرار 22 من الاشخاص المصابين بنوع حصى ستريوفيت مقارنه مع 22 من الاشخاص الاصحاء, وقد اعطت النتائج التحليل الأحصائي زياده معنويه عاليه ($P < 0.0001$) لفعاليه انزيم اليوريز في ادرار المصابين مقارنه مع الاصحاء.
- استخلاص حرير الذره باستخدام المذيبات (99.9 % ايثانول و80% ايثانول والماء) وتقدير تركيز الفلاوفونيدات الروتين والكافيرول والكوارستين بواسطه HPLC ,فكانت النتائج 0.012 ملغم/لتر من الكوارستين في مستخلص 99.9% ايثانول. 0.1398 و 0.15 , 0.11 ملغم/لتر من الروتين والكافيرول والكوارستين بالتتابع في مستخلص ال 80% ايثانول . 0.071 و 0.091 ملغم/لتر من الروتين والكافيرول بالتتابع في المستخلص المائي.
- دراسة تاثير هذه المستخلصات في تثبيط انزيم اليوريز في البكتريا *Klebsiella* فكانت نتائج IC50 = 235.9و 305.5و 247.3 ملغم/لتر لمستخلص 99.9% ايثانول ومستخلص 80%ايثانول والمستخلص المائي بلتتابع وان المثبط القياسي لأنزيم اليوريز (الثايويوريا) اعطى IC50 =138.1ملغم/لتر

نفس المستخلصات استخدمت لتثبيط انزيم اليوريز النقي فكانت نتائج IC50 = 133.9و 169.5و 157.7 ملغم/لتر لمستخلص 99.9% ايثانول ومستخلص

80% ايثانول والمستخلص المائي . وان المثبط القياسي لأنزيم اليوريز (الثايويوريا) اعطى IC50= 137.2 ملغم/لتر

- دراسه العوامل الحركيه للانزيم مع وجود المثبط (مستخلص ال 80 % ايثانول) وبدونه من خلال معادلة Linweaver- Burk. وقد كان نوع التثبيط تنافسي وذلك لثبوت VMAX و تغير KM .

جمـــــــــــــــــــهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعـــــــــــــــــة النهريـــــــن
كليـــــــــــــــــة العـــــــــلوم
قـــــــــــــــــسم الكيميـــــــــاء

مستخلصات حرير الذرة كمثبط للأنزيم اليوريز في بكتريا *Klebsiella* في مرضى حصى الكلية العراقيين

رسالة

مقدمة الى كلية العلوم- جامعة النهرين

وهي جزء من متطلبات نيل درجة الماجستير في علوم الكيمياء

من قبل

رفاه هلال اسماعيل

بكالوريوس علوم كيمياء/كلية العلوم للبنات/جامعة بغداد

(2014)

المشرف

الاستاذ المساعد الدكتور سلمان علي احمد

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