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"ISOLATION, CHARACTERIZATION AND ANTIMICROBIAL SCREENING OF MDR-UTI PATHOGENS AND THEIR SUSCEPTIBILITY TO SECONDARY PLANT METABOLITES"

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ABSTRACT

Urinary tract infection (UTI) is one of the most common microbial infections affecting all age groups across the life span. The present study was aimed to gain knowledge about the type of pathogens responsible for urinary tract infections and evaluation of antimicrobial activity of various plant extracts against the pathogens. 11 samples were collected and processed. In that samples were confirmed as urinary tract infection. Bacterial species isolated from urine samples were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus sp*., From the UTI sample *Shigella sp*., are also isolated usually produce self-limited gastrointestinal infection that rarely result in extraintestinal complications. Present study showed that E.coli and Staphylococcus aureus isolates were the predominant pathogens. Among the tested sample various bacteria isolated from urine samples (88.4%) were sensitive to the 5 tested antibiotics i.e Vancomycin, Ceftazidine, ampicillin, Chloramphenicol, pyrodoxin. Vancomycin and chloramphenicol showed maximum activity against Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumonia, E.coli. The results also indicated that the most of urinary tract infection diseases were by Gram negative bacteria. Currently, the clinically available treatment is not effective against the antibiotic resistance developed by some bacterial species. However, plant-based antimicrobials have immense potential to bacterial, fungal, protozoal and viral diseases without any known side effects. Such plant metabolites include medicinal plants were selected to evaluate antimicrobial activity of methanol, ethanol, acetone and chloroform extracts against 7 bacterial isolates of major urinary tract pathogens by well diffusion method

and MIC. The acetone extract of *Foeniculumvulgare seeds, Punica granatum, Syzygium aromaticum, zingiber officinales, Psidium Guajava* in which *Foeniculumvulgare* and *Punica granatum* showed maximum activity against *E.coli, Bacillus Cereus, Staphyloccocus*. The main purpose of the study was to identify the pathogens from urinary tract infection patients and detect the effectiveness of selected species of plant extracts against these pathogens.

INTRODUCTION

Urinary tract infection are serious health problems affecting millions of people each year. They are the second most common type of infection in the human body. This problem occurs more often in women than men because woman urethra is shorter. The shorter urethra makes makes it easier for bacteria from the anus or genital area to reach the bladder. Transmission occur in four ways through sexual intercourse, through poor personal hygiene and from mother foetus via placenta. The most common symptoms are burning with urination, fever and having to urinate frequently in absence of vagina discharge and significant pain. The incidence of urinary tract infection by bacteria is problematic. Antibiotics provide the main basis for the therapy of microbial (bacteria and fungal) infections. According to Willey *et al*. (2016), UTIs occurs as a result of interactions between the uropathogen and host and their pathogenesis involves several processes such as attachment to the epithelial surface, colonization and dissemination through the mucosa causing tissue damage. After the initial colonization period, pathogens can ascend into the urinary bladder resulting in symptomatic or asymptomatic bacteriuria and if treated it causes up to 30 percent of mothers to develop pyelonephritis and increases risk of low birth weight and preterm birth.

Since the discovery of antibiotics and their uses as chemotherapeutic agents, there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However over use antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several group of microorganisms (Horbottle and White, 2006). The worldwide emergence of *Escherichia coli, Klebsiella pneumonia,* Haemophilus and many ß-lactamase producers has become a major therapeutic problem. Multi-drug resistant strains of *E.coli* and *K.pneumonia* are widely distributed in hospitals and are increasingly being isolated from community acquired infections. Candida albicans are also nosocomial pathogen, has been reported to account for 50-70% cases of invasive candidiasis (Paula *et al*., 2006). Alarmingly, the incidence of nosocomial candidemia has

risen sharply in the last decade (Kao *et al*., 1999). All this has resulted in severe consequences including increased cost of medicines and mortality of patients.

Many drugs have been introduced for UTI such as Vancomycin, ciprofloxacin, Chloramphenicol, ampicillin etc. but the problem of drug resistance and drug dependence and toxic manifestations of long term use of drugs are common. Unfortunately, decades of antibiotic use has rise to antibiotic resistance. The results shows that *E.coli*, the most common pathogen causing UTIs, has resistance rates of 41.5% to ampicillin, 18.9% to Vancomycin, 7.4% to ceftazidine, and 1.2% to ciprofloxacin . There was limitation to even our best antibiotic family, the fluoroquinolones, which include ciprofloxacin and no Administration of fluoroquinolones to immature animals has cause damage to the developing cartilage, and therefore, these agents are currently contraindicated in children, adolescents, and pregnant or nursing women. Therefore, it would be prudent course of action to investigate alternative treatments for UTIs. Keeping in view these features and the fact that ayurveda has guidelines to preserve positive health and to provide relief from diseases. In ayurveda a large number of drugs have been a long term and well recognized problem. Resistance has been observed in multiple genera including *Escherichia, Enterobacter, staphylococcus, Proteus, Salmonella and Pseudomonas* (Khan *et al*., 2009)

However a wide spectrum of treatment can be ranging from a single dose of antibiotic treatment to rescue nephrectomy for pyonephrosis in diabetic patient with septic shock (Kang *et al*, 2011). According to World Health Organization (WHO) more that 80% world population relies on traditional medicine for their primary health care needs (Vashist and Jindal 2015).

Plant produce a wide variety of secondary metabolites which are used either directly as precursor. It is expected that plant extract showing target site other than those used by antibiotics will be active against drug resistant microbial pathogens. Different plant extract of *Punica granatum, Syzygium aromaticum (clove), Zingiber Officinales, Psidium guajava, Foeniculum vulgare* showed maximum antibacterial activity against some microbes.

Punica granatum commonly known as pomegranate is a member of monogenric family. It is a small tree which measure between 5-8m tall and mainly found in Iran, Himalayan. It can also divided into several anatomical compartments include seed, juice, peel, leaf, flower with each processing intresting pharmacological and toxicological activities.

Zengiber officinales commonly known as ginger is a spice consumed world wide for culinary and medicinal purposes. The plant has number of chemical responsible for its medicinal properties such as anti-inflammatory, antibacterial, antifungal etc.

Psidium guajava is a shrub or a small tree usually growing 1-6m tall but the older stem are covered in smooth, light reddish brown widely cultivated in tropical and subtropical region around the world. It is used in inflammation, diabetes, hypertension, wounds, bacterial infection etc.

Foeniculum vulgare is a medium size to large, biennial to or perennial, stout, glabrous aromatic herb. The plant grows up to 150-180cm in height. fennel require a mild climate and is cultivated as a cold whether crop in parts of north India.

Syzygium aromaticum (Cloves) are the aromatic flower buds of a tree in the family Myrtaceae, *Syzygium aromaticum*. They are native to the Maluku Islands (or Moluccas) in Indonesia, and are commonly used as a spice. The clove tree is an evergreen that grows up to 8–12 m tall, with large leaves and crimson flowers grouped in terminal clusters. it is used in formulas for impotence or clear vaginal discharge from *yang* deficiency, for morning sickness together with ginseng and patchouli, or for vomiting and diarrhea due to spleen and stomach coldne.

MATERIAL AND METHODS

Isolation of clinical sample

7 urine sample were collected from Saurabh Diagnostic laboratory Natraj Kankerkhera Meerut and 4 urine sample from Kailashi hospital Bypass kankerkhera, Meerut. The sample were collected from patient having urinary tract infection.

Maintenance of clinical isolates

The microorganisms isolated from the patient were maintained for further use and tests. So the first and foremost thing even before secreening procedures was maintaining the pure cultures of isolates. The bacterial cultures were maintained on NAM (nutrient agar medium) at 37°C.

2.3 Identification of microorganism

To check morphological characteristics, Gram-staining and motility test performed. To check the growth pattern different media including NAM, MacConkey , Mannitol salt agar, Eosin

methyl blue, Salmonella salt agar were used for biochemical characteristics sugar fermentation (Lactose, Dextrose, Sucrose) IMViC (Indole production, MR- VP, Citrate utilization) Urease, Amylase, Caseinase, Catalase test were performed.

Antibiotic against UTI pathogens

UTI, or urinary tract infection, is a common bacterial infection that can be easily treated wi depends on the bacteria that are causing the infection, the severity of symptoms, the possibility of complications and the ability of the patient to take medicine by mouth. There are several types of antibiotics to treat a UTI. In the of presence of any potential growth, antibiotic sensitivity testing was done by the Modified Kirby- Bauer disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines. The antibiotic strength of 10 antibiotics were observed against the most frequent UTI pathogens cultured. The antibiotics tested for sensitivity were vancomycin (30mg), ampicillin (30mg), amphotericin (150mg), ceftriaxone (25µg), chloramphinical (50µg), Nitrofurantoin (300µg), tetracycline (30mg).

Plant Material

The leaves of *Psidium guajava, Foeniculum vulgare seeds, Zingiber officinales, Syzygium aromaticum and Punica grantum* were used for antimicrobial study.

Preparation of extract

For this purpose shade dried powdered of plant materials were used for extraction with different solvents (ethanol, acetone, methanol) were used as solvent to extract the bioactive compounds of *Psidium guajava, Foeniculum vulgare seeds, Zingiber officinales, Syzygium aromaticum* and *Punica grantum* were used as solvent to extract the bioactive compounds.

Antimicrobial Activity Test

Microorganisms used for antimicrobial activity

Staphylococcus aureus, Escherichia coli, shigella dysentrai, epidermis, enterobacter, Klebsiella.

Determination of the antimicrobial activity of medicinal plants

Agar disc diffusion method

The given plant extracts were tested by disc diffusion method. In this method we spread the 100µL of overnight inoculums of the test microorganism on the surface of a Muller Hinton

agar plate with the help of a glass spreader. After spreading the innoculum was allowed to be absorbed on the surface of agar for 15-20minutes. Then with the help of sterilized forceps disc dipped into standardized concentration of the plant extracts and placed on to the agar plates and incubated at 37°C. Plates were examined for inhibition zone around the disc measured by Hi-antibiotic zone scale.

Determination of Minimal inhibitory concentration (MIC)

The MIC estimated and serial dilutions of broth and various concentrations of herbal extracts were made to 3.0 ml in test tube. Then cultures were added. The test tubes were incubated at 37 C for each type of microbial culture. The lowest concentration of the crude drug that inhibited the growth of microorganisms completely was considered as MIC.

Determination of Minimum Bacterialcidal concentration (MBC)

To determine the MBC (Minimum Bacterialcidal Concentration) the selected plants metabolites i.e. oil of fennel oil, the suspended medium were recultured (from MIC concentration) on the nutrient agar medium. Each sample from microtiter well separately streaked on separate NAM plate and incubates at 37°C for one to two days. After one day observe whether the bacterial growth is positive or negative.

RESULTS AND DISCUSSION

In this study, isolation of different bacterial species from various clinical samples from out patient from Kailashi Hospital, Saurabh diagnostic laboratory Kankerkhera, Meerut. 11 clinical samples were isolated in which 6 samples from female and 5 samples from male. therefore bacterial species isolated and selected for further study.

For further characterization of selected bacteria both at morphological and biochemical level was performed. After identification of bacteria, selected test microorganism viz., E.coli isolate-1, E.coli isolate-2, E.coli isolate-3, E.coli isolate-4, E.coli isolate-5, Staphylococcus isolate-1, Staphylococcus isolate-2, Shigella sp., enterobacter sp., Proteus, Klebsiella. E.coli was present in maximum percentage in the urine samples and minimum percentage proteus.

The selected bacterial sp., were also tested through antibiogram test for antibiotic testing and found resistance against various synthetic antibacterial compounds viz., vancomycin, Ampicillin, Amphotericin, Chloramphenicol, Ceftazidine, Pyrodoxin.

During further antibacterial screening of various plant extracts/ secondary metabolites against MDR-UTI tested pathogens. The seed of *Foeniculum vulgare* and *Punica grantum* was found to be most effective and thus selected for further study and determination of minimum inhibitory concentration of bioactive plant.

To determine the MIC (minimum inhibitory concentration) of the selected plant secondary metabolites, the microtitre plate based broth dilution method was used (Eloff, 1999) with some minor modification.

Antimicrobial activity of different plant extracts against pathogens

Psidium guajava, Foeniculum vulgare seeds, Zingiber officinales, Syzygium aromaticum and Punica grantum showed significant antimicrobial activity against the different pathogens of UTI patients. All the extracts were assessed for their antimicrobial properties by agar well diffusion method and MIC also performed to see the minimum concentration of plant extract which inhibit the growth of pathogens completely. In our study, we have achieved isolation, identification of pathogens from urine samples with help of normal media, differential media, and selective media and biochemical tests. Evaluation of antimicrobial activity also performed with different plant extracts such as acetone extract of plants.

The study implicated 7 microorganisms as possible aetiological agents of UTI cases observed. These organisms were *Escherichia coli, Enterobacter, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus sp., Bacilus cereus.* They are common causative agents of urinary tract infection mentioned by other researchers also. (Meers et al 2010, Kolawole et al, 2011, Mansour et al, 2011). The uropathogens identified in our study are similar to those of many other studies conducted in different countries either in the region or internationally. (Astal et al, 2016). However different results have been reported. The similarities and differences in the type and distribution of uropathogens may results from different environmental conditions and host factors, and practices such as healthcare and education programmes, socioeconomic standards and hygiene practices in each country.

In the present study, most common causative organisms of UTI was *Escherichia coli* (31.25%) followed by *Staphylococcus aureus* (16.66%), *Streptococcus sp.* (10.41%) and least common pathogens in *Staphylococcus epidermidis*. This higher prevalence of *E.coli* may be due to the faecal contamination, the prediction of the organisms from the toilets and the shortness of the female urethra. Other research works also supporting the same result

(Foxman B, 2014). The prevalence of Gram-positive cocci was not high in some studies (Mansour et al, 2016), this is dissimilar to our studies in different countries. *Enterobacteriaceae,* are the commonest organisms isolated from UTI with uncomplicated cases *E.coli* is the most frequent etiological agent causing community and hospital acquired UTIs. Infection of the urinary tract due to *Shigella dysentrai* is an uncommon but well-described complication of modern therapeutics.

In the study, the frequency of UTI was greater in women as compared to men. (68.75%) of the patients were females and (31.25%) were males principally owing to anatomic and physical factors. This is in agreement with other reports which stress that UTI is prevalent in females than in male during youth and adulthood. That conclusion supported by other research works done in international level. (Abu, 2015). In the present research work, the cases of UTI chances examined based on age and gender. It was observed that majority of the positive cases fall between ages 21to 30 years. In the recent years development of multi drug resistance in the pathogenic microorganisms is high.

During this study 4 plants were selected which were used for the treatment of UTI. Undoubtedly the plant kingdom still holds many species of the plant containing substances of medicinal values that are yet to be discovered, though large numbers of plants are constantly being screened for this antimicrobial properties but more pharmacological investigation is necessary.

We found that out of 5 plants extract the maximum zone of inhibition was observed in the acetone extract of *Psidium guajava* against *Shigella dysentrai* (28 mm), *Staphylococcus aureus* (16 mm), *Escherichia coli* (25 mm) respectively.

A urinary tract infection represents one of the most common diseases occurring today. UTI is most serious global health issues in 21st century. Recurrent urinary tract infections (UTIs) present a significant. It is problem for women and a challenge for the doctors who care for them. Correct identification of pathogens from the clinical samples also important.

Antimicrobial susceptibility patterns varied in isolates from different categories. Bacterial pathogens have evolved numerous defence mechanisms against antimicrobial agents; hence resistance to old and newly produced drugs is on the rise.

Table 1: The distribution of UTI patients in relation to their age group.

S. No.	Age group	No. of male	No. of female
1.	1-10	1	0
2.	11-20	0	2
3.	21-30	0	4
4.	31-40	0	0
5.	41-50	1	0
6.	51-60	1	2

Table 2: Percentage of Gram positive and Gram negative Bacteria isolated from UTI Patients.

Bacterial isolates	Colony morphology	Total organism	Total % of UTIs
Gram (-ve)		9	
Escherichia coli	Small, circular, slightly raised, smooth	5	49.8%
Klebsiella pneumoniae	Circular, mucoid convex, small colonies, capsulated	1	12.2%
Shigella dysentriae	Small, circular, smooth, pale pink colony	2	20%
Enterobacter aerogenes	Rod, small, convex	1	12.4%
Gram (+ve)		6	
Streptococcus lactis	Circular with entire margin, often raised with depressed centres	2	21.1%
Bacillus cereus	Opaque, abundant, white waxy growth	2	26.3%
Staphylococcus aureus	Circular, pinkheaded colonies, convex with entire margins	2	22.34%

Table 3: Identification of micro-organisms.

S. No	Culture	Microscopy		Motility	Catalase	Starch	Casinase	Urease	IMVIC				Carbohydrate		
		Shap	Stain						Ind	MR	VP	Cit	Lac	Suc	Dex
1	S_2N_C	Escherichia coli-	rod	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	AG	AG
2	S_4N_b	Escherichia coli-2	rod	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	AG	A
3	S_2P_a	Klebsiella pneumoniae	rod	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve*	+ve	AG	AG
4	S_7N_a	Enterobacter aerogenes	rod	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	AG	AG
5	S_7N_C	Streptococcus lactis-1	coccus	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	A	A
6	S_1P_c	Bacillus cereus-1	rod	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-	A
7	S_5N_b	Staphylococcus aureus-1	rod	+ve	+ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve	A	A
8	S_4P_a	Staphylococcus aureus-2	rod	+ve	+ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve	A	A
9	S_6N_C	Bacillus cereus-2	rod	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-	AG
10	S_8M_C	Streptococcus lactis-2	coccus	+ve	+ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	A	A
11	S_9Ma	Escherichia coli-3	Rod	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	AG	AG
12	S_6N_b	Escherichiacoli-4	rod	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	AG	AG
13	S_8N_a	Shigella dysentriae-1	rod	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-	A
14	S_8N_b	Escherichia coli-5	rod	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	AG	AG
15	S_8M_a	Shigella dysentriae-2	rod	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	-	A

Table 4: Antimicrobial screening of plant extract against clinical isolates.

S. no.	Name of plant	Zone of inhibition (in mm)										
		E.coli-1	E.coli-2	E.coli-3	E.coli-4	E.coli-5	S.aureus-1	S.aureus-2	B. cereus	E. aerogenes	Shigella dy-1	Shigella dy-2
1.	Punica grantum	20mm	18mm	15mm	18mm	10mm	22mm	20mm	18mm	12mm	16mm	15mm
2.	Syzygium aromaticum	11mm	8mm	20mm	10mm	12mm	17mm	15mm	13mm	13mm	16mm	10mm
3.	Zingiber officinales	15mm	12mm	14mm	20mm	20mm	12mm	12mm	---	5mm	---	----
4.	Psidium guajava	18mm	17mm	20mm	28mm	22mm	---	17mm	16mm	14mm	25mm	28mm
5.	Foeniculum vulgare	25mm	20mm	18mm	23mm	15mm	25mm	20mm	19mm	15mm	20mm	18mm

CONCLUSION

Drink plenty of water daily, wipe from front to back to prevent bacterial around the anus from entering the vagina or urethra, avoid smoking, clean genital area before sexual intercourse, avoid using feminine hygiene sprays and scented douches which may irritate urethra Antibiotics for 1-2 days, longer treatment needed by patients infected with Mycoplasma which is treated with ampicillin, kidney infections requires several weeks of antibiotic treatment, and heating pads may help in some cases.

The leaves of *Psidium guajava, Punica grantum* and *foeniculum vulgare* was found most effective against isolated multi-drug resistance strains of UTI causing pathogens viz., *E.coli isolate-1, E.coli isolate-2, E.coli isolate-3, E.coli isolate-4, E.coli isolate-5, Klebsilla pneumonia, shigella dysentrai, staphylococcus, Enterobacter* respectively.

To determine the MIC (minimum inhibitory concentration) of the selected plant secondary metabolites, the microtitre plate based broth dilution method was used (Eloff, 1999) with some minor modification. The inhibitory concentration of the *foeniculum vulgare* found $1.5625x\ 10^{-6}$, $3.125x10^{-6}$, $1.5625x10^{-6}$, $6.25x10^{-4}$, $3.125x10^{-5}$ (mg/ml) against *E.coli isolate-1, E.coli isolate-2, E.coli isolate-3, E.coli isolate-4, E.coli isolate-5, Klebsilla pneumonia, Shigella dysentrai, Staphylococcus* respectively.

To determine the MBC (Minimum Bactericidal Concentration) of the selected plant metabolites, the suspended medium were recultured (from MIC concentration) on the nutrient agar medium. The minimum bactericidal concentration of the oil was found $3.125x10^{-5}$, $3.125x10^{-5}$, $6.25x10^{-4}$, $12.5x10^{-3}$ and $12.5x10^{-5}$ (mg/ml) against *E.coli isolate-1, E.coli isolate-2, E.coli isolate-3, E.coli isolate-4, E.coli isolate-5, Klebsilla pneumonia, Shigella dysentrai, Staphylococcus, Enterobacter* respectively.

REFERENCES

1. A.O.A.C. Official methods of recommended practices of American oil chemistry society. 4thed, 1995; 2.
2. A.O.A.C., Association of Official Analytical Chemists. Official Methods of Analysis of the Association of Official Analytical Chemists. 18thed. Washington, DC, USA, 2005; 3.
3. Abed, KF. Antimicrobial activity of essential oils of some medicinal plants from Saudi Arabia. Saudi J. Biol. Sci., 2007; 14(1): 53-60.

4. Abubakar, M.C., Ukwuani, A.N. and Shehu, R. A. Phytochemical screening and Antibacterial activity of Tamarindus indica pulp extract. Asian J. Biochem, 2008; 3(2): 134-138.
5. Adebolu T and Oladirneji S. Antimicrobial activity of leaf extracts of Punica grantum on isolated diarrhea causing bacteria in south western Nigeria. African Journal Biotechnol, 2005; 4: 682-684.
6. Ahmad I, Mehmood Z, Mohammad F Screening of some Indian medicinal plants for their antimicrobial properties. J.Ethnopharmacol, 1998; 62: 183-189.
7. Akintobi, OA, Onoh, CC, Ogele, JO, Idowu, AA, Ojo, OV and Okonko, IO, 2013
8. Antimicrobial Activity of Zingiber Officinale (Ginger) Extract against Some Selected Pathogenic Bacteria. Nature and Science, 11(1): 6.
9. Antimicrobial Activity of Zingiber Officinale (Ginger) Extract against Some Selected Pathogenic Bacteria. Nature and Science, 11(1).
10. Bayati, FA. Synergistic antibacterial activity between Thymus vulgaris and Pimpinella anisum essential oils and ethanol extracts. JEthnopharmacol, 2008; 116(3): 403-406.
11. Bayati, FA. Synergistic antibacterial activity between Thymus vulgaris and Pimpinella anisum essential oils and ethanol extracts. JEthnopharmacol, 2008; 116(3): 403-40.
12. Bingeman, 2016B.A.H. Bingeman Evaluating normative epistemic frameworks in medicine: EBM and causistic medicine J. Eval. Clin. Pract, 2016; 22: 490-495.
13. Boldsen and Milner, J.L. Boldsen, G.R. MilnerAn epidemiological approach to palaeopathology A.L. Grauer (Ed.), A Companion to Palaeopathology, Blackwell, Oxford, 2012; 114-132.
14. Bowen, J.L. BowenEducational strategies to promote clinical diagnostic reasoning, 2006; 2217-2225.
15. Braga LC, Leite AAM, Xavier KGS, Takahashi JA, Bemquerer MP, Chartone SE, Nascimento AMA Synergic interaction between pomegranate extracts and antibiotics against staphylococcus aureus Can.J. Microbiol, 2005; 51: 541-547.
16. Coates A, Hu Y, Bax R, Page C The future challenges facing the development of new antimicrobial drugs. Nat Rev. Drug Discov, 2002; 1: 895-9 10.
17. Collee JG, Duguid JP, Fraster AG and Marimion BP Laboratory strategies in medical microbiology, 1996; 14: 53-56.
18. Darout I, Cristy A, Skaug N and Egeberg P Identification and quantification of some potentially antimicrobial anionic components in miswak extracts. Indian Journal Pharm, 2000; 32: 11-14.

19. Demirci, Baser HC, Calis IE, Gokhan. Essential oil and antimicrobial evaluation of the Pistacia eurycarpa. Chemistry of Natural Compounds, 2001; 37(4): 332-335.
20. K.abu and Bonjara M.L analysis and antimicrobial activity of the essential oil of Syzigium aromaticum (clove). African Journal of Microbiology Research, 2009; 2: 162.
21. Kareemi, K.K. Inhibitory Effect of Parsley (Petroselinum crispum Juiceagainst Some Urinary Pathogens in-vitro. The Iraqi Postgraduate Medical J., 2012; 11: 3-10.
22. Kareemi, K.K. Inhibitory Effect of Parsley (Petroselinum crispum Juiceagainst Some Urinary Pathogens in-vitro. The Iraqi Postgraduate Medical J., 2012; 11: 3-10.
23. Sharif, F.M. Urinary Tract Infection with Escherichia coli and Antibacterial Activity of Some Plant Extracts. Inter. J. Microbiol. Res., 2011; 2(1): 1-7.
24. Sharif, F.M. Urinary Tract Infection with Escherichia coli and Antibacterial Activity of Some Plant Extracts. Inter. J. Microbiol. Res., 2011; 2(1): 1-7.
25. Esienberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van Rampay M, Kessler RC. Trends in alternative medicine use in the United States, 1990-1997: results of follow-up national survey. JAMA, 1998 Nov 11; 280(18): 1569-75.
26. Eloff A sensitive and quick microplate method to determine the minimum inhibition concentration of plant extracts for bacteria, Plant extracts for bacteria, Plant Med, 1998; 711-713.