



## Evaluation of Antibacterial Effect of Green Tea Extract on Bacterial Isolated from Patients With Vaginal Infection

Masoud Dadashi<sup>1</sup>, Mina Amjadi Kashani<sup>1</sup>, Gita Eslami<sup>\*1</sup>, Hossein Goudarzi<sup>1</sup>, Fatemeh Fallah<sup>2</sup>, Parviz Owlia<sup>3</sup>, Nafiseh Adhami<sup>4</sup>, Tahoor Mousavi<sup>5</sup>

1-Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

2-Pediatric Infections Research Center, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

3-Molecular Microbiology Research Center (MMRC), Shahed University, Tehran, Iran

4-Msc Student of Microbiology, Islamic Azad University of Pharmaceutical Sciences, Tehran, Iran

5-Ph.D Student in Molecular and Cell Biology, Student in Research Committee, Molecular and Biology Research Center, Faculty of Mazandaran, Mazandaran University of Medical Sciences, Sari, Iran

### ARTICLE INFO

#### Article history:

Received 14 October 2015

Accepted 23 November 2015

Available online 1 December 2015

#### Keywords:

Vaginitis, Green tea ,extract,  
Antibiotic susceptibility, infection

### ABSTRACT

Vaginitis is an inflammatory and infectious disease in human host that involved vaginal mucosa. Increase of resistance to various antibacterial agent has been very significant in three or four recent decades and this important subject reveals the use of new and natural sources such as plants with medical and antimicrobial property for eradication of these infections. Therefore, the aim of this study is to evaluate the antibacterial effect of green tea plant on vaginitis agents. This experimental study was performed on 60 samples obtained from patients with genital infection. Then, samples were introduced to transport medium and transported to microbiology laboratory of Shahid Beheshti University of Medical Science. Incubation was performed after culturing the samples in specific medium. Microorganisms were identified responsible for vaginitis and isolated after growth of colonies and performing the confirmation tests. Finally, disk diffusion and MIC tests were performed for detection of susceptibility and resistance of infections to 6 mentioned antibiotics in comparison with green tea extract. The antibacterial effect of green tea extract on lactobacillus and listeria was more effective in comparison with current antibiotics except ciprofloxacin. Green tea extract had antibacterial effect on B group streptococcus as like as gentamicin and ampicillin. Green tea extract can be used as an effective antimicrobial agent in conjunction with other antibiotics for the treatment of vaginal infections in women.

### 1. Introduction

Genitalia in women is an ecosystem that is influenced by many factors causing the disease. It is constantly exposed to various microbes due to close proximity to the digestive system and sexual intercourse which many of them can create a complication if conditions to be favorable and the microbial flora loses (Nardis et

al., 1946). Vaginitis is an infectious or non-infectious and inflammatory disease that affects the vaginal mucosa. The main symptom of vaginitis is the increase of vaginal discharges or leukorrhea which often associated with pus and odor (Sang Hee Kim et al., 2008). Species of *Lactobacillus*, *Streptococcus pneumoniae*, *Mycoplasma genitalium*, *Prevotella*, *Clostridium* species, *Candida albicans*, *Trichomonas*

\*Corresponding author: Prof. Gita Eslami  
E-mail address: G\_eslami@yahoo.com

*vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhoea* and *Gardenella vaginitis* along with aerobic bacteria are the most vaginalis factors (Larsen et al., 2001; Marrazzo et al., 2002; Redondo-Lopez et al., 1990; Srinivasan et al., 2008). Since medicinal plants have long been used in treatment of diseases (Eslami et al., 2013), the role of plants in relation to diseases that occur due to infection with microbial factors can be investigated. Green tea has many effective impacts including antimicrobial effects and has a synergistic effect with many antibiotics. Green tea that obtained from the young leaves of *camellia sinensis* is very safe at high doses and contains various polyphenols including epicatechingallate (ECG), epigallocatechin (EGC), gallate (EGCG) - epigallocatechin3 epicatechin (EC), galocatechin (GC) and catechin (Amarowicz et al., 2000; Banerjee et al., 2005; Cooper et al., 2005; Ju et al., 2005). There is no study about the effects of green tea on vaginal infections in Iran, but another study which is significant was conducted by Hosseini Jazani and colleagues in Orumieh in 2007 (Hosseini et al., 2007).

Researchers have done studies on green tea in recent years and came to conclusion that green tea can play a role by its polyphenols particularly EGCG, ECG through the effectiveness of cell membranes structure and affecting on its performance, and inhibit the growth of a wide range of gram-positive and gram-negative bacteria (Mabe et al., 1999; Yang et al., 2002). Evidence suggests that these molecules are effective to control the common mouth infections such as tooth decay and periodontal diseases and also vaginal infections.

In addition to boosting effects of green tea on cardiovascular system, it has the antioxidant properties as well (Banerjee et al., 2005). As reducing the sensitivity and increasing the resistance to different antibiotic materials have been very dramatic in three or four recent decades, the need for new resources is felt to eradicate the causes of this disease more than ever before. The natural products are a very important source in development of new drugs. So, the aim of this study is to evaluate the antimicrobial effects of green tea, so that we might use of green tea as a supplemental therapy in treatment of vaginal diseases with knowledge of healing properties of it.

## 2. Materials and Methods

After collecting 60 samples of vaginal by obstetrician, samples were introduced to TSB transport medium and incubated at 37 ° C for 24 hours after transferring to the microbiology laboratory of Shahid Beheshti University of Medical Sciences and then slides were prepared from the TSB containing the sample, and gram staining was conducted about the cocci and gram-positive or gram-negative bacillus for the early identification of bacteria. Then, samples were cultured in blood agar and Mac Cancan medium and the slides were again produced from the created colonies and performed gram staining after putting plate containing the bacteria in incubator at 37°C for 24 hours. Finally, microorganisms were cultured in special and differential mediums that the specific methods and mediums including Palkam medium for growth of listeria, MRSAgar for growth of lactobacilli, blood agar medium for growth streptococcus beta-hemolytic along with CAMP tests and sodium hippurate hydrolysis were used. After identifying the bacteria, their sensitivity and resistance against the 6 received antibiotics consist of cephalixin, ciprofloxacin, cotrimoxazol, amoxicillin, ampicillin and gentamicin were evaluated by the disk diffusion method according to CLSI rules.

### 2.1. Preparation of Green Tea Extract

Drying was conducted in the open air after preparation of green tea leaves, and then cut into smaller parts by choppers blender. The water extraction method and purification with Ethyl acetate was used to prepare the aqueous extract. Accordingly, the resulting mixture was transferred into a beaker after mixing 200 grams of green tea powder and 1 liter of water at 95°C, and extraction practice was conducted by the heater with temperature 45 ° C for 8 hours. After isolating and smoothing the mixture, 500cc Ethyl acetate was added to the aqueous phase for 15 minutes to purify the phenolic compounds from the other substances contained in aqueous phase. After this time, the ethyl acetate phase was separated and then the dry extract was obtained after eliminating the ethyl acetate.

### 2.2. Preparation of McFarland Standard

1% solution of pure sulfuric acid and 1.175% solution of Barium chloride have been used which create different opacity based on the extent that these two mix together (McFarland 1907).

### 2.3. Preparation of Microbial Inoculation

Microbial inoculation was conducted by Kirby-Bauer method to perform the Antibiogram test after preparing a half solution of McFarland. In such a way that several isolated colonies are removed from the bacteria culture medium by a sterile loop and dissolved in 10 ml broth and placed in drying cabinet at 37 °C and the obtained opacity was compared by using the 0.5 score of McFarland after 2-3 hours.

### 2.4. Antibiogram Test by Disk Diffusion Agar Method

Paper disc contains a specific antibiotic and a blank disc impregnated with an extract of green tea was placed on a suitable culture medium that has already been redirected in microbial inoculation at appropriate intervals (Coleman et al., 2013). Thus, a disk was placed in the center of plate and the other disks around it and the distance of disks from the edge of plate was determined 15 mm and the distance of disks from each other was 32-34 mm. Mueller-Hinton Agar (MHA) medium is suitable culture medium for this purpose. Then, measuring the diameter of growth inhibition was conducted by a millimeter ruler and sensitivity or resistance of bacteria to antibiotics and green tea extract were evaluated by using light and results were accurately reported. It is worth noting that the antibiotic disks (Mast Group, Merseyside, UK) used include: cephalexin (30 µg), ciprofloxacin (30 µg), cotrimoxazol (10 µg), amoxicillin (10 µg), ampicillin (10 µg) and gentamicin (10 µg) which were examined on 3 microorganisms of listeria, streptococci beta hemolytic and lactobacilli. Also, the sensitivity of studied bacteria to green tea extract and common antibiotics was analyzed statistically by the Chi test 2.

## 3. Results

This experimental study was conducted on 60 patients with vaginitis. The antimicrobial effect

of green tea extract compared to 6 common antibiotics in treatment of vaginitis was studied on 3 microorganisms. Sensitivity of *Lactobacillus* to green tea extract was reported 17% at a concentration of 100 mg and 18.67% at a concentration of 200 mg. As well as, sensitivity of *Lactobacillus* to the antibiotics of gentamicin, ampicillin, amoxicillin, cotrimoxazol, ciprofloxacin, cephalexin was reported 16.33%, 5%, 13.67%, 0%, 21.33%, and 0% respectively (Diagram1). The sensitivity of listeria and B group streptococcus to the green tea extract and 6 antibiotics studied was provided in diagrams 2 and 3.

## 4. Discussion

The present study suggests that the green tea extract has a significant antibacterial effects on lactobacillus, B group streptococcus and listeria bacteria and can be said that it has showed more antimicrobial properties than to some common antibiotics like amoxicillin and cotrimoxazol. Vaginitis is infectious or non-infectious and inflammatory disease that affects the vaginal mucosa. The main symptom of vaginitis is the increase of vaginal discharges or leukorrhea. As taking antibiotics in treatment of diseases is always accompanied to antimicrobial resistance and creating the adverse effects in patients, therefore, many experts consider the medicinal plants as a desirable method to treat the disease because of the importance of plants in the treatment of disease. According to this matter, the antimicrobial effect of green tea plant on the bacteria causing vaginitis was studied and evaluated. Green tea which obtains from young leaves of *Camellia sinensis* plant contains various polyphenols that are considered as an effective substance to have the medicinal properties (Gupta et al., 2012). There is no study about the effects of green tea on vaginal infections in Iran, but another study which is significant was conducted by Hosseini Jazani and colleagues in Orumieh in 2007 (Hosseini et al., 2007). In this study, they have examined the synergistic effects of green tea soluble in water on ciprofloxacin in urinary infections contain the *E.coli*. The results of this study showed that green tea, as a supplementary medicine and also as a synergism with ciprofloxacin antibiotic, has a unique antibacterial effect in treatment of vaginitis diseases that these results are consistent

with the findings the study. Another study that confirms the antibacterial properties of green tea at high level was conducted by SG Upta and colleagues in Delhi of India in 2012 under title review and development of green tea properties as micro-emulsion on vaginal infections (Mbata et al., 2008) that the results of this study also confirm the antimicrobial properties of green tea and is consistent with our research results. In another study which performed in 2008 by Mbatha, TI and his colleagues in Africa, the antibacterial effect of Chinese crude extract of green tea on *Listeria monocytogenes* bacteria was approved as well (Divjani et al., 2010). In 2012, another study was conducted by Devijani Chakraborty and his colleagues at the research center of Gujarat in India in which the antibacterial effect of methanol extract of tea leaves on 4 bacteria of *Pseudomonas aeruginosa*, *Bacillus cereus*, *Escherichia coli*, and *Staphylococcus aureus* was tested (Lamont et al., 2011) that the results of this study also suggested the antimicrobial effect of green tea extract and were consistent with the results of present study. In general, the antibacterial effects of green tea have been well proven in studies carried out on different regions (Sang Hee Kim et al., 2008; Ravel et al., 2011). But, a study based on comparison the antimicrobial effects of green tea with six common antibiotics, in our country, i.e. Iran that green tea is its native plants, was not found that we can clearly understand the role of green tea to the other antibiotics. For this reason, the present study tried to measure the medicinal properties of green tea to the other six common antibiotics.

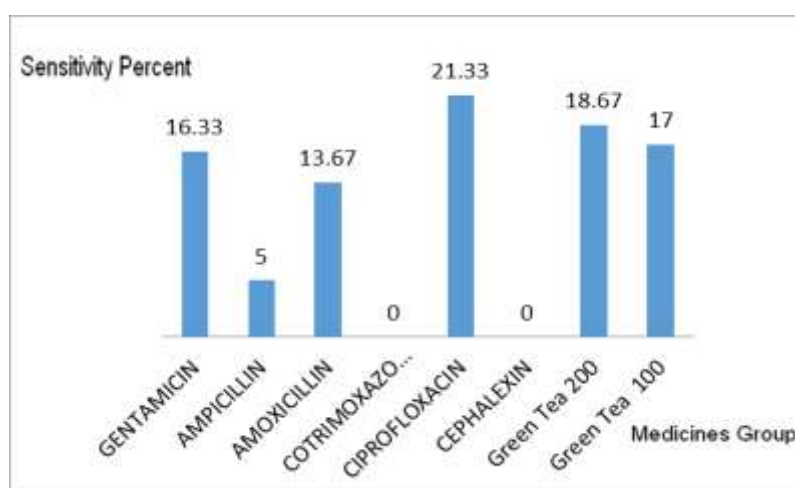
This experimental study was conducted by the disk diffusion method and green tea extract had a significant antibacterial effects, so that it has shown a better antimicrobial performance than even the common antibiotics in some cases. This means that *Lactobacillus* and *Listeria* spp were more sensitive to the green tea extract compared to 6 antibiotics tested except ciprofloxacin. The extract with two concentrations of 100mg/ml and 200mg/ml had also a similar effects with two antibiotics of ampicillin and gentamicin in the case of B group streptococcus and acted stronger of the other antibiotics.

## Conclusion

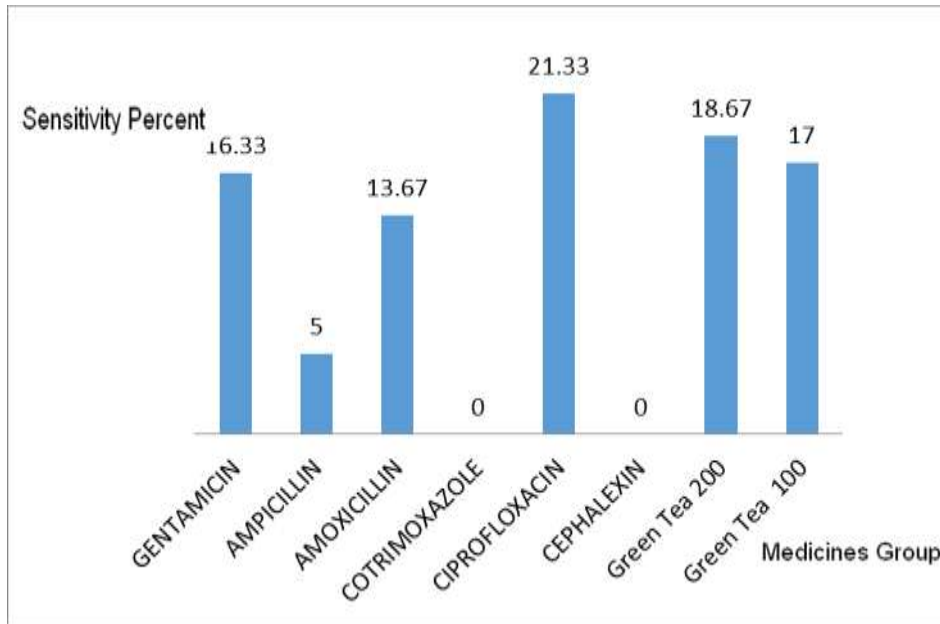
The results of this study suggest that green tea extract can be used as an effective antimicrobial agent in conjunction with other common antibiotics and perhaps more effective than some of them for the treatment of vaginal infections in women and if the patients do not tolerate each of antibiotic compounds to them due to side effects or incidence of allergy, they can use of herbal products such as green tea as a complementary drug in the treatment and antimicrobial substance in vaginitis infections caused by bacteria. The need to examine its antibacterial range is felt well in the case of other microorganisms.

## Acknowledgments

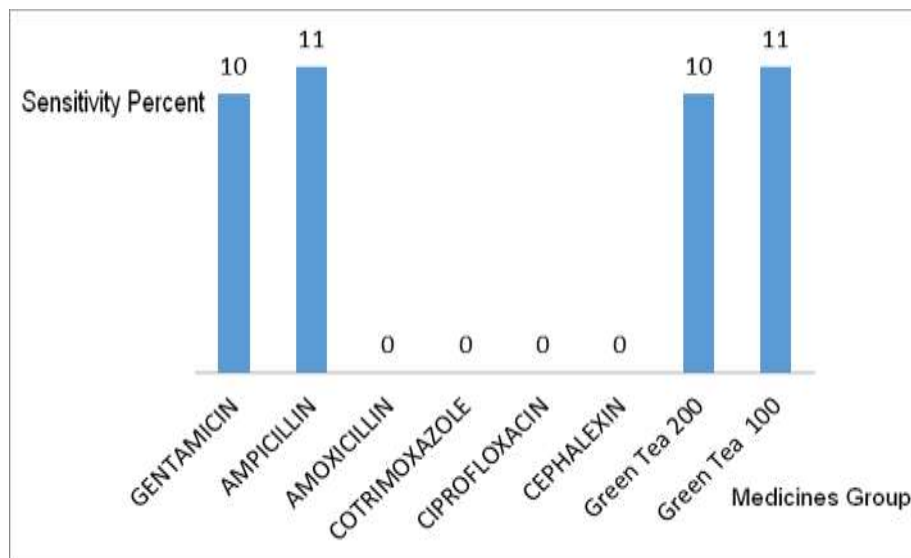
We sincerely appreciate from the respectable Management of Microbiology Department for assistance in conducting this study.



**Diagram1.** Distribution of patients with vaginitis based on sensitivity of lactobacillus to green tea extract and antibiotics.



**Diagram2.** Distribution of patients with vaginitis based on sensitivity of listeria to green tea extract and antibiotics.



**Diagram 3.** Distribution of patients with vaginitis based on sensitivity of B group streptococcus to green tea extract and antibiotics.

## Refereces

- Amarowicz, R., Pegg, B.R., Bautista, A.D., 2000. Anti-bacterial activity of green tea polyphenols against *Escherichia coli* K12. *Die Nahrung*. 44: 60-62.
- Banerjee, S., Manna, S., Saha, P., Panda, C.K., Das, S., 2005. Black tea polyphenols suppress cell proliferation and induce apoptosis during benzo-

(a) pyrene-induced lung carcinogenesis. *Eur J Cancer Prev*. 14: 215-21.

- Chakraborty D, Chakraborti S., 2010. Bioassay-guided isolation and identification of antibacterial and antifungal component from methanolic extract of green tea leaves (*Camellia sinensis*) *Res J Med Plant*. 4:78-86.
- Coleman, J.S., Gaydos, C.A., Witter, F., 2013. *Trichomonas vaginalis* vaginitis in obstetrics and gynecology practice: new concepts and controversies. *Obstet Gynecol Surv*. 68: 43-50.

- Cooper, R., Morre, D.J., Morre, D.M., 2005. Medicinal benefits of green tea. II. Review of anticancer properties. *J Altern Complement Med.* 11: 639-52.
- Eslami, G., Fallah, F., Taheri, S., Navidinia, M., Dabiri, H., Dadashi, M., et al., 2013. Evaluation of antibacterial effect of cinnamon extract on *Helicobacter pylori* isolated from dyspeptic patients. *Research in Medicine.* 37 (2): 85-89.
- S. Gupta, J.K. Sahni, J. Ali, R. Gabrani and S. Dang., 2012. "Development and Characterization of Green Tea Loaded Microemulsion for Vaginal Infections", *Advanced Material letters.* 3(6): 493-497.
- Jazani NH, Zartoshti M, Shahabi S, Yekta Z, Nateghi S., 2007. Evaluation of the Synergetic Effects of Water Soluble Extracts of Green Tea (*Camellia Sinensis*) on the Activity of Ciprofloxacin in Urinary Isolated *E. Coli*. *Journal of Biological Sciences* 7(8):1500-1503.
- Ju, J., Hong, J., Zhou, J.N., Pan, Z., Bose, M., Liao, J., Yang, G.Y., Liu, Y.Y., Hou, Z., Lin, Y., Ma, J., Shih, W.J., Carothers, A.M., Yang, C.S., 2005. Inhibition of intestinal tumorigenesis in *Apcmin/+* mice by (-) - epigallocatechin-3-gallate, the major catechin in green tea. *Cancer Res.* 65: 10623-31.
- Lamont, R.F, Sobel, J.D., Akins, R.A., et al., 2011. The vaginal microbiome: new information about genital tract flora using molecular based techniques. *BJOG.* 118: 533-49.
- Larsen, B., Monif, G.R., 2001. Understanding the bacterial flora of the female genital tract. *Clin Infect Dis.* 32: e69-e77.
- Mabe, K., Yamada, M., Oguni, I., Takahashi, T., 1999. In vitro and in vivo activities of tea catechins against *Helicobacter pylori*. *Antimicrob Agents Chemother.* 43: 1788-91.
- Marrazzo, J.M., Koutsky, L.A., Eschenbach, D.A., Agnew, K., Stine, K., Hillier, S.L., 2002. Characterization of vaginal flora and bacterial vaginosis in women who have sex with women. *J Infect Dis.* 185: 1307-13.
- Mbata, T.I., Debiao, L.U., Saikia, A., 2008. Antibacterial activity of the crude extract of Chinese green tea (*Camellia sinensis*) on *Listeria monocytogenes*, *African Journal of Biotechnology.* 7 (10): 1571-1573.
- McFarland, J., 1907. Nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *Journal of the American Medical Association.* 14: 1176-1178.
- Nardis, C., et al., 2013. Vaginal microbiota and viral sexually transmitted Diseases. *Ann Ig.* 25: 443-456 doi: 10.7416 / ai.2013.1946.
- Ravel, J., Gajer, P., Abdo, Z., 2011. Vaginal microbiome of reproductive-age women. *PNAS.* 108 (Suppl 1): 4680-87
- Redondo-Lopez, V., Cook, R.L., Sobel, J.D., 1990. Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora. *Rev Infect Dis.* 12: 856-72.
- Sang Hee Kim, et al., 2008. Antimicrobial and antifungal effects of a green tea extract against vaginal pathogens. *Journal of Women's Medicine.* 1(1): 27-8.
- Srinivasan, S., Fredricks, D.N., 2008. The human vaginal bacterial biota and bacterial vaginosis. *Interdiscip Perspect Infect Dis.* 750: 479.
- Yang, C.S., Maliakal, P., Meng, X., 2002. Inhibition of carcinogenesis by tea. *Annu Rev Pharmacol.* 42: 25-54.