Effect of propolis extract in inhibiting the growth of bacteria isolated from UTI in patients Of AL-Hussein Teaching Hospital, In Thi-Qar, city

Dr. Ahmed H. Mohammad

Dept of Pharmacology / coll. Medicine \ Thi-qar university

Email: drahmed.hamza@yahoo.com

Key word : Antibiotic-Resistant, Propolis , urinary tract ,Antimicrobial Activity

Abstract :

Among the most common diseases that afflict the human urinary tract is a bacterial infections (**UTIs)** is one more common of these injuries involve the lower urinary tract, the bladder and urethra , UTIs are much more common in elderly than other individuals for a different reasons, and frequently occur both in community and hospital environments .The study was aimed at determining the causal microbial agents of urinary tract infection UTI infections and the susceptibility of isolates to propolis as a natural antimicrobial substance compared with the antibiotcics used . A total of 25 urine sample was obtained from patients (10 male and 15 female) who were diagnosed with UTI attending AL- Hussein Teaching Hospital ,Thi-Qar city, Samples were collected between June and July 2015, cultured and the isolates were characterized by standard microbiological procedures. Of the 25 samples,10 male had positive cultures *with E.coli having the highest prevalence* followed by 3 *Klebsiella* species and 2 isolates were identified as *Staphylococci* species and 15 female positive results with 7 *E.coli* followed by *Klebsiella* and *Staphylococci* species same, and evaluating of the inhibitory effect of crud propolis against bacterial isolates. Four graduated concentrations were prepared propolis 12.5, 25, 50, and 100 mg/ml and its activity was checked up by agar well diffusion method. The concentration of propolis exhibit proportionality with zone of inhibition of bacterial isolates. The propolis at concentration 50and 100 mg/ml has significant activity in comparison with antibacterial used in this study at ($P<0.001$) for each ,Ceftriaxone 30 µg, Amikacin 30 µg and Gentamycin 10 µg respectively which were exhibited best activity from each other antibacterial which have been used.

تأثير مستخلص العكبر في تثبيط نمو الجراثيم المعزولة من إصابات المسالك البولية في مرضى مستشفى الحسين التعليمي، مدينة ذي قار، العراق

احمد حمزة محمد

كلية الطب /جامعة ذي قار /فرع الادوية

الخلاصة:

من بين الأمراض الأكثر شيوعا التي تصيب المسالك البولية في الانسان هي العدوى البكتيرية (عدوى المسالك البولية) وهو أحد أكثر الامراض شيوعا وتشمل اصابات المسالك البولية السفلى ، والمثانة ومجرى البول، عدوى المسالك البولية هي أكثر شيوعا في كبار السن من الأفراد الآخرين لأسباب مختلفة وكثيرا ما تحدث في كل من البيئات المجتمع والمستشفى . تهدف الدراسة إلى تحديد المسببات الميكروبية لالتهابات المسالك البولية وحساسية العزلات للعكبر باعتباره مادة طبيعية مضادة للميكروبات مقارنة مع المضادات الحيوية المستخدمة. تم الحصول على ١٥ عينة ادرار من المرضى (١٠ ذكور و ١٥ إناث) الذين تم تشخيص التهاب المسالك البولية المترددين على مستشفى الحسين التعليمي في مدينة ذي قار، تم جمع عينات بين يونيو ويوليو ٢٠١٥، زرعت العينات المأخوذة واجري لها الاختبارات المكروبيولوجية القياسية. وكانت النتائج من ٢٥ عينة، كان خمس عزلات من الذكور ايجابية الزرع مع الاشريشيا القولونية وجود أعلى نسبة انتشار لها ، تليها ثلاث عزل من كليبسيلا و عزلتان من الأنواع المكورات العنقودية و اما عزل الاناث فكانت سبعة عزل نتائج إيجابية الزرع للاشريشيا القولونية ، يليه اربعة عزل لكل من الأنواع كليبسيلا و المكورات العنقودية ، وتضمن تقييم تأثير مثبط للعكبر الخام ضد البكتريا المعزولة. حضرت أربعة تراكيز متدرجة من العكبر الخام ١٢.٥، ٢٥، ٥٠، و ١٠٠ ملغ / مل وفحصت فعاليتها ضد البكتريا المعزولة بطريقة الانتشار وتبين ان هناك علاقة طردية بين تراكيز البروبولس وقطر تثبيط النمو ضد انواع البكتريا ،اظهر العكبر عند تركيز(٥٠ و١٠٠) ملغم/مل فعالية معنوية مقارنة مع المضادات البكيريه المستخدمة قيد الدراسة عند مستوى(P <0.001) لكل من ، سيفترياكسون ٣٠ ميكروغرام، الأميكاسين ٣٠ ميكروغرام و الجنتاميسين ١٠ ميكروغرام على التوالي التي أظهرت أفضل تأثير للمضادات البكتيرية المستخدمة في الدراسة

INTRODUCTION

Urinary tract infection (UTI) Considered is one of the most important bacterial infections in different ages which cause acute and chronic morbidity such as hypertension and chronic kidney disease (1). Most of these infections include the, mainly the bladder and the urethra .UTIs are observed in younger individuals lesser than elderly for a variety of reasons and frequently occur in both hospital environments, and community,(2). Urethritis, cystitis, acute pylonephritis, prostatitis, and intra -renal and peri-nephric abscesses are the common types of UTIs [3-4]. Because of their short straight anatomy of the women recorded infection Rate more than three times greater risk for UTI than the men ,and termination of female urethra beneath the labia resulting in colonization by colonic gram-negative bacilli (5). most Symptoms and signs of urinary tract infections may include fever, dysuria, urinary urgency, cloudy, dysuria, and frequency or malodorous urine ,bacteria in the distal urethra and the periurethral flora which causes a Infections are nearly always ascending in origin. These bacteria colonize the perineal area and inhabit the distal gastrointestinal (GI) tract . the pathogens that cause UTI are different (6) ,which include . *Klebsiella* species, *E.coli, P.aeruginosa*, and *Enterococcus* species were the most common bacterial pathogens isolated from the urinary tracts of infected patients (7) . Propolis is considered one of such products that are being tested on pathogens is a natural resinous substance collected by bees from tree exudates and secretions. Its antimicrobial activity has been investigated and inhibitory action , have been widely reported and have a long history . **The aim of the present study** was to investigate the effects of antimicrobial of ethanolic propolis (EEP) against pathogens isolated from patients with UTIs compare with other antibacterial agents .

Materials and methods

propolis:

The material was purchased from a shop selling honey in Thi-Qar city, the material was dried and the ground by an electric grinder. **Antibiotic discs**: (company Merseyside .U.K) n: Amikacin 30 µg ,Ceftriaxone 30 µg , Gentamycin 10 µg.

Culture Media: MacConkey agar, Nutrients, Mueller Hinton agar , blood agar base, E.M.B agar were all Oxoid Products (Oxoid, Bassingtoke, U. K).

Collection of Samples

In total, ٢٥ patients with clinical symptoms of UTI referred to AL- Hussein Teaching Hospital , Thi-Qar. Urine of the them was collected in a sterile tube (4-5 ml) and immediately transported to the laboratory and inoculated firstly into blood and nutrient agar incubated at 37Cº, for 24hrs,after that transfer to selective media. The diagnosis depends on traditional methods (morphological and biochemical test)

Antibiotic susceptibility:

The antibiotic sensitivity of the isolates test was performed using disc diffusion method as described by Clinical and Laboratory Standards Institute (CLSI) (8), colonies taken from overnight growth on selective media which mentioned (24h. at 37°C) were re-suspended in Mueller-Hinton broth . The turbidity of the suspension is adjusted to an equivalent 0.5 McFarland .This suspension was used to inoculate on Mueller-Hinton agar plates. Amikacin 30 µg (AM) ,Ceftriaxone 30 µg (CFN) , Gentamycin 10 µg (GEN) were placed on Mueller Hinton agar and were interpreted after incubation for 24 h at 37°C. The inhibition zones diameters (mm) measured around each disk were interpreted on the basis of guidelines published by the Clinical and Laboratory Standards Institute (9).

Ethanolic Extraction of Propolis:

Propolis which prepared was diluting 25 g crude propolis in 100 ml of 70% ethanol, and extracted at room temperature. After three days the extract was filtered (What man paper) and kept at refrigerator temperature. The obtained ethanol extract of propolis (EEP) was used for antimicrobial tests.

Preparation of Standard Dilution of Propolis.

Dilution of propolis were prepared by using of ethylene glycol as diluents, which considered good solvent and its inactive against microorganisms growth(10), stock solution of propolis has been prepared from this extract (mg/ml) and dilution was done into five final concentration n(100,50,25,12.5 mg/ml) respectively as the same procedure.

Antimicrobial Activity of Propolis

Agar well diffusion method, (11), was used to evaluation the general effect of propolis on the growth of bacterial isolate , 0.3 ml of standardized bacterial stock suspensions (1.5 ×10^8) cell/ml of isolate thoroughly mixed to each 25 ml of sterile Muller Hinton agar for each plate , The agar was left two hours to set and in each of these plates. Three well were then made on the surface of the medium in each plate for each concentration by using sterile stainless steel borer. The wells were filled with 0.1ml of different concentration of propolis (12.5, 25, 50, and 100) mg/ml respectively as well as fill 0.1 of ethylene glycol in one of them wells as control. The plates were incubated microearophilically in 35- 37°C for 18-24 h. The diameters of the inhibitory zones were measured in millimeters.

Result and Discussion:

The study focused on the microbial pathogens of UTI in patients and their sensitivity to propolis. The prevalence of bacteria and isolated from urine of patients in different sex groups was shown in **Table (1)** All analyzed samples (٢٥) had positive cultures. The identified bacterial isolates included five isolate *E. coli ,three Klebsiella* species and two *Staphylococci* species **Figure(1)** of isolates for males , while the bacterial isolates for females were seven isolated *E. coli* , four isolated *Klebsiella* species and four isolated *Staphylococci* species . in present study showing a predominance of females (60%) with UTI that is analogous with those of other reported studies . Host factors such as changes in normal vaginal flora and differences between male and female genitourinary systems in anatomy it is one of the reasons that led elevated incidence of infection among females (12) .

The most common uropathogens in our study were *E. coli* (50%) and *Klebsiella* (٣٠%) in male and 26.6% in female genders , it supports the previous studies indicating that *E. coli* is the principal etiological agent of UTI, of the screened cases (13) . From my point of view the predominance of *E. coli* observed in those patients because direct fecal contamination of urinary tract from the anus especially when common hygiene practices are not followed, such as, hand washing before and after catheterization and keeping the underwear dry.

Sex	*E. coli*	*Klebsiella* species	*Staphylococci* species	Total
Males n(10)	5	3	2	10
Females n(15)	7	4	4	15
Total (25)	12	7	6	25

Table 1:sex distribution of 25 patients suffering from urinary tract infection

In this study, The isolated bacteria showed wide range of differences in their susceptibility pattern to the tested antibiotics as the result indicated, high proportions of the test organisms were sensitive to ceftriaxone to all bacterial isolates (28.12 ± 0.9 ,26.22 ± 0.82 , 24.44 ± 0.61)mm ($p>0.05$) against *Klebsiella* species ,*E coli*, *Staphylococcus* species respectively followed by Gentamicin which more effective against *Klebsiella* species 19.62 ± 0.12mm and then Amikacin 18.35 ± 0.25mm for *Klebsiella* species **Table (2) and Figure(1-A).**This observed actions of ceftriaxone more activity against *Klebsiella* species growth are in agreement with Hwang K P *et al*., 2009 who studied show Ceftriaxone and is stable to beta-lactamases, particularly those produced by Gram-negative bacteria has high potency against all the *Enterobacteriaceae*, . Act as bactericidal against susceptible organisms by inhibiting the third and final stage of bacterial cell wall synthesis by preferentially binding to specific penicillin-binding proteins (PBPs) that are located inside the bacterial cell wall , this drug attack the enzymes responsible for joining glycine and the peptide . The transpeptidases, better known as PBPs, also inhibit the extracytoplamic phase by making the pentapeptide precursor unavailable to the PBPs by binding to acyl-D-alanyl-D-alanine, ultimately leads to cell lysis. Lysis is mediated by bacterial cell wall autolytic enzymes (i.e., autolysins),Because it is that of the third generation of cephalosporine , which considered highest activity against penicillins and cephalosporins which product from gram negative bacteria.

In my study also observed the bacterial isolates good susceptibility to gentamicin and Amikacin in mention concentrations which considered act as bactericidal against this organisms by irreversible binding to the bacterial ribosome and inhibiting protein synthesis. Gentamicin and Amikacin, disrupts protein synthesis, and eventually causes cell death through leakage of essential bacterial constituents and active ag

positive and many Gram-negative organisms and also uses for treatment of urinary tract infections. The results of the current study were agreed with Badaruddin (2007) who stated that some species *E.coli* was predominant isolate from UTI patients and treated with Gentamicin is appearing good quality response against the isolates of Escherichia spp (80%) Klebsiella spp (60%) and Proteus spp. (50%), (15).

The activities of propolis on the bacterial isolates were examined in this study. Statistical analysis showed significant differences ($P<0.05$) between different concentration effect of propolis on bacterial isolates. Propolis was completely active against all isolates since the inhibition zones were ranged from (15-28mm) in diameter. Furthermore, the density of bacterial colony culture were reduced after treated with propolis there were (12.5 , 25, 50, and 100 mg/ml) for (*E. coli*, *Klebsiella* species and , *Staphylococci* species) respectively. but there's no complete effect of propolis on the *Staphylococci* species , *Klebsiella* species and *E. coli* respectively at concentration 12.5 mg/ml **Table (3) and Figure(2-B)**. In the present study, the agar-well diffusion and the disc diffusion methods were used because they have the properties of showing both inhibition and control growth .

This results show, the Propolis is the bee product with the highest antimicrobial activity. Numerous scientific studies confirm The antibacterial activity of propolis, this activity has been demonstrated against both gram Negative and positive both aerobic and anaerobic types reference, Although the composition of propolis differs considerably depending on its botanical origin, all examined types of propolis revealed a strong antibacterial activity (16). Although clarity the inhibitory effect of propolis on Gram-positive bacteria , the activity of bee glue against Gram-negative bacteria is a matter of controversy for example, propolis has shown good activity against Haemophilus influenzae and Moraxella catarrhalis, because of Presence one or some of the propolis constituents caused a significant inhibition of bacterial mobility, besides ion permeability alteration on the inner bacterial membrane that is considered a possible explanation for propolis action mechanism(18).This effect of ethanolic extract of propolis reflects its antibiotic action on bacterial isolates , suggesting its possible use as an alternative control of this infection.

In the current study when observed the result of propolis compare with antibiotic used we showing significant differences ($P<0.05$) in different concentration they were (23.60 ± 0.61 at 50mg/ml and 25.41 ± 0.76 at 100mg/ml)mm for propolis respectively against all *E.coli* isolate , while against *Klebsiella* species which isolate from male and female they were result(21.88 ± 0.36 at 50mg/ml and 24.25 ± 0.54 at 100mg/ml)mm respectively **Table (4)** .and the

results for propolis against all *Staphylococci* species were (23.55 ± 62mm at 50mg/ml, 27.63 ± 0.85 at 100mg/ml)mm , Amikacin 30µg and Gentamycin against *E.coli* 10 µg (17.12 ± 0.18 , 18.42 ±0.22)mm respectively and against *Klebsiella* species they were result (18.35 ± 0.25 and 19.62 ± 0.12)mm respectively. Ceftriaxone 30µg campier propolis we show the result proportional with increase concentration they were (26.22 ± 0.82 against *E. coli ,* 28.12 ± 0.93)mm against *Klebsiella* species and 24.44 ± 0.61mm against *Staphylococci* species while the zone of inhibition for propolis in 50mg/ml and 100mg/ml they were (23.55 ± 62 , 27.63 ± 0.85)mm respectively **Table (4)** ,from this result we observed the isolated bacterial more sensitive to ceftriaxone than gentamycine , amikacine and propolis , this is due to the ceftriaxone very effective aginst gram negative and positive and more penetrable the cell wall of bacteria than other agent ,that agree with Luke,2011 who reported the Ceftriaxone and cefotaxime, are considered to be the drugs of choice for many infections caused by members of the *Enterobacteraciae.* relationship between the activity of propolis against of bacterial isolates and zone of inhibition showed proportionality with the concentration of propolis(19). This may be attributed to increase the inhibitory effect of active ingredients that these have antimicrobial effect especially the flavonoid, phenolic acid, pinocembrin, caffeic acid, cinnamic acid and pinobanksin (20).

	Antibiotics Mean diameter zone (mm)		
Bacterial isolate	**Amikacin 30 µg**	**Ceftriaxone 30 µg**	**Gentamycin 10 µg**
E. coli	17.12 ± 0.18 A	26.22 ± 0.82 B	18.42 ±0.22 C
***Klebsiella* species**	18.35 ± 0.25 A	28.12 ± 0.93 B	19.62 ± 0.12 C
***Staphylococci* species**	15.22 ± 22 A	24.44 ± 0.61B	17.82 ± 0.88C
D.W	0.00 ± 0.00 C	0.00 ± 0.00 C	0.00 ± 0.00 C

Table (2) Mean diameter zone of inhibition of different antibacterial agents against Bacterial isolate.

-The values represent Mean ± SE

-Different capital litter refer to significant differences between concentration horizontally P<0.05

	EthanolicExtraction of Propolis (EEP) Means diameter zone (mm)			
Bacterial isolates	**12.5 mg/ml**	**25 mg/ml**	**50 mg/ml**	**100 mg/ml**
E. coli	**17.31 ± 0.31A**	**19.52 ± 0.31B**	**23.60 ± 0.61C**	**25.41 ± 0.76 D**
***Klebsiella* species**	**17.15 ± 0.15A**	**18.51 ± 0.36A**	**21.88 ± 0.36C**	**24.25 ± 0.54D**
***Staphylococci* species**	**15.56 ± 0.62A**	**17.41 ± 18B**	**23.55 ± 62 C**	**27.63 ± 0.85D**
Ethylene glycol	**0± 0.000A**	**0± 0.00A**	**0± 0.00A**	**0± 0.00A**

Table (3) Diameter zone of inhibition of Propolis for different concentrations against Bacterial isolates

-The values represent Mean ± SE

-Different capital litter refer to significant differences between concentration horizontally P<0.05.

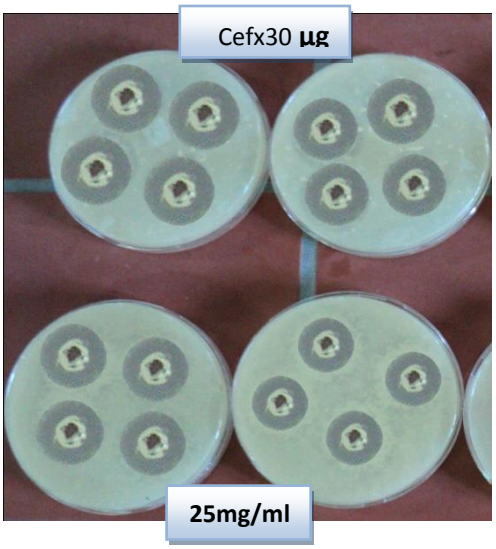
	Means diameter zone (mm) for EEP and other antibacterial				
Bacterial isolates	Ami 30 µg	Cefx 30 µg	Gen 10 µg	EEP 50mg/ml	EEP 100mg/ml
E. coli	17.12 ± 0.18 A	26.22 ± 0.82 B	18.42 ±0.22 C	23.60 ± 0.61 D	25.41 ± 0.76 E
Klebsiella species	18.35 ± 0.25 A	28.12 ± 0.93 B	19.62 ± 0.12 C	21.88 ± 0.36 D	24.25 ± 0.54 E
Staphylococci species	15.22 ± 22 A	24.44 ± 0.61 B	17.82 ± 0.88 C	23.55 ± 62 D	27.63 ± 0.85 E

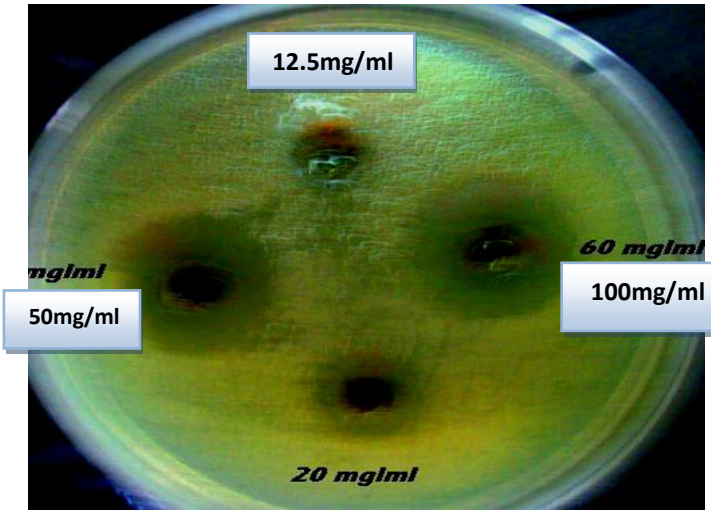
Table (4) Diameter zone of inhibition of Propolis(EEP) and other antibacterial against Bacterial isolates

-The values represent Mean ± SE

-Different capital litter refer to significant differences between concentration horizontally

P<0.05.Ami: Amikacin **, Cefx:** ceftriaxone **,Gen:** gentamycine **,EEP: ethanolic extract of propolis**





Figure(2) A-Sensitivity of *E .coli* to ceftriaxone30µg B- sensitivity *Staph.* Spices to EEP

and gentamycine10µg:

conclusion

We concluded that the ethanolic extract of propolis was relatively effective as antibacterial agent in vitro and its effect was directly proportional with its concentration , This result must be given to them so as to reduce the antibiotic clinical doses and their marked side effects

References:

1- KİREÇCİ,E; Sleman , D.M ; Ahmed, D.Y;Rahman,D.B and Yazdee ,F.S.(2015), Identification of the bacterial types that cause urinary tract infection and antimicrobial susceptibility in Erbil, Iraq , Sky Journal of Microbiology Research. Vol. 3(1), pp. 011 –014.

2- Bendall, M.J;(1984), A review of urinary tract infection in the elderly. J Antimicrob Chemother (13):69–78.

3- Dipiro , J.T; Talbert, R.L; Yee, G;C ; Matzke, G.R; Wells, B.G ; Posey, L.M.(2011) Pharmacotherapy: A Pathphysiologic Approach. 8th edition. New York: McGraw-HILL.

4- Fauci, A.S; Brunwald, E; Kasper, D.L; Longo, D.L; Hauser, S.L. Jameson, J.L;Loscallo, J.(2008). Disorder of the urinary and kidney tract. In Harrison's: Principles of Internal Medicine. 17th edition. Edited by Wiener C. USA: McGraw-Hill Companies; (18).

5- Akinkugbe ,F.M; Familusi, F.B; Akinkugbe, O.(1973). Urinary tract infection in infancy and early childhood. East Afr Med J , 59:514–520.

6- Brkic, S.S ; Mustafic, S ; Nuhbegovic, F; Ljucam and L, Gavran, (2010).Clinical and epidemiology characteristics of urinary tract infections in childhood. Med. Arh., 64: 135-138.

7- Kolawole, A.S; Kolawole, O.M ; Kandaki-Olukemi, Y.T ; Babatunde, S.K; Durowade, K.A; Kolawole, C.F: (2009).Prevalence of urinary tract infections

(UTI) among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria. Int J Med Sci (1):163–167.

8- Wikler, M.A.(2006).Performance standards for antimicrobial susceptibility testing: Sixteenth informational supplement.: Clinical and Laboratory Standards Institute.

9- Charles, O.W.; Oie, G.; Robert, F. (1969): Textbook of organic Medicinal and pharmacological chemistry. 5th ed. Lipincott Philadelphia and torento2.

10- Hwang K P, Tang Y F and Shen Y H (2009), Activity of etrapenem, ciprofloxacin, Ceftriaxone, piperacillin-tazobactum and ampicillin-salbactam against 12 common clinical isolates of community acquired bacteremia", J Microbiol Immunol Infect., Vol. 42, No. 5, pp. 433-438.

11- Perz, C.; Paul, M. and Bazergue, P. (1990): An antibiotic assay by the agar well diffusion method. J Acta Biologiae et Mdicine Experimentalis; 15: 113-1152.

12- Strom, B.L; Collins, M; West ,S.L; Kreisberg, J; Weller, S. (1987). Sexual activity, contraceptive use and other risk factors for symptomatic and asymptomatic bacteriuria; A case-control study. Ann Intern Med 107:816-23.

13- Jha, N; Bapat, S.K (2005). A study of sensitivity and resistance of pathogenic microorganisms causing UTI in Kathmandu valley. Kathmandu Univ Med J 3:123-9.

14- Badaruddin, A.M. (2007). Predominant and common cause of urinary tract infection in Sukkur city. Clin. Infect. Dis., 29: 745-746.

15- BANKOVA, V; POPOVA, M; TRUSHEVA, B (2007) Plant origin of propolis: Latest developments and importance for research and medicinal use, *In* Marghitas, L A; Dezmirean, D (eds) *Apicultura - De la stiinta la agribusiness si apiterapie*, Editura Academic Pres; Cluj Napoca; pp 40-46.

16- Drago, L.; Mombelli, B.; De Vecchi, E.; Fassina, M. C.; Tocalli, L. & Gismondo, M. R. (2000): In vitro antimicrobial activity of propolis dry extract. J Chemother; 12: 390–3952.

17- MIRZOEVA, OK.; Grishanin, RN. and CALDER PC. (1997). Antimicrobial action of propolis and some of its components: the effects of growth, membrane potential and mobility of bacteria. *Microbiological Res.* 152: 239-46.

18- Luke, C. H. (2011) . Studies on *Salmonella enterica* and *Escherichia coli* with a focus on ceftiofur and the genetic resistance determinant blaCMY-2. PhD Dissertation. Graduate Program in Veterinary Preventive Medicine ,Ohio State University , US.

19- Koo, H.M.; Rosalen, P.L.; Cury, J.A.;Park, Y.K.;William,H.and Bowen, H. (2002): Effect of compounds found in propolis on treptococcus mutans growth and on glucosyltransferase Activity. Amr. Soc.for Microbiol; 5: 1302-1309 2.