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IN VITRO AND IN VIVO EFFECT OF THREE AQUEOUS PLANT EXTRACT ON PATHOGENICITY OF KLEBSIELLA PNEUMONIA ISOLATED FROM PATIENT WITH URINARY TRACT INFECTION

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ABSTRACT

This study was aimed to evaluate the antibacterial activity of four aqueous plants extract (pomegranate, apple, lemon & orange peel) on the survival and virulence factor of *Klebsiella pneumonia* isolated from urine *in vitro* and *in vivo*. Antimidrobial assay relied on the estimation of the colony forming unit number present the pomegranate was higher effect than a anther plant. when study the hidrophobicity of cells precent plant extract have different effect from very strong hydrophopic (apple , lemon) to hydrophilic (orange) . Bacterial strain loss their ability of hemagglutination in present lemon and apple but orange & pomegranete extracts did not show any alteration of hemagglutination. In curli expression thes found only bacteria incubated in the presence of all concentrations of pomegranete extract and 8000mg/mL of orange& lemon extracts formed white colonies which indicated the loss of curli fimbriae. Other extracts did not inhibit the synthesis of curli fibers. The antihemolytic activities were obtained in the present of pomegranate in 500 mg/ml and orange peels in concentration 1000 mg/ml and higher. Bacteria growing in the presence of apple extracts did not show effect on heamolytic. Bacteria growing in the presence of apple, lemon and pomegranet extracts did not show effect on viscosity in concentration 62.5mg/ml. The histopathological change in mice kidney infect with bacterial suspension only is acute cellular degeneration and degenerated glomeruli and severe degeneration of renaltubules aggregation of inflammatory cells and bleeding in the renal tubule while mice kidney infected with bacterial suspension and pomegranate not observed any histopathological change.

Keyword:- antihemolytic, antihemolytic, hemagglutination.

INTRODUCTION

A urinary tract infection is a bacterial infection that affects any part of urinary tract. In most cases bacteria travel to the urethra and multiply causing kidney infection if not treated (Bethesda, 2005, David *et al*, 2008). Urinary tract bacterial infections are common in women because they have a shorter urethra than men**.** Approximately 50% to 70% of women will have UTI during their lifetimes, and 20% to 30% of women will have recurrent episodes. UTI has become the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections, and it is the second most common cause of bacteraemia in hospitalized patients (Kolawole *et al.*, 2009). Despite the advances in various field of medicine, urinary tract infections are still considered as serious public health problems and inflict a major burden to health care services around the world and especially in developing countries (Hooton *et al*., 2012).

K. pneumoniae is an opportunistic, Gram-negative bacterium responsible for both communal and hospital acquired infections It is a frequent cause of urinary tract infections and pneumonia, and subsequent systemic infections. Mortality rates of nosocomial *K. pneumoniae* bacteremia range from 20%– 50%, with no improvement in outcomes within the last decade (Tumbarello *et al* .,2007 ; Yu *et al* ., 2007 ; Wu *et al.*, 2012). *K. pneumoniae* is known worldwide for its rapid acquisition of resistance to traditional and new antimicrobial drugs, including resistance to third-generation cephalosporins, fluoroquinolones, carbapenem, and extended-spectrum β-lactamases. (Keynan & Rubinstein,2007). It is also involved in surgical site infections, peritonitis, pyogenic liver abscess, thoracic empyema and psoas muscle abscess (Chang *et al.*, 2005; Maltezou *et al.*, 2009).

Development of resistance against antibiotics and antiseptics is a growing cause of concern which has limited the preventive measures. Therefore, there is a containing need to search for new antimicrobial agents, over the last decade, plant antimicrobial activity has been studied in different regions of the world (Sedighinia *et al*., 2012). An antimicrobial is a substance which kills or inhibits the growth of much type of microorganisms such as bacteria, fungi or protozoan's. Antimicrobial drugs either kill or prevent the growth of microbes. The antimicrobial is substances which are used on nonliving objects are disinfectants (Mathur *et al*., 2012). The use of higher plants and their extracts to treat infections is an ancient practice of traditional medicine. Human are using natural products from plants for thousands of years either in the pure forms or crude extracts. About 80% of individuals from developed

countries use traditional medicine, with origin from plants. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are chiefly synthesized during secondary metabolism of the plant (Bakht *et al*,.2011). The use of plants for curing diseases was inevitable as is already proven by seeing the problems associated with synthetic antibiotics. Peels of some plants as *Punica granatum* (having antibacterial properties) which are generally treated as wastes are true antibiotics as they are available for no cost, have no side effects and the most important benefit is that antibiotic resistant pathogens will be easily killed by these new and natural antimicrobials because they will take at least a few decades to get mutated and resistant to them. Pomegranate peel (*Punica granatum)* is a rich source of tannins, flavonoids, polyphenols and some anthocyanins as delphinidins, cyanidins, etc. (Li *et al*., 2006). Antioxidant and antibacterial properties of pomegranate peel in in-vitro model systems have been reported (Opara *et al*., 2009; Alzoreky, 2009). All the compounds of pomegranate peels are reported to have therapeutic properties. Extracts of peels of pomegranate show antibacterial property against bacterial strains
(Hegazy & Ibrahium, 2012).

Citrus fruit (lemon & orange) products act as antimicrobial agents against the bacteria and the fungus. The citrus product has an important and physiological role because of its commercial value in food and pharmaceutical industries of the entire world (Mathur *et al*., 2012). The antioxidant property is presence the plant materials due to many active photochemical which include the vitamins, flavonoids, terpenoids, carotenoids, cumarins, lignin, saponin, plant sterols etc. The Citrus fruits and their juices are an important source of the bioactive methanol, the compound are an important to human nutrition which including the antioxidants such as ascorbic acid, phenolic compounds, flavonoids and pectins (Hegazy & Ibrahium, 2012).

As children, many of us were told to "eat your vegetables because they are good for you", and the adage "an apple a day keeps the doctor away" is still quite popular. Consumption of apples has been linked to the prevention of chronic disease. Apple intake has been negatively associated with lung cancer incidence in two separate studies (Le Marchand *et al*., 2000). Apples are a good source of phenolic compounds Eberhardt *et al* .,2000) .The total extractable phenolic content has been investigated and ranges from 110 to 357 mg/100 g of fresh apple (Podsedek *et al*.,2000 ; Liu *et al*.,2001). Compared to other tree fruits, apples

contain a higher content of bioactive compounds (Leontowicz *et al*., 2007) and phytochemicals in apples seem to be responsible for most of the reported health benefits (Thilakarathna & Rupasinghe, 2012). Quercetin glycosides are the major flavonoids in apples and are exclusively located in apple peel (Boyer&Liu, 2004). Quercetin glycosides are knownas free radical scavengers and chelators of transition metal ions (Kamada *et al*., 2005). Triterpenes are the largest and most widely spread class of secondary metabolites inn plants (Zhang *et al*., 2006), and is another important group of bioactives in apple peel (He & Liu, 2008). Previous studies have demonstrated that triterpenes possess anti-atherogenic properties (Zhang *et al*., 2006). The aim of this study was to evaluate the antibacterial activity of four aqueous plants extract (pomegranate, apple, lemon & orange peel) on the survival and virulence factor (hydrophobicity, viscosity, haemolysin production curli & hemaagglutination) of *Klebsiella pneumonia* isolated from urine *in vitro* and *in vivo*.

MATERIAL & METHOD

Collection of Material

Fresh orange, lemon, pomegranate, apple were collected from the local market in the Hilla city. The fruits were washed well using tap water. The peel is separated, then the pulp of fruits was separated by cutting them into small pieces and peel is also cut into small pieces then it was dried in the oven for a period of 6-7 days, at an ambient temperature of 30˚C. The dried samples were grinded properly using a mortar and pestle and later using a grinder, to obtain the powdered form. The powder of the peels were stored separately in air tight bottles (Mamta & Parminder, 2013)

Preparation of Plant Extracts

Purchased dry peels were ground into powder in an electric blender. 20 g of each peels powder was dissolved in 180 mL of distilled water in a glass bottles, heated to 85 ˚C in a water bath and kept at this temperature with shaking for 8 h. After cooling, the liquid was filtered through the man No. 1 filter paper. The filtrates were then condensed and dried in smaller glass bottles at 37 ˚C for 48 h. Then, the dried extracts were dissolved in distilled water to obtain concentrations ranging from 0.62 to 8000 mg/mL (Dorota *et al*., 2012) .

Bacterial Strain

Klebsiella pneumonia clinical strain was isolated from the urine of patient with urinary tract infection, hospitalized in the Hilla Hospital Education in Hilla city. Cultural, morphological characteristics of isolates were studied, some biochemical tests were performed. The species

affiliation of the examined strain was confirmed using the API-20E test kit. The strain was maintained on Mueller–Hinton agar slopes at 4 ˚C. (Koneman, 2008).

In Vitro Assay the Effect of Plant Extract

1-Antibacterial Activity

The antimicrobial activity of plant extracts was determined as described below. Briefly, the strain was grown overnight, and then bacterial cells were transferred to fresh Mueller–Hinton broth and incubated at 37 ˚C for 30 min. Following incubation, the bacterial cells were centrifuged (4,000 rpm for 20 min) and suspended in phosphate-buffered saline (PBS) to reach the final density 0.5 in McFarland scale. Bacterial suspension and plant extracts were mixed together to obtain following concentrations: 8000, 4000, 2000,1000,500,250,125,62.5 mg/mL of extract in sample. All samples were incubated at 37 ˚C for 24 h, then diluted and cultured on nutrient agar plates. After 24-h incubation at 37 ˚C, the number of colony forming units (c.f.u.) was counted. Control sample contained no plant extracts was taken as 100 % survival (Dorota *et al.*, 2012).

2- Hydrophobicity of Bacterial Cells

Bacterial cells were incubated with plant extracts for 24 h at 37˚C. After incubation, they were washed three times in PBS. After last centrifugation, samples were diluted to obtain final optical density (measured at 470 nm) of 1.0. Untreated bacterial strain was assessed as a control. The salt aggregation test of ammonium sulfate was used. The control and treated suspensions (20 µL) were mixed with a series of dilutions of ammonium sulfate (20µL) ranging from 0 to 3.2 M. The lowest concentration of ammonium sulfate at which bacterial aggregation was visible was determined. Based on the salt aggregation test values, the bacterial cell surface was classified as: > 0.2 M—very strong hydrophobic, 0.4–1.0 M—strong hydrophobic, 1.2–1.6 M—hydrophobic, >1.8 M—hydrophilic (Dorota *et al.*, 2012) .

3- Hemagglutination and Expression of P Fimbriae

Assays were performed on each strain grown overnight with plant extracts. After washing thrice in PBS, the final density of bacterial suspension was adjusted to 0.5 in McFarland scale. P fimbriae expression was confirmed by the hemagglutination of 3 % erythrocytes from human with blood group O in the presence or absence D-mannose (Latham & Stamm , 1984).

4- Curli Expression

Effect of plant extracts on curli expression was assessed according to (Hammar *et al*., 1995). Bacterial suspension was prepared as described in paragraph devoted to hydrophobicity determination. 10 µL of suspension was inoculated onto plate containing triptic soy agar supplemented with congo red (CRI) and the plant extract. Curli-producing *K.pneumonia* bound congo red dye and formed red colonies, whereas curli-negative bacteria formed white colonies. Control culture contained no plant extracts.

5-Hemolytic Activity

Ten microliters of bacterial suspension was spotinoculated onto sheep-blood agar plates contain plant extract. Alpha-hemolysin production was confirmed by the appearance of a hemolysis zone around the spot (Ghenghesh *et al*., 2009). Control culture contained no plant extracts.

6- Modified String Test for Hypermucoviscosity (HMV) Test

A variation of the string test was used to determine the HMV phenotype (Fang *et al*., 2005; Lee *et al*., 2006) *K. pneumoniae* strains were inoculated on BHI plates contain plant extract and incubated overnight at 37˚C. A standard bacteriological loop was used to vertically stretch a mucoviscous string from a single colony. The formation of a mucoid string >5 mm was regarded as a HMV+ phenotype (Fang *et al*., 2005). Control culture contained no plant extracts.

In Vivo Test

1- Bacterial Inoculum Preparation

Bacterial inoculum was prepared from a pure culture according to *K.pneumonia* was grown in nutrient agar at 37˚C for 24 hours under aerobic condition. By using phosphate buffer saline prepare bacterial suspension was adjusted with Macfarland tube No. 5 to a concentration of approximately 5×10^8 CFU/ml (Mohammad *et al*., 2014).

2- Experimental infection of mouse

Six BALB/c mice were 7-8 weeks old, when challenged. Body weight was 25-30 g. They were obtained from animal house, College of science, Babylon University. The mice were housed in an air conditioned room with 12 hour light, 12 hour dark cycle. They were fed a commercial rodent diet with free access to drinking water. Six mice were divided into three groups:

Group one: two mice were injected intraperitoneal (I. P) with (0.7ml) which contain 10^8 cfu from bacterial isolate. Group two: two mice were injected I. P with same dose from the same bacterial isolate but after adding pomogranete peel extract. Group three*:* two mice were injected I.P with phosphate buffer saline as a control (AL-saady 2010).

3- K.Peumonia Infected Animal Isolation From The

Two week after administration of the dose, the mice from each group killed. Blood, kidneys were taken. Blood and homogenized kidney tissues cultured on blood agar and MacConkey agar at 37˚C for 24hr to bacterial cell detected. Growing bacteria were identified by Gram staining, morphology and other biochemical tests (Mohammad *et al*, 2014).

4- **Tissue Preparation**

Histological section preparation of infected animals was achieved according to Mohan (2007) method, which includes Fixation, Dehydration, Clearing, Impregnation, Embedding and blocking, Section cutting and staining.

RESULT

1-Antibacterial Activity of Plant Extracts

Results obtained in the present study showed that the tested plant extracts possessed different antimicrobial activities (Fig. 1). The number of bacterial cells (c.f.u/mL) in control sample as 4.5 * 108. Increased concentrations of plant extracts caused decrease in survival of bacterial cells. The extracts of pomegranate showed the highest bactericidal activity in concentrations of 1000 mg/mL and higher. Very strong reduction of *K. pneumonia* growth was also observed during incubation of bacteria in apple extract concentrations of 4000 mg/mL. Effect of lemon and orange extract was slightly less efficient in comparison with pomegranate and apple Exposure of pomegranate peel extracts inhibited their growth from 56 % (at concentration of 62.5 mg/mL) to 0 % (at concentration of 1000 mg/mL and higher) of the control sample. The percent of survival of bacteria incubated in the presence of apple peel extracts was reduced from 70 % (at concentration of 62.5 mg/mL) to 0 % (at concentration of 4000 mg/mL and higher) of the control. Figure1. Clearly shows that pomegranate peel extract were more effective than apple, lemon and orange peel Orange peel extract had the weakest antimicrobial activity.

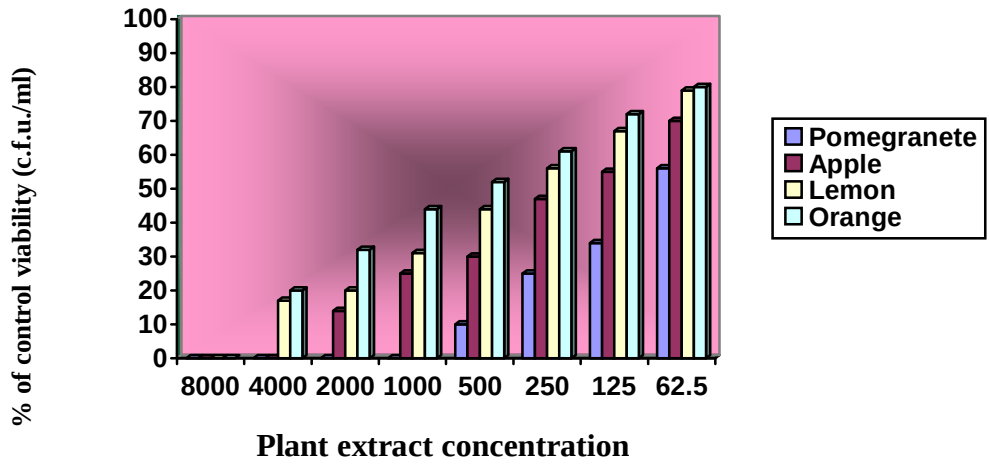

Figure -1- The Percentage of *K.Pneumonia* Survival after Exposure to Plant Extract

2-**Effect of Plant Extracts on Hydrophobicity of Bacterial Cells**

The results showing the effect of plant extracts on cell surface hydrophobicity are shown in Table 1 Surface hydrophobicity of autoaggregating *K. pneumonia* changed after exposure to plant extracts. The cell surface very strong hydrophobicity changes were observed in bacteria treated with apple peel extracts at concentrations of 62.5, 125 and 250 mg/mL and lemon in concentration 62.5 mg/ml. Cells surfaces were classified as very strong hydrophopic because they aggregated in orange peel resulted in bacteria aggregation in the lower concentrations of ammonium sulfate (0.4–0.8 M) indicating strong hydrophobic bacterial cells surface. All concentrations of lemon excepte 62.5 mg/ml 2000, 4000, 1000 mg/ml of orange and 1000, 2000 mg/ml of pompgranat peel extracts showed hydrophobic cells surface, because they aggregated in 1.2–1.6 M ammonium sulfate. The lower concentrations of orange peel extracts resulted in bacteria aggregation in 0.1–0.2 M, hence their cell surface was considered to be hydrophillic.

Table-1- The Effect of Plant Extract on Hydrophobicity of *K. Pneumoniae*

Plant Extract concentration (mg /ml)	Pomegranate	Apple	Lemon	Orange	Control
62.5	strong hydrophopic (0.8)	Very strong hydrophopic (0.1)	Very strong hydrophopic (0.2)	strong hydrophopic (0.4)	autoaggregative
125	strong hydrophopic (0.8)	Very strong hydrophopic (0.2)	Hydrophopic (1.2)	strong hydrophopic (0.4)	autoaggregative
250	strong	Very strong	Hydrophopic	strong	autoaggregative

	hydrophopic (0.8)	hydrophopic (0.2)	(1.4)	hydrophopic (0.8)	
500	strong hydrophopic (0.8)	strong hydrophopic (0.4)	Hydrophopic (1.4)	strong hydrophopic (0.8)	autoaggregative
1000	Nt	strong hydrophopic (0.8)	Hydrophopic (1.4)	strong hydrophopic (0.8)	autoaggregative
2000	Nt	strong hydrophopic (0.8)	Hydrophopic (1.4)	Hydrophopic (1.4)	autoaggregative
4000	Nt	Nt	Hydrophopic (1.6)	Hydrophilic (1.8)	autoaggregative
8000	Nt	Nt	Nt	Nt	autoaggregative

3- Effect of Plant Extracts on Hemagglutination and Expression of P Fimbriae

The effects of plant extracts on hemagglutination and expression of P fimbriae are shown in Table (2) .They depended on the type and concentration of the plant extract used. The lowest extract concentration causing no agglutination was 1000 mg/mL and it was observed in case of lemon peel extract. Bacteria incubated with apple lost their hemagglutination ability at extract concentrations of 2000 mg/mL and higher. Bacteria growing in the presence of orange and pomegranete extracts did not show any alteration of the analyzed properties.

4-Effect of Plant Extracts on Curli Expression

The impact of plant extracts on the occurrence of the curli fibers is shown in Table 3. Only bacteria incubated in the presence of all concentrations of pomegranete extract and 8000mg/mL of orange& lemon extracts formed white colonies which indicated the loss of curli fimbriae. Other extracts did not inhibit the synthesis of curli fibers.

5- Effect of plant extracts on hemolytic activity

The effects of of plant extract on hemolysin production are shown in Tables 2.The antihemolytic activities were obtained in the present of pomegranate in 500 mg/ml and orange peels in concentration 1000 mg/ml and higher. Bacteria growing in the presence of apple extracts did not show effect on heamolytic activity.

Table -2- Effect of Plant Extracts on Hemagglutination And Expression of Fimbriae(P), Curli Expression (C) And Hemolytic Activity(H).

Plant Extract concentration (mg /ml)	pomegranate			apple			lemon			orange			control		
Test	P	C	H	P	C	H	P	C	H	P	C	H	P	C	H
62.5	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
125	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
250	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
500	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
1000	Nt	Nt	Nt	+	+	+	-	+	-	+	+	-	+	+	+
2000	Nt	Nt	Nt	-	+	+	-	+	-	+	+	-	+	+	+
4000	Nt	Nt	Nt	Nt	Nt	Nt	-	-	-	+	+	-	+	+	+
8000	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	+	+	+

5- Test for Hypermucoviscosity (HMV)Test

The effects of plant extracts on Hypermucoviscosity of *K.pneumonia* are shown in Fig (2) they depended on the type and concentration of the plant extract used. Bacteria growing in the presence of apple, lemon and pomegranet extracts did not show effect on viscosity in concentration 62.5mg/ml.

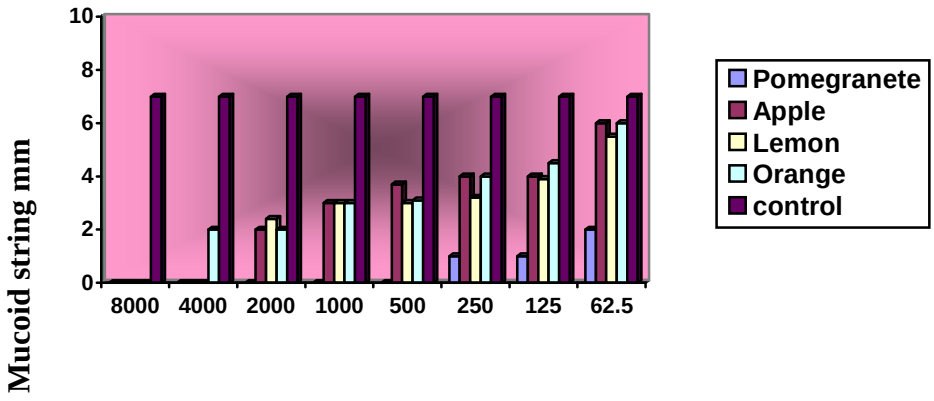

Figure-2- Effect of Plant Extract on Viscosity of K. Pneumonia

In Vivo Assay of Plant Extract

When study the effect of four plant extract on virulence factor of *K. pneumonia in vitro* present the pomegranate is more effect than another type of plant extract (apple , lemon &orange) for this resoene it was chose to study the effect plant extract on K. pneumonia *in vivo* by using laboratory mice . 2 week after infected the mice with bacterial suspension and

bacterial suspension with plant extract obtain the fallowing change in the some mice kidney tissue (Fig-3).

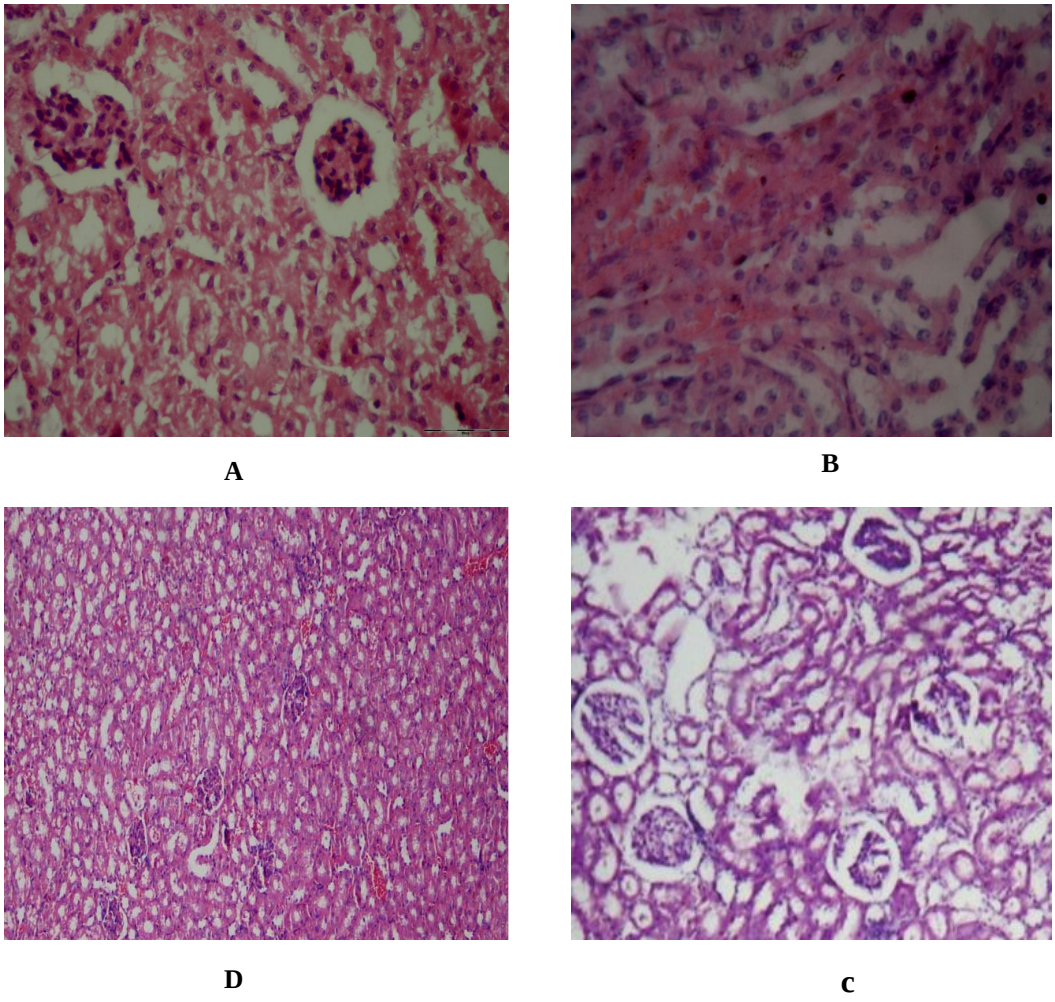

Fig - 3 - Histological section of mouse kidney staining with H & E

1. Injection with bacteria only showing acute cellular degeneration (400X) .
2. Injection with bacteria only showing degenerated glomeruli and severe degeneration of renal tubules (X 400).
3. Injection with bacteria and pomegranate showing no histopathological change (400X).
4. Injection with phosphate buffer saline (control) (100X)

DISCUSSION

The widespread use of commercially available antimicrobials led to the consequence of emergence of antimicrobial resistant pathogens that ultimately led to the threat to global public health. Since 1980 the introduction of new antimicrobials has declined due to the huge expense of developing and testing new drugs. All commercially available antibiotics with prolonged use may have negative effect on human health because they kill gut flora, so

human beings need to take probiotics to replace the killed gut flora. All the above points make a clear way for herbal antimicrobials. The use of plants for treating diseases is as old as the human civilization. There are many plants which have been in use as traditional medicine, so they are called as medicinal plants (Li *et al*., 2006). *K.pneumonia* is one of the most common bacteria capable of causing infection in humans, particularly urinary tract infection (Iroha *et al*.,2009).It is known that in the prevention and treatment of urinary tract infections, one should use medicinal herbs as supplement of the daily diet. Is relationship between the chemical structures of the most abundant compounds in the plant extracts and microbial activity of tested plants (Ejrnaes *et al*., 2011).

Cranberry is one of the most recommended plants by both, doctors and pharmacists due to its properties, this fruit prevents adhesion of pathogenic bacteria to uroepithelial tissue, what results in inhibition of their growth and multiplication. Apart from cranberry; however, many other plants are used in folk medicine to prevent or to treat bacterial infections (Aljamali, 2013). As an example of such plants may serve Pomegranate, apple, lemon & orange peels which are used in traditional medicine. Many reports describe their medical properties (diuretic, diastolic, diaphoretic activities and anti-inflammatory effect) which are caused by their chemical composition typical for each species. Phytochemical investigations have shown that these plants contain the flowing component.

1. The lemon peels (*Citrus limonum*): vitamin C, flavonoids, essential oils, metals: Fe, K, terpens, phenole.
2. The organ peels (*Citrus sinensis*): vitamin A, B1, B2, metals: Fe, Ca, Iodine, Manganese nitrate, stearic acid, essential oils, flavonoids, and terpenes.
3. The pomegranate peels (*Punica granatum*): tannins, fats, alkaloids, metals: K, Fe, phenols.
4. The apple (*Pyrus malus*): vitamin C, sacarides, fibers, metals, phenols **(**Iqbal *et al*., 2010).

According to the results presented in this paper, Pomegranate, apple, lemon & orange peel extracts should also be remembered and added to the list of herbs which can be used in UTIs. Unfortunately, only a few research groups described the antibacterial activity of birch, horsetail, woodruff, rupturewort, nettle and lingonberry extracts (Singh & Sharma, 2012). For this reason, the purpose of our study was to determine the effect of these extracts on bacterial survival *in vitro* & *in vivo*. The findings of the present study clearly indicate that the tested extracts exhibit significant differences in their antimicrobial activities against *K. pneumonia*.

the compounds such as, flavonoids, essential oils, metals, terpens, phenole. stearic acid, essential oils, flavonoids, terpenes. Tannins, fats, alkaloids, The sacarides, phenols **(**Iqbal *et al*., 2010). reported that tannins fractions purified from leaves of some plants show antimicrobial activity against Gram negative strains: *K. pneumoniae Pseudomonas aeruginosa, E. coli, S. typhi*, and *P. mirabilis* (Ho *et al*, 2001). In our experiments. The growth of bacteria was totally inhibited by extracts concentrations ranging from 1000, 2000, 4000 & 8000 mg/mL for pomegranate, 8000, 4000 mg /mL in case of apple & 8000 in case of lemon and orange. It was found that the ethanolic extract of the *Citrus medica L.* peel extract possess a broad spectrum of activity against *K. pneumonia, St.aureus, P. vulgaris, , E. coli* and *B. subtilis, P. aeruginosa* (Kabra *et al*., 2012). (Gülay kirbaşlar *et al.,* 2009) reported that *Citrus sinensis* peel oil showed zone of inhibition of 13mm and 17mm against *E. coli and K. pneumonia* respectively. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body (Akinmoladun *et al.,* 2007). The preliminary phytochemcial investigation revealed the presence of various constituents of citrus peels. The presence of phenol further indicated that *Citrus limon* and *Citrus sinensis* peels could act as antiinflammatory, anticlotting, antioxidant, immune enhancers and hormone modulators. *Citrus lemon* and *Citrus sinensis* peels have high quantity of saponin which has hemolytic activity and cholesterol binding properties Therefore, in addition to their use as drugs, citrus peels can be used as a food preservative or even as food supplement as many literature says that they are highly nutritive (Ashok kumar *et al* .,2011). EL-Farmawi *et al*., 2014 present cinnamon and green tea exhibited the highest antibacterial activity against *K. pneumoniae*.

The limited number of publications describing the influence of plant extracts on bacterial virulence factors prompted us to perform research in this area. The most important pathogenic factors involved hydrophobicity ,heamolycin production , haemoaglutination of red blood cell , viscosity . In our study the changes in the cell surface properties were observed for *K.pneumonia* incubated in pomegranate ,apple, orange and lemon peels extracts rang from very strong hydrophopic to hydrophilic . The authors established that the hydrophobicity of *St.mitis, St.sanguinis* and *Actinomyces sp*. incubated in the presence of Psidium guajava extract was reduced (Razak *et al.*,2006).

The presence of fimbrial adhesins promotes the attachment of the bacterial cells to the host tissues and protects them against removing from the urinary tract with urine We established

that extracts of Garlic extract inhibited erythrocyte hemagglutination by *P. mirabilis* strain, which indicates the dysfunction of P fimbriae. It is known that these plants are rich in tannins—compounds with the structure very similar to receptors found on bladder and kidney cells (Dorota *et al*.,2013). Gupta *et al*., 2012 found that *E. coli* rods growing in the presence of the cranberry juice lost the expression of P fimbriae leading to a loss of the ability to epithelial cells colonization Therefore, these compounds act by binding to fimbriae and thereby preventing their attachment to the host tissue. Curli fibers play an important role in biofilm formation by rods belonging to Enterobacteriaceae family (Zogaj *et al* .,2003) . It has been also shown that the reduction of pili correlated with the loss of the ability of uropathogenic *E. coli* strains to colonize bladder cells and to form biofilm (Aberg & Almqvist, 2007). According to some researchers, recurrent UTIs are caused by microorganisms that invade the urinary tract and form a biofilm structures (Anderson *et al*.,2004). The results of our study indicate that the exposure of *K. pneumonia* to plant extracts significantly reduced or inhibited the biofilm production. Such activity of these plant extracts can be explained by the presence of flavonoids. It is known that flavonoids such as quercetin, kaempherol, naringenin and apigenin reduce biofilm synthesis because they can suppress autoinducer-2 activity which is responsible for cell-to-cell communication (Vikram *et al*.,2010). Lee et al.,2011 The results obtained in our study suggest that anti-biofilm effect of plant extracts can be caused by modifications in the bacterial surface structures responsible for binding to the occupied surface.

In the present study found the plant extract inhibition heamolytic activity this result similar the result obtain from Dorota *et al*.,2013 when study the effect of asiatic & ursolic acids from plant on producing heamolysin by *E.coli*. Bacteria growing in the presence of pomegranate , apple, lemon & orange peels extracts did not show effect on siderophor production. The effects of plant extracts on Hypermucoviscosity of *K.pneumonia* are depended on the type and concentration of the plant extract used. Bacteria growing in the presence of apple , lemon and pomegranet extracts did not show effect on viscosity in concentration 62.5mg/ml. It was belong to the chemical component such as vitamin C, flavonoids, essential oils, terpens, phenole, tannins, fats, alkaloids, metals: K, Fe, present in these plant.

In order to examine the effect of water extracts of pomegranate peel *in vivo* on laboratory mice were used and infected i.p. with 5×108 CFU/mL suspension of *K. pneumoniae* . (Provan *et al*., 2004). present histopathological change in mice kidney infected with *K. pneumoniae*

only but no any effect in mice kidney treated with pomegranate indicated that pomegranate extract inhibition the growth of *K. pneumoniae*.These findings have cleared demonstrated that the clearance of *K. pneumoniae* from the blood of infection mice by water extract was zero, as compared with the infected untreated mice even after 16 days from infection the number was 5×102 cell mL. this result similar the result obtain frome (Ebtehal *et al*., 2010). Furthermore it was more effective than other treatments. The effect of pomegranate peel extract may be due to that is rich in tannin and other components and the antimicrobial activity of tannin is well documented (Abu-shanab *et al*., 2005; Gulmez *et al*., 2006). The ethanolic extract displayed broad spectrum of activity, science G+ and G- bacteria were inhibited with *R. coriaria* extracts (Abu-Shanab *et al*.,2005).

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