SAR Journal of Pathology and Microbiology

Abbreviated Key Title: *SAR J Pathol Microbiol*
Home page: https://sarmedjournals.com/sarjpm/home

ISSN 2707-7756 (P)
ISSN 2709-6890 (O)

Original Research Article

In Vitro Susceptibility Patterns of Aqueous Extract of Green Tea against Bacteria That Cause Urinary Tract Infection in Pregnant Women Attending Shendi Hospitals

Khalid Saeed Hammad[1], Amani Osman Shakak[1], Mosab Nouraldein Mohammed Hammed[2*]

[1]Department of Microbiology, University of Shendi, Shendi Town, Sudan
[2]Department of Medical Parasitology Faculty of Health Sciences, Elsheikh Abdallah Elbadri University, Berber, Sudan

***Corresponding Author**
Mosab Nouraldein Mohammed Hammed

Article History: | Received: 25.05.2021 | Accepted: 04.07.2021 | Published: 12.07.2021 |

Abstract: The aim of study is to determine the activity of aqueous extract of *Camellia Sinensis* (Green tea) on different types of bacteria isolated from 191 pregnant women attended to Shendi locality hospitals, suffering from urinary tract infection between February 2015 to February 2017 following informed consent. One hundred thirty seven bacteria were isolated, different bacteria with different Gram reactivity was isolated then identified biochemically, in vitro sensitivity testing using well diffusion technique against aqueous green tea extract was done. The largest zone of inhibition appeared in Gram positive bacteria *Enterococcus fecalis* (17.6 ± 1.9 mm)**.**the concentration of green tea aqueous extract able to suppress the growth of bacteria was 48.6 milligram per ml against *Staphylococcus aureus* and 280.2 milligram per ml against *Escherchia coli***.**

Keywords: *Camellia Sinensis,* Aqueous extract, Green tea, pregnant woman, Shendi, Sudan**.**

BACKGROUND

In the human females, the urinary tract has an important relation with the reproductive organs. In the non-pregnant state, the uterus lies behind and partly over the bladder while in the pregnant state the enlarging uterus effects on urinary tract (Mittal & Wing, 2005).

Urinary Tract Infections has become common hospital-acquired infection rating of about 35 % of nosocomial infections, and it is the second common cause of bacteraemia in hospitalized patients (Epoke, *et al*., 2000).

UTI is a common problem during pregnancy (Mittal & Wing, 2005). This usually begins in first trimester and peaks during seconds and third trimester (Van Brummen, *et al*., 2006).UTI can lead to serious complications if left untreated, (Mazor-Dray, *et al*., 2009).Also it has been observed that asymptomatic bacteriuria can lead to cystitis and pyelonephritis (Barnick & Cardozo, 1991), which can lead to acute respiratory distress, transient renal failure, sepsis and shock during pregnancy (Gilstrap & Ramin,2001).

Screening of pregnant women for UTI can minimize it, and the associated complications (Millar & Cox, 1997). Recently various risk factors of UTI during pregnancy have been reported; perhaps these are varied according the geographical, social and biological settings (Haider, *et al*., 2010). Escherichia coli with its multidrug resistant strains- has been found to be the commonest cause of UTI among pregnant women (Dalzell & Lefevre, 2000; Kariuki, *et al*., 2007).

In recent years, the health benefits of consuming green tea, including the prevention of cancer and cardiovascular diseases, the anti-inflammatory, antiarthritic, antibacterial, antiangiogenic, antioxidative, antiviral, neuroprotective, and cholesterol-lowering effects of green tea and isolated green tea constituents are under investigation. However, adding green tea to the diet may cause other serious health concerns (Chacko, *et al*., 2010).

The health-promoting effects of green tea are mainly attributed to its polyphenol content, particularly flavanols and flavonols, which represent 30% of fresh leaf dry weight. Recently, many of the forementioned beneficial effects of green tea were attributed to its most abundant catechin, (-)-epigallocatechin-3-gallate (EGCG). Green tea extracts are more stable than pure epigallocatechingallate, one of the major constituents of green tea, because of the presence of other antioxidant constituents in the extract.

In general, herbal medicines are complex mixtures of different compounds that often act in a synergistic fashion to exert their full beneficial effect (Chacko, *et al*., 2010).

Tea is one of the most popular beverages consumed worldwide. Tea, from the plant *Camellia sinensis*, is consumed in different parts of the world as green, black, or Oolong tea. Among all of these, however, the most significant effects on human health have been observed with the consumption of green tea (Cabrera, et al., 2006).The association between tea consumption, especially green tea, and human health has long been appreciated (Weisburger, 2000; Sato & Miyata, 2000).Some studies have suggested an inverse association between green tea consumption and the risk of kidney stone formation (McKay & Blumberg, 2002; Ishizuk, *et al*., 2003).

A series of good conducted studies show spatially in Japan that tea extracts have several useful antibacterial effects that can inhibit and kill Staphylococcus aureus, Staphylococcus epidermidis which are urinary pathogenes, Salmonella typhi, Salmonella typhimurium, Salmonella enteritidis, Shigella flexneri, Shigella dysenteriae, and Vibrio spp, including Vibrio cholera (Hamilton-Miller, 1995).

MATERIALS AND METHOD

Study design

Hospital based cross sectional study.

Study duration

From February 2015 to February 2017

Study population

Pregnant females in Shendi hospitals - Sudan

Sample size

Sample was calculated from free online web site http://www.calculator.net by using confidence level 95% and confidence interval 8%, One hundred ninety one.

Sample size = $Z^2 \times (p) \times (p-1) / C^2$
Z = Z value – confidence level 95% (1.96),
C = Confidence interval (.08 = ± 8).
P = standard division (0.5).

The calculated sample size based on this formula was 191.

Ethical approval

The proposal was reviewed and approved ethic ally by the scientific and the ethical committee of Shend i University.

DATA COLLECTION

Data was collected by using questionnaire.

Study setting

Shendi locality, River Nile State, Sudan. Shen di is a town in northern of Sudan beside the River Nile a nd Almatamah town also far 150 km northeast from Kh artoum the capital of Sudan (16°41'N 33°25'E). The are a is inhabited by the Ga'aleen Tribe.

Specimen collection

The patient was given a sterile non-opened, wi de mouth, leak proof container and requested by the la b to collect at least 10 ml of urine specimen. The contai ner was labeled with the date, the name and number of t he patient, and the time of collection. When immediate delivery to the laboratory was not possible, the patient was requested to refrigerate the urine at 4–6 °C until del ivery not more than 24 hours.

Culture of urine specimen

Urine sample were mixed well by rotating urine container several times. Beside opened Bunsen burner urine container was opened and Nichrome loop was inserted after sterilization by flaming and cooling. Small amount of urine sample was taken by loop and inoculated by making firstly well in Cysteine lactose electrolyte deficient agar (CLED) media then making primary lines from the well then secondary lines from primary lines then tertiary lines from secondary lines finally zigzag from last line of tertiary lines. The inoculated plates were incubated in incubator at 37ºC for 24h under aerobic condition.

Interpretation of culture growth

The plates were observed for any bacterial colonies grow signicantly. The bacteria were well isolated and then identified by colonial morphology, Gram stain and biochemical tests.

Preparation of plant extract

The plant extracts were prepared using the solvent water. 15g of green tea dry leaves (Veitnam green tea) minced to powder were taken and homogenized with100 ml of the distil water **(**Kumar, *et al.*, 2012). The mixture was left in hot air oven under 80ºC for 2 hours **(**Uzunalic, et al., 2006**).** After that left to cool and filter the mixture by piece of gauze then by filter paper of Whatman No.1 manufacture. The extract was heated in hot air oven under 60ºC to make stock crude material. The crude was weight by sensitive balance and 0.5g then dissolved in 1ml of sterile distil

water to obtain concentration 500mg/ml then diluted in tubes by using two fold dilution to make concentration 250.125,62 and 31 mg/ml.

Procedure of inoculation in Mueller Hinton agar plates and applying green tea extract

By the loop 3–5 colonies were taken and also must be similar in appearance, then transfer the colonies tube containing sterile normal saline and mixed then compared the tube with the standard turbidity and adjusted the turbidity of the test suspension to that of the standard by adding more bacterial colonies or more sterile normal saline. The plates were inoculated by dipping a sterile swab into the tube containing bacterial suspension.

The excess of bacterial suspension was removed by gentle pressing and rotating the swab against the sides of the tube above the level of the suspension. The swab was masked all over the surface of the plate media with rotating each time to ensure complete covering. Finally. The plate was left to dry for a 1- 2 minutes at room temperature with the lid closed. By using glass porer of size 6 mm in diameter, 5 pores were made in agar plate then the pores were filled by green tea extract by using automatic pipette in volume 50 µ.l of concentrations 500mg/ml (Stock concentration), 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31 mg/ml.The plates were incubated for 24h in incubator under aerobic condition in 37ºC.

Interpreting the sensitivity of green tea extract

The diameter of each inhibited growth zone had been measured by graduated ruler under-surface of the plate without opening the cover and recorded in mm.

DATA ANALYSIS

Data was analyzed by using online web site https//www.graphpad.com to determine the mean and standard deviation of the data. Proportional data were presented as frequencies and percentages**.**

RESULT

A total of one hundred ninety one pregnant women with UTI were contributed in this study. The age mean was (25.4 ± 6.7) years. Most of them were within the age group less than 24 and 25 – 34 years (47.7%, 47.1%) respectively (Table 1).More than 50% were in their 3d trimester (Table 2). One hundred forty six (76%) of the pregnant women drinking tea while they were pregnant and 145 (99.3%) drink black tea showed in (Table 3). The main causative agent of UTI in the study population was S. aureus (24.8%), followed by E. coli (21.9%) and the least causative agents were Hafnia alvei and Proteus vulgaris (0.7 %) (Table 4). The Gram negative bacilli isolated were E. coli represented the main causative agent between Gram negative bacilli bacteria 30 (50) and lowest causative Gram negative bacilli were Proteus vulgaris and Hafnia alvei 1 (1.6%) (Table 5). The Gram positive cocci isolated was S. aureus 34 (44.2%) and it is the main causative agent between Gram positive cocci bacteria and lowest causative Gram positive cocci was Enterococcus fecalis 5 (6.5 %) (Table 6). In 1st trimester Citrobacter freundii was the main causative agent of UTI 5 (29.4%), E. coli and Klebsiella pneumonae were least isolated agents 1 (5.9%).In 2nd trimester S. aureus was the main causative agent of UTI 10 (27.1%), Enterobacter cloacae, S. saprophyticus and

E. fecalis were least isolated agents 1 (2.7%).In 3d trimester also S. aureus was the causative agent of UTI 21 (25.3%), Proteus vulgaris, Hafnia alvaei, Citrobacter freundii and Enterobacter cloacae were least isolated agents1 (1.2%) (Table 7). Antimicrobial susceptibility of green tea extract has largest zone of inhibition against Enterococcus fecalis was (17.6 ± 1.9 mm) and least zone of inhibition against Proteus vulgaris and Enterobacter cloacae was (8 ± 0 mm) (Table 8).The results of antimicrobial activity of extract was compared with Standard drugs (Gentamicin) as positive control. Antibacterial activities of tea extract were tested by well diffusion method. The concentrations of green tea water extract used was 500, 250, 125, 62.5 and 31 mg/ml. MIC results of water extracts of green tea on test organisms which the lowest concentration of green tea extract able to suppress the growth of bacteria was (48.6 mg/ml) appear against S.aureus followed by Staphylococcus saprophyticus (58 mg/ml), Enterococcus fecalis (62.8 mg/ml), Micrococcus luteus (66.8 mg/ml) Staphylococcus epidermidis (68.2 mg/ml), Hafnia alvei (250 mg/ml), E. coli (280.2 mg/ml), Citrobacter freundii (312.5 mg/ml), Citrobacter diversus (325 mg/ml), Enterobacter cloacae. Klebsiella pneumonae and Proteus vulgaris (500mg/ml) (Figure 1).

Table-1: Show population distribution according to ages

Ages groups	Number	%
Less than 24	91	47.7%
25 – 34	90	47.1%
35 – 44	9	4.7%
More than 44	1	0.5%
Total	**191**	**100%**

Age (mean ± SD) = (25.4 ± 6.7)

Table-2: Show distribution of the population accordi ng to trimester

Trimester	Number	%
First	24	12.6%
Second	60	31.4%
Third	107	56.0%
Total	**191**	**100**

Table-3: Show distribution of population according to tea type used

Type of tea used	Number	%
Black	145	99.3%
Green	1	0.7%
Total	**146**	**100%**

Table-4: Show percentage of bacteria isolated from urine of study population

Bacteria name	Number	%
Citrobacter diversus	10	7.30%
Citrobacter freundii	6	4.40%
Escherchia coli	30	21.9%
Enterobacter cloacae.	2	1.50%
Enterococcis fecalis	5	3.60%
Hafnia alvei	1	0.70%
Klebsiella pneumonae	10	7.30%
Micrococcus luteus	15	11%
Proteus vulgaris.	1	0.70%
Staphylococcus aureus	34	24.8%
Staphylococcus epidermidis	16	11.70%
Staphylococcus saprophyticus	7	5.10%
Total	**137**	**100**

Table-5: Show Gram negative Bacteria isolated from urine of study population

Bacteria isolated	Number	%
Citrobacter diversus	10	16.7%
Citrobacter freundii	6	10%
Escherchia coli	30	50%
Enterobacter cloacae.	2	3.4%
Hafnia alvei	1	1.6%
Klebsiella pneumonia	10	16.7%
Proteus vulgaris	1	1.6%
Total	**60**	**100%**

Table-6: Show Gram positive Bacteria isolated from urine of study population

Bacteria isolated	Number	%
Staphylococcus aureus	34	44.2%
Staphylococcus epidermidis	16	20.7%
Staphylococcus saprophyticus	7	9.1%
Enterococcus fecalis	5	6.5%
Micrococcus luteus	15	19.5%
Total	**70**	**100%**

Table-7: Show percentage of isolated bacteria from urine of pregnant woman according to trimester

Bacteria name	Total No.	1st trimester		2nd trimester		3d trimester	
		n	%	n	%	n	%
Citrobacter diversus	10	0	0%	4	10.8%	6	7.2%
Citrobacter freundii	6	5	29.4%	0	0%	1	1.2%
Escherchia coli	30	1	5.9%	9	24.3%	20	24.1%
Enterobacter cloacae.	2	0	0%	1	2.7%	1	1.2%
Enterococcis fecalis	5	4	23.6%	1	2.7%	0	0%
Hafnia alvei	1	0	0%	0	0%	1	1.2%
Klebsiella pneumonae	10	1	5.9%	3	8.1%	6	7.2%
Micrococcus luteus	15	3	17.6%	5	13.5%	7	8.5%
Proteus vulgaris.	1	0	0%	0	0%	1	1.2%
Staphylococcus aureus	34	3	17.6%	10	27.1%	21	25.3%
Staphylococcus epidermidis	16	0	0%	3	8.1%	13	15.7%
Staphylococcus saprophyticus	7	0	0%	1	2.7%	6	7.2%
Total	**137**	**17**	**100%**	**37**	**100%**	**101**	**100%**

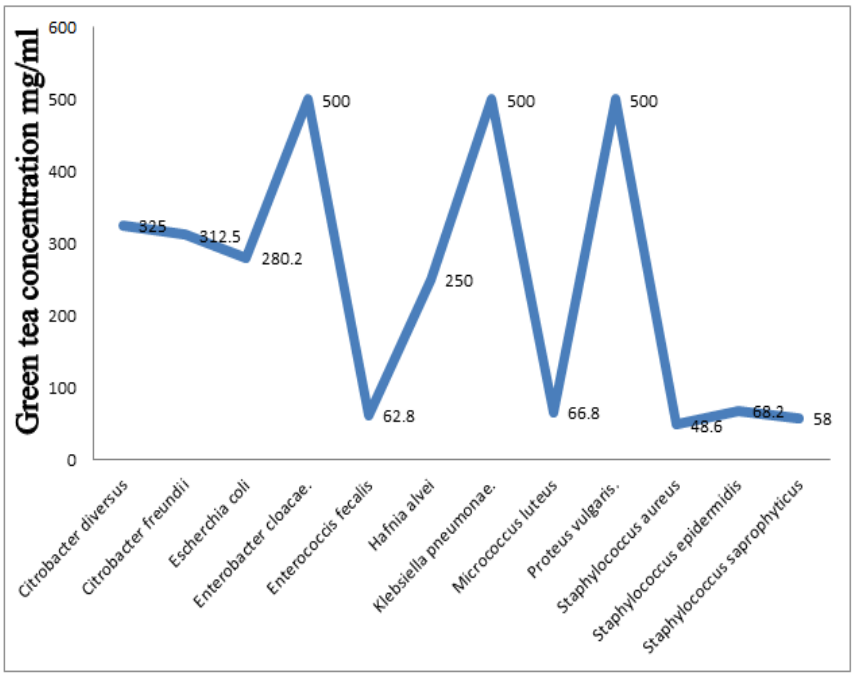

Fig-1: MIC of aqueous extracts of green tea against tested bacteria (mg/ml)

Table-8: Show antimicrobial susceptibility of green tea extract compared to Gentamicin

Bacteria isolated	**Inhibition zone diameter (mm)**	
	Aqueous extract (Green tea 500 mg/ml)	**Positive control (Gentamicin 10 mcg)**
Citrobacter diversus.	9.1 ± 0.9	19.5
Citrobacter freundii	9.1 ± 2.1	19
Escherchia coli.	10.8 ± 3.0	18
Enterobacter cloacae.	8 ± 0	24
Enterococcis fecalis.	17.6 ± 1.9	29
Hafnia alvei.	9 ± 0	20
Klebsiella pneumoniae.	8.6 ± 2.5	23
Micrococcus luteus.	16.5 ± 1.9	28.5
Proteus vulgaris.	8 ± 0	20
Staphylococcus aureus.	18.0 ± 2.4	29.1
Staphylococcus epidermidis.	16.8 ± 2.1	28.9
Staphylococcus saprophyticus.	16.8 ± 2.5	30

Table-9: Antimicrobial activity of green tea extracts on bacteria isolated

Bacteria Isolated (n=137)	**Inhibition zone (mm)**
Gram positive bacteria	17.1 ± 0.6
Gram negative bacteria	8.9 ± 0.9

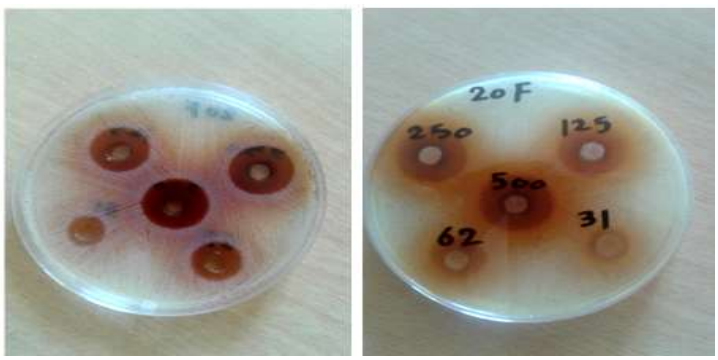

Plate-1: Green tea water extract inhibition zones of S.aureus as example at different concentrations (500, 250, 125, 62 and 31 mg/ml)

DISCUSSION

Infection of urinary tract is a common problem during pregnancy (Alemu, *et al*., 2012). The prevalence of UTI among Sudanese pregnant women was 14.0% (Hamdan, *et al*., 2011). This usually begins in first trimester and peaks during seconds and third trimester of pregnancy due to a number of factors and physiological changes including urethral dilatation, increased bladder volume (Van Brummen, et al., 2006). The main causative agent of UTI in the study population was S. aureus (24.8%), which reported as commonest pathogen among pregnant women, this is in agreement with report from Nigeria **(**Akinloye, *et al*., 2006), followed by E. coli (21.9%). The green tea water extract had an suppressive effect on the growth of E. coli strains isolated from UTI**s** (Reygaert & Jusufi, 2013). In current study antimicrobial susceptibility of aqueous green tea extract show highest area of inhibitory growth against Enterococcus fecalis (17.6 ± 1.9 mm) and lowest zone of inhibition against Proteus vulgaris and Enterobacter cloacae (8 ± 0 mm).The antibacterial activity of tea is due to presence of catechins and polyphones which disrupt bacterial cell membrane (Kumar, et al., 2012).Ikigai and colleagues reported that tea catechins have less activity on Gram negative bacterial cell membrane due to the fact that Lipopolysaccharide outer membrane of Gram negative is negatively charged (IKigai, et al., 1993), Kumar and colleagues reported that the daily drinking of green tea can kill Staphylococcus aureus also including many other harmful bacteria (Kumar, et al., 2012), Alizadeh and Mohebalian reported that the maximum area of growth suppression was observed against S. aureus (A Gram positive organism) and the minimum was against pseudomonas (Alizadeh & Mohebalian, 2016). Many studies have shown that 500 µg of tea polyphenols can suppress the growth of E. coli, and ≥5000 µg are considered bactericidal. This effect is believed to be due tea polyphenols down regulate the production of proteins such as EF-2 (elongation factor for protein translation); proteins involved in phospholipid, carbon, and energy metabolism; and production of proteins involved in amino acid biosynthesis (Reygaert & Jusufi, 2013).The MIC results of green tea water extract on tested bacteria showing that lowest concentration able to suppress the growth of bacteria is (48.6 mg/ml) appear against S. aureus followed by Staphylococcus saprophyticus (58 mg/ml), Enterococcis fecalis (62.8 mg/ml), Micrococcus luteus (66.8 mg/ml) Staphylococcus epidermidis (68.2 mg/ml), Hafnia alvei (250 mg/ml), Escherchia coli (280.2 mg/ml), Citrobacter freundii (312.5 mg/ml), Citrobacter diversus (325 mg/ml), Enterobacter cloacae. Klebsiella pneumonae and Proteus vulgaris (500mg/ml). Neyestani and colleagues investigated microbiologic effects of tea extract on some antibiotics against E. coli in vitro. They used bacterial control strain ATCC 25920 and tea extracts (green and black tea) of different concentrations (6.25 mg/mL, 12.5 mg/mL, 25 mg/mL, 50 mg/mL, and 100 mg/mL).They use disc diffusion protocol for bacterial sensitivity tests. Green tea at 20 mg/mL concentration inhibited E. coli growths completely (Neyestani, et al., 2007) which disagree with the study results. We suggested that green tea that prepared with liquid chromatography show high performance than prepared with dry oven.

CONCLUSION AND RECOMMENDATIONS

The water based green tea extract has ability to suppress the growth of most bacteria that cause urinary tract infection. The lowest concentration of green tea aqueous extract able to suppress growth of bacteria that cause UTI is 48.6 mg/ml the suppressive activity of green tea aqueous extract is better in Gram positive bacteria than Gram negative according to MIC and zone of inhibition results. Further studies recommended with different ethnic groups.

ACKNOWLEDGEMENT

The authors appreciate to ethical review committee of the selected health facilities and the pregnant women who consented to taking part in the study and microbiology staff in University of Shendi. Funding for this study was from personal contributions from the authors, no external funding.

REFERENCES

- Akinloye, O., Ogbolu, D. O., Akinloye, O. M., & Terry Alli, O. A. (2006). Asymptomatic bacteriuria of pregnancy in Ibadan, Nigeria: a re-assessment. British journal of biomedical science, 63(3), 109-112.
- Alemu, A., Moges, F., Shiferaw, Y., Tafess, K., Kassu, A., Anagaw, B., & Agegn, A. (2012). Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at University of Gondar Teaching Hospital, Northwest Ethiopia. BMC research notes, 5(1), 1-7.
- Mohebalian, H., & Alizadeh, S. (2016). The antimicrobial activity of green tea extract against the major food born bacteria. Journal of Advances in Health and Medical Sciences, 2(2), 54-60.
- Assefa, A., Asrat, D., Woldeamanuel, Y., Abdella, A., & Melesse, T. (2008). Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at Tikur Anbessa Specialized Hospital Addis Ababa, Ethiopia. Ethiopian medical journal, 46(3), 227-235.
- Barnick, C. G. W., Cardozo, L. D., & Studd, J. (1991). The lower urinary tract in pregnancy, labour and puerperium. Progress in obstetrics and gynaecology, 9, 195-204.
- Cabrera, C., Artacho, R., & Giménez, R. (2006). Beneficial effects of green tea—a review. Journal of the American College of Nutrition, 25(2), 79-99.
- Chacko, S. M., Thambi, P. T., Kuttan, R., & Nishigaki, I. (2010). Beneficial effects of green tea: a liter

ature review. Chinese medicine, 5(1), 1-9.
- LeFevre, M. (2000). Urinary tract infections during pregnancy. American family physician, 61(3), 713 -720.
- Enzveiler, L., Gressler, G., Heckler, E., Picoli, S., & Suyenaga, E. S. (2011). Evaluation of antimicrob ial activity of aqueous extract of white tea (Camelli a sinensis) L. Kuntze (1887). Pharmacologia, 2(5), 2011.
- Epoke, C. O., Anyanwu, G. O., & Opara, A. A. (20 00). The prevalence of significant bacteriuria in dia betic patients. Diabetic International, 10, 16-17.
- Gilstrap III, L. C., & Ramin, S. M. (2001). Urinary tract infections during pregnancy. Obstetrics and gy necology clinics of North America, 28(3), 581-591.
- Haider, G., Zehra, N., Munir, A. A., & Haider, A. (2010). Risk factors of urinary tract infection in preg nancy. JPMA. The Journal of the Pakistan Medical Association, 60(3), 213.
- Hamdan, H. Z., Ziad, A. H. M., Ali, S. K., & Adam , I. (2011). Epidemiology of urinary tract infections and antibiotics sensitivity among pregnant women at Khartoum North Hospital. Annals of clinical mic robiology and antimicrobials, 10(1), 1-5.
- Hamilton-Miller, J.M. (1995). Mini review Antimic robial Properties of Tea (Camellia sinensis L.).Anti micobial agents and chemotherapy. Nov; 39(11); 2 375–77.
- Hosseni, N., & Zartoshti, M. (2007). Evaluation of synergic effect of water soluble extracts of green te a on the activity of Ciprofloxacin in urinary isolate d E.coli. Journal of biological sciences, 7(8); 1500-1503.
- Ikigai, H., Nakae, T., Hara, Y., & Shimamura, T. (1 993). Bactericidal catechins damage the lipid bilaye r. Biochimica et Biophysica Acta (BBA)-Biomemb ranes, 1147(1), 132-136.
- Ishizuka, H., Eguchi, H., Oda, T., Ogawa, S., Naka gawa, K., Honjo, S., & Kono, S. (2003). Relation o f coffee, green tea, and caffeine intake to gallstone disease in middle-aged Japanese men. European jo urnal of epidemiology, 18(5), 401-405.
- Kariuki, S., Revathi, G., Corkill, J., Kiiru, J., Mwit uria, J., Mirza, N., & Hart, C. A. (2007). Escherichi a coli from community-acquired urinary tract infect ions resistant to fluoroquinolones and extended-spe ctrum beta-lactams. The Journal of Infection in De veloping Countries, 1(03), 257-262.
- Kumar, A., Kumar, A., Thakur, P., Patil, S., Payal, C., Kumar, A., & Sharma, P. (2012). Antibacterial activity of green tea (Camellia sinensis) extracts ag ainst various bacteria isolated from environmental s ources. Recent Research in Science and Technolog y, 4(1), 19-23.
- Mazor-Dray, E., Levy, A., Schlaeffer, F., & Sheine r, E. (2009). Maternal urinary tract infection: is it in dependently associated with adverse pregnancy out come?. The Journal of maternal-fetal & neonatal m edicine, 22(2), 124-128.
- McKay, D. L., & Blumberg, J. B. (2002). The role of tea in human health: an update. Journal of the A merican College of Nutrition, 21(1), 1-13.
- Millar, L.K., & Cox, S.M. (1997). Urinary tract inf ections complicating pregnancy. Infectious Disease s Clinics of North America. Mar; 11(1);13-26.
- Mittal, P., & Wing, D.A. (2005). Urinary tract infec tions in pregnancy. Clinics in Perinatology. Sep; 32 (3);749–764.
- Neyestani, T. R., Khalaji, N., & Gharavi, A. A. (20 07). Selective microbiologic effects of tea extract o n certain antibiotics against Escherichia coli in vitr o. The Journal of Alternative and Complementary Medicine, 13(10), 1119-1124.
- Reygaert, W., & Jusufi, I. (2013). Green tea as an e ffective antimicrobial for urinary tract infections ca used by Escherichia coli. Frontiers in microbiology , 4, 162.
- Sato, T., & Miyata, G. (2000). The nutraceutical be nefit, part I: green tea. Nutrition. Apr; 16(4);315-7
- Perva-Uzunalić, A., Škerget, M., Knez, Ž., Weinrei ch, B., Otto, F., & Grüner, S. (2006). Extraction of active ingredients from green tea (Camellia sinensi s): Extraction efficiency of major catechins and caf feine. Food chemistry, 96(4), 597-605.
- Van Brummen, H. J., Bruinse, H. W., van der Bom, J. G., Heintz, A. P. M., & van der Vaart, C. H. (20 06). How do the prevalences of urogenital sympto ms change during pregnancy?. Neurourology and U rodynamics: Official Journal of the International C ontinence Society, 25(2), 135-139.
- Weisburger, J.H. (2000). Approaches for chronic di sease prevention based on current understanding of underlying mechanisms. The American Journal of Clinical Nutrition, 71(6);1710s-1714s