

Antibacterial Activity Of *Zingiber officinale* (Ginger) Against Clinical Bacterial Isolates

Abstract

Aims: The objective of the present study was to investigate the phytochemical constituents and antibacterial activity of ginger extracts against some pathogenic bacteria responsible for Urinary tract infection.

Study design: A total of 35 samples were collected from patients with UTIs and wound infections.

Place and Duration of Study: The study was conducted at 2 hospitals in Baghdad from 1/7/2017 to 1/9/2017

Methodology: The urine sample was collected using a sterile container, while a swap from the infected wound was also taken. The classical methods for diagnosis pathogenic bacteria in urine and wound are based on culture on different microbiological media including. Blood agar, nutrient agar, then incubated at 37°C for 24 hrs. The diagnostic procedures consisted of direct microscopy observation, Gram staining, Biochemical tests, Catalase and coagulase tests.

Results: Results show that 30.55%, 38.8%, 19.46% and 11.11% isolates gave typical morphological characteristics and biochemical test for *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* respectively. Antibiotic susceptibility test reveals that *Escherichia coli* isolates were 100% sensitive to gentamicin, tetracycline, streptomycine. *Pseudomonas aeruginosa* isolates reveal that 100% of them were sensitive to gentamicin, Imipenem, ampicillin and streptomycine. *Staphylococcus aureus* isolates reveal that 100% of them were sensitive to Gentamycine, tetracycline and streptomycine. *Klebsiella pneumoniae* isolates reveal that 100% of them were sensitive to nitrofurantoin and Imipenem. Ginger roots extract at high concentration (250,500 mg/ml) have strong antibacterial activity against pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*).

Conclusion:

This study has shown that ginger extracts possess medicinal properties, antibacterial activity and that the inhibition of bacterial growth was dose dependent. The results of the present study show that ginger extracts are more effective against all tested bacterial strains. The results of present study have provided the justification for therapeutic potential of ginger and also used as dietary supplement for food preservation.

Introduction

Ginger (*Zingiber officinale*), Roscoe belongs to the Family Zingiberaceae. It is a perennial herb with thick tuberous rhizomes. The erect leafy aerial stem grows up to approximately 1 meter in height and has purple flowers (Ali *et al.*, 2008). Its roots are used as a spice in cooking throughout the world. The ginger plant has a long history of cultivation known to originate in China and then spread to India, South East Asia, West Africa and the Caribbean. Ginger contains up to 3% of essential oil that causes the fragrance of the spice. The main constituent is zingiberene. Other components include β -sesquiphellandrene (O'Hara *et al.*, 1998).

Antibiotic resistance has limited ability of physicians to treat some infectious diseases. Incidence and spread of drug resistance in major infectious diseases lead to use a plant extract. The water extract of ginger roots has direct anti-microbial activity, thus can be used as a source of medicines for the treatment of various diseases including parasitic diseases, neurological and respiratory diseases, headache and UTI, burns wound infections and many other diseases (Afshar, 2007).

Urinary tract infection (UTI) also known as acute cystitis when infection affects the lower part of the urinary tract and when it affects the upper urinary tract it is known as pyelonephritis. Urinary tract infections caused by different pathogenic bacteria occur in common. Rarely it may be due to viral or fungal infections (Gupta *et al.*, 2010).

Wounds infection have been a recognized as the most critical problem especially in the presence of foreign materials that increases the risk of serious infection even with a relatively small bacterial number (Farrag *et al.*, 2016).

Material and Methods

Isolation of bacteria

For isolation of bacteria causes UTI infection and wound infection, (35) samples were collected from patients with UTIs and wound infections from (2) hospital in Baghdad from 1/7/2017 to 1/9/2017.

The urine sample was collected using a sterile container, while a swap from the infected wound was also taken. The classical methods for diagnosis pathogenic bacteria in urine and wound are based on culture on different microbiological media including. Blood agar, nutrient agar, then incubated at 37°C for 24 hrs.

For further diagnosis of gram-negative bacteria *E. coli* and *Klebsiella*, *Pseudomonas aeruginosa*, samples were cultured on MacConkey Agar, also selected isolated colonies were further streaked on EMB agar plates for diagnosis of *E. coli*, also non –lactose fermenting colonies on MacConkey agar were streaked on King A, King B medium for isolation of *Pseudomonas aeruginosa*. For isolation of gram-positive *Staphylococcus spp* in samples, selected colonies from blood agar were subcultured on Mannitol salt agar and incubated at 37°C for 24 hrs.

Identification of bacteria

The diagnostic procedures consisted of direct microscopy observation, Gram staining, Biochemical tests, Catalase and coagulase tests.

A. Gram stain

Colonies that were able to grow on the selective media were further identified by studying their morphological characteristics (Gram stain, shape, spore formation).

B-Identification of Gram-negative bacteria by API 20E system

Gram-negative bacteria were identified by sub-culturing pure colonies from MacConkey Agar plates on the API 20E microtubes system. This system contains 20 standard biochemical tests. Each gallery contains 20 microtubes. 5ml of tap water were put into the incubation tray. A single colony from plating medium was transferred, and suspended in 5ml of saline and mixed thoroughly. With a sterile Pasteur pipette, the twenty microtubes were inoculated according to the manufactures instruction, both the tubes and couple section of CIT, VP and GEL microtubes were filled to the end of the tube, also couple section of the ADH, LDC, ODC, H₂S and URE microtubes were completely filled with sterile mineral oil. After inoculation, the plastic lid was placed on the tray and the galleries were incubated for 18-24 hours at 37°C.

Identification of the isolates using the analytical profile index (Numerical Coding) for rapid identification of species that were done as supplied by the manufacturer and the identification is determined.

C-Identification of *Staph aureus*

A selective isolated colony from blood agar were further identified by Catalase test and Coagulase test

Antimicrobial susceptibility Test

All aspects of this procedure were standardized according to NCCLS to ensure consistent and accurate results.

A- Preparation of bacterial samples

Inoculum from test bacteria (*Klebsiella pneumoniae*, *E.coli*, *Pseudomonas* and *Staphylococcus aureus*) were cultured in a broth media and incubated at 37°C for 24hrs, then a broth culture was diluted to match

a 10^5 McFarland turbidity standard, which is roughly equivalent to 150 million cells/ mL.

B- Incubation Procedure

Media used in this test was Mueller-Hinton agar, poured into Petri dishes at only 4 mm depth. The pH level of the agar must be between 7.2. Using an aseptic technique, place a sterile swab into the broth culture of a specific organism and then gently remove the excess liquid. Using the swab, streak on the Mueller-Hinton agar plate to form a bacterial lawn. To obtain uniform growth, streak the plate with the swab in one direction, rotate the plate 90° and streak the plate again in other direction. Repeat this rotation 3 times. Allow the plate to dry for approximately 5 minutes, then used a flame-sterilized forceps and gently press each disc containing specific antibiotics to the agar to ensure that the disc is attached to the agar and incubated overnight at 37°C before reading the results.

Preparation of aqueous extract of ginger roots

Dry roots of ginger were obtained from the market and grained to powder for preparation of an aqueous extract of ginger by mixing 10.0gm of dry powder with 100ml of boiled sterile distilled water in a 250ml sterile conical flask with occasional shaking at 37°C for 24hrs. The extract was filtered through a gauze for coarse residue and finally filtered through Whatman filter paper No.1. The filtrates were then concentrated by using rotavapor, then filtered through a Millipore filter (0.45)mm and (0.22) mm, then stored in universal bottles at 4°C in the refrigerator prior to use.

Detection of antibacterial Susceptibility test for aqueous extract of ginger roots

Antibacterial Susceptibility testing for an aqueous extract of ginger roots was done by using well diffusion method. Sterile Muller Hinton agar plates were prepared and autoclaving at 121°C for 15 minutes. After solidification, with Pasteur pipette 3 wells were made in which the centre well was used to put the negative control (normal saline), while others wells were used to put the ginger roots water extract, then 0.2 ml of inoculum suspension was inoculated with micropipette and spread uniformly with a sterile cotton swab over agar surface, the inoculum was allowed to dry for 5 minutes. 0.1 ml of different concentrations of aqueous extracts (250,500) mg/ml were loaded in individual wells, while 0.1 ml of normal saline were used as negative control in the centre well. Plates were observed after 24hrs of incubation at 37°C for the appearance of zones of inhibition around the wells. Antibacterial activity was evaluated by measuring the diameter of zones of inhibition in(millimetres) of bacterial growth.

Results and discussion

Isolation and Identification of *Enteropathogenic Escherichia coli*, *Klebsiella pneumoniae*, *staph aureus* and *pseudomonas aeruginosa*.

Results showed that among the total of (35) samples that were collected, only (11) isolate (30.55%) were gave typical morphological characteristics and biochemical test that related to *Escherichia coli* while the (14) isolates (38.8%) gave typical morphological characteristics and biochemical test that related to *Pseudomonas aeruginosa* and (7) isolates (19.46%) were related to *Klebsiellapneumoniae*, while the rest (4) isolates (11.11%) related to *Staphylococcus aureus*.

Diagnosis of *Escherichia coli* is depended upon isolation and laboratory identification of the bacterium. The samples were streaked on MacConkey and EMB agar and incubated at 37°C for 24 hrs. On

MacConkey agar, lactose fermented colonies are produced, as the organism is lactose-positive, and fermentation of this sugar will cause the medium's pH to become acid. Growth on EMB agar produces colonies with a greenish metallic sheen. Also, *Klebsiella pneumoniae* were given large, mucoidness, lactose fermented colonies on MacConkey agar. Further diagnosis of *Pseudomonas aeruginosa* loopful of non-fermented colonies was streaked further on selective medium (King A, King B) agar plates and incubated at 37°C for 24 hours. Bacteria form blue-green pyocyanin pigment on King A, also produced pyoverdine pigments on King B media that Fluorescence under ultraviolet light.

Gram-negative bacteria revealed as the rod or Slenders shaped, pink coloured that arranged in single or in pairs, while *Staphylococcus aureus* appears as gram-positive, blue grape-like clusters.

In API 20E system, *Escherichia coli* colonies give negative results in Arginine Dihydrolase (ADH), Citrate utilization (CIT), H₂S production, urease production (URE), Tryptophane deaminase (TDA), Voges Proskauer (VP), Gelatin liquefaction (GEL). Isolates were given positive results in Indole production (IND), B-lactamase test (ONPG), lysine decarboxylase (LDC), ornithine decarboxylase (ODC),) Glucose, mannitol, Sorbitol, rhaminose, melibiose fermentation but give negative results in sucrose, inositol, amylase fermentation.

pseudomonas aeruginosa colonies were identified by API 20E system. Its give positive results in Arginine Dihydrolase (ADH), Citrate utilization (CIT), Arabinose fermentation (ARA). It gives variable results in Gelatin liquefaction (GEL). Strains were gave negative results in B-lactamase test (ONPG), Lysine Decarboxylase (LDC), ornithine Decarboxylase (ODC), H₂S production, urease production (URE), Indole production (IND), Tryptophane Deaminase (TDA), Voges-Proskauer (VP),

Mannitol, inositol, Sorbitol, Rhamnose, sucrose, Melibiose, Amygdaline, Glucose Fermentation. *Klebsiella* also results were in agreement with those mentioned by Warren *et al* ., (2000). *Staphylococcus aureus* were given positive results for coagulase and catalase enzymes.

Antibiotic susceptibility test

Results of Antibiotic susceptibility test for pathogenic bacteria isolated from UTI and wound infections reveals that *Escherichia coli* isolates were 100% sensitive to gentamycin, tetracycline, streptomycin, also 50% of them were sensitive to imipenem and trimethoprim as shown in the table (1). Results also reveal that 100% of isolates were resistant to ampicillin, Aztreonam, and nitrofurantoin.

Results of Antibiotic susceptibility test for *Pseudomonas aeruginosa* isolates reveals that 100% of them were sensitive to gentamycin, Imipenem, ampicillin and streptomycin. Results also reveal that 100% of isolates were resistant tetracycline, nitrofurantoin, Aztreonam, and Trimethoprim as shown in the table (1).

Results of Antibiotic susceptibility test for *staph aureus* isolates reveals that 100% of them were sensitive to gentamycin, tetracycline and streptomycin also 50% of them were sensitive to Imipenem, and nitrofurantoin. Results also reveal that 100% of isolates were resistant to ampicillin, Trimethoprim and Aztreonam.

Results of Antibiotic susceptibility test for *Klebsiella pneumoniae* isolates reveals that 100% of them were sensitive to nitrofurantoin and Imipenem also 50% of them were sensitive to ampicillin, Aztreonam, and streptomycin. Results also reveal that 100% of isolates were resistant to Trimethoprim, Gentamycin, and tetracycline.

Table(1): Antimicrobial susceptibility test for *E.coli*,*Klebsiella Pneumoniae*,*pseudomonas aeruginosa* .and *Staph aureus*isolates.

Bacteria	CN 10mg/m	TE 10mg/ml	F 100/ mg ml	IPM 10mg/ml	ATM 30mg/ml	TMP 10mg/ml	AM 25 mg/ml	S 25 mg/ml
E1	I	S	R	I	R	I	R	S
E2	S	S	R	R	R	R	R	I
PS1	S	R	R	S	R	R	S	I
PS2	I	R	R	S	R	R	S	S
SA1	S	S	I	R	R	R	R	S
SA2	S	I	R	S	R	R	R	S
K1	R	R	I	S	R	R	R	R
K2	R	R	S	I	I	R	I	I

NO. number ;PS. *Pseudomonas*;SA. *Staph aureus*;E. *Escherichia coli*, S. sensitive,R.resistant,I.intermediate.

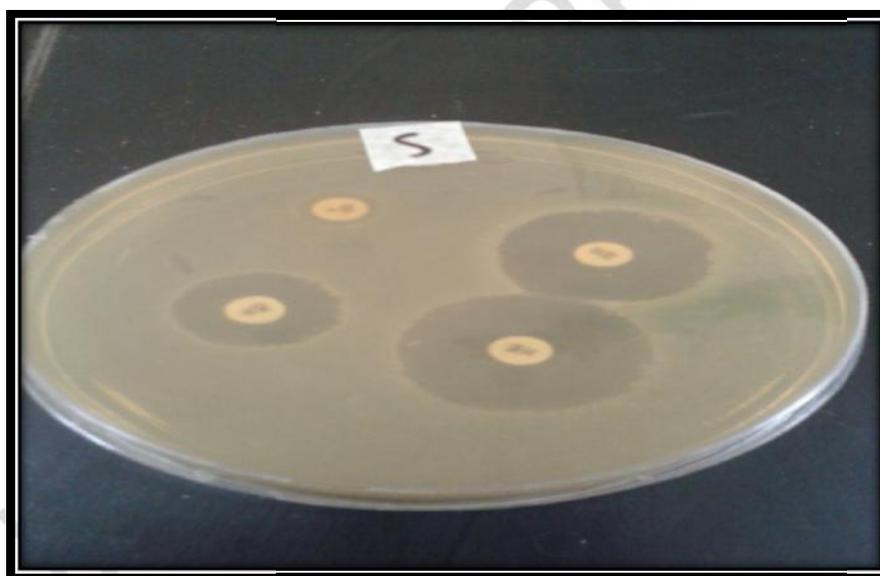


Plate (1): Antibiotic susceptibility test for *Staph aureus* isolate.

In another survey, a total of 17 *Escherichia coli* isolates were collected from urine specimens of patients with urinary tract infection. Antibiotics sensitivity test indicated that amikacin followed by chloramphenicol and ciprofloxacin are the most effective antibiotics (Lane&Akhar, 2011).

The susceptibility studies showed 37% *E. coli* strains isolated from urine samples were resistant to amoxicillin+clavulanate, 33% to cotrimoxazole and 22% to ciprofloxacin. 30% strains were resistant to cefuroxime but only (1%) to fosfomycin. Also, 13% of *Staphylococcus aureus* isolated from urine samples, were methicillin-resistant (Rosa *et al.*,2001). Other studies have shown higher resistance to ampicillin(86%)for staph *spp* isolated from UTI infections (Andrade & Gales,2006).

Antibiotic-resistant *E. coli* may also pass on the genes responsible for antibiotic resistance to other species of bacteria, such as *Staphylococcus aureus*, through a process called horizontal gene transfer. *E. coli* bacteria often carry multiple drug-resistance plasmids, and under stress, readily transfer those plasmids to other species. Indeed, *E. coli* is a frequent member of biofilms(Hotton, T. M. (2000). Thus, *E. coli* and the other enterobacteria are important reservoirs of transferable antibiotic resistance (Hanselman *et al.*,2009).

Only a few antibiotics were effective against *Pseudomonas aeruginosa* isolated from UTI infections including fluoroquinolones, gentamicin and imipenem, and even these antibiotics are not effective against all strains (Nadia *et al.*,2004). The low susceptibility to antibiotics is attributable to a concerted action of multidrug efflux pumps with chromosomally encoded antibiotic resistance genes (*mexAB*, *mexXY*) and the low permeability of the bacterial cellular envelopes. Moreover, *Pseudomonas* maintains antibiotic resistance plasmids, both R-factors and RTFs (Ravichandra *et al.*, 2012).

In addition to this intrinsic resistance, *P. aeruginosa* easily develops acquired resistance either by a mutation in chromosomally-encoded genes

or by the horizontal gene transfer of antibiotic resistance determinants(Rahual *et al.*,2009).

Study the antibacterial activity of gingerroots water extract against pathogenic bacteria isolated from UTI and wound infection

Detection antibacterial activity of ginger roots water extract against different pathogenic bacteria isolated from patients with UTI and wound infection was done by using well diffusion methods and different concentration of ginger water extract (250,500) mg/ml was used. Results reveals that ginger roots water extract at different concentrations have strong antibacterial activity against pathogenic bacteria (*Staphylococcus aureus*, *E.coli* and *Klebsiella pneumoniae* isolated from UTI and wound infections in which the diameter of zone of inhibition was increased respectively with the increased concentration of ginger water extract that ranged from (12-26)mm for *Klebsiella pneumoniae* as shown in Plate (2)and from (14-25) mm for *Staphylococcus aureus* and from (15-19) mm for *E.coli*. While the ginger water extract at two concentrations (250,500)mg/ml have no antibacterial activity against pseudomonas isolates. Also, PBS has no antibacterial activity against all isolates as shown in the table (2).

Table(2): Antimicrobial activity of ginger roots on the growth of *E.coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* isolates.

Bacteria <i>Spp</i>	Conc. Of ginger roots water extract		PBS
	250 Mg/ml	500 Mg/ml	
St1	14	25	0
St2	15	18	0
K1	23	26	0
K2	12	23	0
E1	15	17	0
E2	16	19	0
PS1	0	0	0
PS2	0	0	0

St: Staph aureus ,K: Klebsiella pneumoniae,E1: Escherichia coli, PS: Pseudomonas aeruginosa

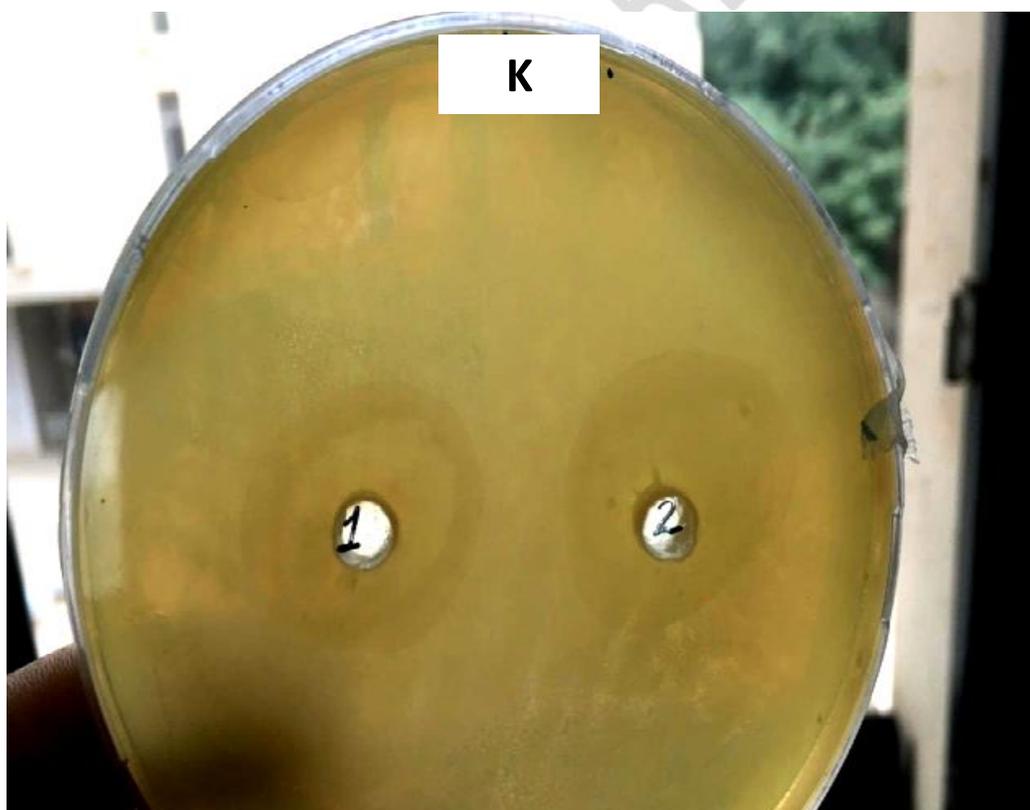


Plate (2): Antimicrobial activity of ginger water extract against *Klebsiella pneumoniae*.

In a study ginger was extracted at boiling temperature, then the antibacterial activity of the extract was detected against many pathogenic bacteria isolated from clinical cases. Results indicate the thermostable antibacterial property of ginger extracts protect the antimicrobial activity of ginger at high temperature and as a result, good antimicrobial activity was found against *Klebsiella pneumoniae*, *Escherichia coli* (Suhad *et al.*,2012). Synergistic antimicrobial effect of soybean and ginger at boiling temperature against foodborne pathogens indicates the thermostable antibacterial property of ginger extracts (Malu *et al.*, 2009).

Also, the synergistic effect of water extract of ginger and garlic against pathogenic spp revealed that antimicrobial activity of the water extract of ginger and garlic was strong against a broad range of bacteria including *Bacillus spp.*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella spp.* The diameter of the zone of inhibition varied ranging from (18-20) mm for ginger extract as compared to (23.33±3.51 mm) for gentamicin (White, 2007). The minimum inhibitory concentration (MIC) of ginger extracts on *Klebsiella pneumoniae*, *Escherichia coli* ranged from 0.1-0.2gm showing that ginger was more effective and produced marked inhibitory effect on the two test organisms compared to the onion extracts (Preuss *et al.*, 2005). Ginger has the capacity to eliminate harmful bacteria, such as *Escherichia coli*, responsible for most of the diarrhoea, especially in children, Gingerols have analgesic, sedative, antipyretic, antibacterial and gastrointestinal tract motility effects (Afshar,2007).

Conclusion

In conclusion, this study has shown that ginger extracts possess medicinal properties, antibacterial activity and that the inhibition of bacterial growth was dose dependent. The results of the present study show that ginger extracts are more effective against all tested bacterial strains. The results

of present study have provided the justification for therapeutic potential of ginger and also used as dietary supplement for food preservation.

Consent Disclaimer:

As per international standard or university standard written participant consent has been collected and preserved by the authors.

Ethical Disclaimer:

As per international standard or university standard written ethical permission has been collected and preserved by the author(s).

References

- Andrade, S. S.; Sader, H. S. and Gales, A. C. (2006). Increased resistance to first - line agents among bacterial pathogens isolated from urinary tract infection in Latin America. *Antimicrob. Agents. chemother.* 101:741-748
- Hanselman, B. A.; Kruth, S. A. and Weese, R. (2009). Coagulase positive *Staphylococcus* colonization of human. *Epidemiol. Infect.* 136: 953 – 964.
- Gupta, k.; Hooton; T. M.; Naber, K. G. and Schaeffer, A. J. (2010). "International clinical practice guidelines for the treatment of acute uncomplicated cystitis and Pyelonephritis in women. *Am. Eur. Sci. Microbiol.* 52(5). 103-120.
- Hooton, T. M. (2000). Pathogenesis of urinary tract infections. *J. Antimicrob. chemoth.* 46:1-7.
- Lane, D. R. and Akhar, S. (2011). Diagnosis and management of urinary tract infection and pyelonephritis. *Med. clin, Nor. Am.* 29(3):539-552.
- Nadia, G.; Talat, Y.; Mujahid, S. and Samia, A. (2004). Isolation, Identification and antibiotic resistance profile of indigenous bacteria isolates from urinary tract patients. *Pak. J. Biol. Sci.* 17(2): 2051-2054.

- Rahual, M.; Sudhir, A.; Saroj, S.; Sangay, C. and Kusum, H. (2009). Urinary tract infections caused by *Pseudomonas aeruginosa*: Aminireview, J. infect. Pub. Helath. 2: 101-111.
- Ravichandra, R. P.; Rashmi, B.; Neena, K.; Suresh, S. and Vijayanath, V. (2012). Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* strain isolated from clinical sources. J. Pharma. Biomed. Sci. 14(5): 2230-2235.
- Rosa, D.; Jose, G. and Gonzalo, P. (2001). Antibiotic susceptibility of bacterial strains isolated from patients with community acquired urinary tract infections. Antimicrob. chemother. 5(2):16-20.
- Farrag, H.A.; El-Rehim, A.; Hazaa, M. and El-ayed, A.s. (2016). Prevalance of pathogenic bacterial isolates infecting wound and their antibiotic sensitivity. J. Infect. Dis. ther. 4(5):2-7.
- Afshar, A.T. (2007). Effect of ginger on diabetic neuropathy, plasma antioxidant capacity and lipid peroxidation in rat. Food. chem. 10(1);148-153.
- OHara, M.; Keifer, D.; Farrel, K. and Kemper, K. (1998). A review of 12 commonly used medical herbs. Archives. Fam. Med. 7;523-536.
- Ali, B. H.; Blunden, G.; Tanira, G. and Nemmmar, A. (2008). Some phytochemical, pharmacological and toxicological properties of ginger. Food. chem. Toxicol. 46(2);409-420.
- Suhad, A.A.; Iman, J.J., and Hamssah, E.A. (2012). Study the antibacterial activity of Zingibarofficinale roots against some of pathogenic bacteria. 23(3): 63-70.
- Malus, S.P.; Obochi, E.N. and Nyong, B. (2009). Antibacterial activity and medicinal properties of Ginger (Zingibarofficinale). J. pure. Appl. Sci. 15(3): 365-368.
- White, B. (2007). Antimicrobial activity of ginger against different m.o. physician. 75: 1689-1691.

- Preuss, H.G.; Echard, B.; Ening, M and Elliott, T.B. (2005). Minimum inhibitory concentration of herbal essential oils on gram positive and gram negative bacteria. Mol. Cell. Biochem. 72: 29-34.
- National committee for clinical laboratory standard. performance standard for antimicrobial susceptibility testing. NCCLS. 2001

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