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Antimicrobial activities of widely consumed herbal tea’s alone or in combination with antibiotics: An in vitro study

Mayram Tuysuz, Sibel Dosler, Ayse Seher Birteksoz Tan, Gulten Otuk

Background: Because of increasing antibiotic resistance, herbal teas are the most popular natural alternatives, which are gaining even more importance. We examined the antimicrobial activities of 31 herbal teas both alone and in combination with antibiotics or antifungals against the standard and clinical isolates of Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, methicillin susceptible/resistant Staphylococcus aureus and Candida albicans. Methods: The antimicrobial activities of the teas were determined by using the disk diffusion and microbroth dilution methods, and the combination studies were examined by using the microbroth checkerboard and time killing curve methods. Results: Rosehip, rosehip bag, pomegranate blossom, thyme, wormwood, mint, echinacea bag, cinnamon, black, and green teas were active against most of the studied microorganisms. In the combination studies, we characterized all the expected effects (synergistic, additive, and antagonistic) between the teas and the antimicrobials. While synergy was observed more frequently between ampicillin, ampicillin-sulbactam, or nystatine, and the various tea combinations, most of the effects between the ciprofloxacin, erythromycin, cefuroxime, or amikacin and various tea combinations, particularly rosehip, rosehip bag, and pomegranate blossom teas, were antagonistic. The results of the time kill curve analyses showed that none of the herbal teas were bactericidal in their usage concentrations; however, in combination they were. Discussion: Some herbal teas, particularly rosehip and pomegranate blossom should be avoided because of antagonistic interactions during the course of antibiotic treatment or should be consumed alone.
Title: Antimicrobial Activities of Widely Consumed Herbal Tea’s alone or in Combination with Antibiotics

Short title: Antimicrobial Activities of Herbal Tea’s

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Abstract

**Background:** Because of increasing antibiotic resistance, herbal teas are the most popular natural alternatives, which are gaining even more importance. We examined the antimicrobial activities of 31 herbal teas both alone and in combination with antibiotics or antifungals against the standard and clinical isolates of *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, methicillin susceptible/resistant *Staphylococcus aureus* and *Candida albicans*.

**Methods:** The antimicrobial activities of the teas were determined by using the disk diffusion and microbroth dilution methods, and the combination studies were examined by using the microbroth checkerboard and time killing curve methods.

**Results:** Rosehip, rosehip bag, pomegranate blossom, thyme, wormwood, mint, echinacea bag, cinnamon, black, and green teas were active against most of the studied microorganisms. In the combination studies, we characterized all the expected effects (synergistic, additive, and antagonistic) between the teas and the antimicrobials. While synergy was observed more frequently between ampicillin, ampicillin-sulbactam, or nystatine, and the various tea combinations, most of the effects between the ciprofloxacin, erythromycin, cefuroxime, or amikacin and various tea combinations, particularly rosehip, rosehip bag, and pomegranate blossom teas, were antagonistic. The results of the time kill curve analyses showed that none of the herbal teas were bactericidal in their usage concentrations; however, in combination they were.
**Discussion:** Some herbal teas, particularly rosehip and pomegranate blossom should be avoided because of antagonistic interactions during the course of antibiotic treatment or should be consumed alone.

**Keywords:** Herbal tea, antimicrobial activity, combination, checkerboard, time kill curve.

**Introduction**

Although antibiotics are the major drugs used for the treatment of infectious diseases, in recent years, antibiotic resistance has been increasing, and is becoming a serious problem in infection control (Akova, 2016). Some microorganisms may develop a resistance to a single antimicrobial agent and others that are called “multidrug-resistant (MDR) strains” to several agents. Infections caused by these strains, often fail to respond to standard treatment and generate a greater risk of death due to the spread of the resistance to other microorganisms (Giamarello, 2010; Martis, Leroy & Blanc, 2014). In some cases, MDR microorganisms, which are called “pan-resistant organisms”, have become resistant to all the available antibiotics and cannot be treated with any single antibiotic alone (Khosravi & Mohammadian, 2016). The failure of the existing antibiotics to control infections makes it crucial to find alternative agents with new mechanisms of action. One such novel therapeutic strategy involves the use of natural antimicrobial compounds such as plant-derived products such as spices, essential oils, the extracts or the consumption of herbal teas alone or in combination with antibiotics.

Herbal teas, besides their delicious properties, are used for the treatment of human diseases worldwide. Green and black teas, which are consumed by over two-thirds of the world’s population, are the most popular beverages next to water. Approximately 4.50 million tons of tea is produced and consumed yearly, and the largest producers are the Republic of China, India,
Kenya, Sri Lanka, and Turkey (Bansal et al., 2013). The tea that originates from the leaves of the plant *Camellia sinensis* (*L*) exists in four main types according to its harvesting and processing: white, green, black, and oolong. As a beverage, tea is commonly prepared by infusing the *C. sinensis* leaves in hot water. These leaves contain approximately 2000 different phytochemicals such as phenolic compounds, methyl-xanthines, carbohydrates, proteins, free amino acids, L-ascorbic and other organic acids, volatile compounds, lipids, carotenoids, chlorophylls, minerals, and trace elements. Polyphenols are the most important constituents of tea leaves because of their higher relative abundance and bioactive properties (Moderno, Carvalho&Silva, 2009). Fresh green tea leaves are rich in monomeric flavanols known as catechins (Bansal et al., 2013). The most abundant and biologically active catechin is epigallocatechin-3-gallate (EGCG), and the other catechin derivatives are (−)-epicatechin-3-gallate, (−)-epigallocatechin, (−)-epicatechin, (+)-catechin, (+)-gallocatechin, and (−)-gallocatechin-3-gallate (Moderno, Carvalho&Silva, 2009). Tea and its components contain many health-promoting abilities such as protection from cardiovascular diseases, the control of obesity and diabetes, and have anticarcinogenic, antiaging, antihistaminic, antiarthritic, anti-inflammatory, antibacterial, antifungal, and antiviral effects (Patel, 2005).

Although the studies on other herbal teas or components are limited, there is extensive literature suggesting the health benefits of consuming teas prepared from *C. sinensis*. In particular, the antimicrobial activities of catechins against multidrug resistant clinical isolates of *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, enterohemorrhagic *Escherichia coli*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Mycobacterium tuberculosis*, and *Candida* *sp.* have been demonstrated (Anand, Kaul&Sharma, 2006; Gordon&Wareham, 2010; Hu et al., 2002; Isogai et al., 2001; Osterburg et al., 2009; Park et al., 2011).
peppermint, chamomile, sage, thyme, and cinnamon also have antimicrobial activities and other health benefits (McKay & Blumberg, 2006a; McKay & Blumberg, 2006b; Peng et al., 2010; Shan et al., 2007). In the present study, we examined the antimicrobial activities of 31 herbal teas alone and in combination with antibiotics or antifungals against both standard and clinical isolates of *Pseudomonas aeruginosa*, *A. baumannii*, *E. coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, methicillin-susceptible *S. aureus* (MSSA), MRSA, and *C. albicans*, which can cause serious nosocomial or community-acquired infections.

**Materials and methods**

**Microorganisms**

The clinical isolates of eight different organisms were obtained from different specimens the specimens submitted to the Clinical Microbiology Laboratories of Istanbul University, Istanbul Faculty of Medicine, single sample per person. Isolates were identified with Vitek 2 (BioMerieux, France) and verified with API test kits (BioMerieux, France). The standard strains of *P. aeruginosa* ATCC 27853, *A. baumannii* ATCC 19606, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 4352, *E. faecalis* ATCC 29212, MSSA ATCC 29213, MRSA ATCC 43300, and *C. albicans* ATCC 10231 were used in the study.

**Teas**

Aqueous tea infusions of the following teas were prepared by adding 100 ml of boiling water to 10 g of dried tea leaves: green, black, thyme, linden, lemon balm, hibiscus, wormwood, rosemary, nettle, chamomile, bay, yarrow, eucalyptus, lavender, mint, rosehip, pomegranate blossom, galangal, orange, sage, cinnamon, ginger, herb bennet, and echinacea teas. After 30 min of infusion, the teas were filtered through 0.40- and 0.22-µm filters. These 10% tea infusions
were aliquoted and stored at −20°C. The infusions using tea bags of green, black, linden, chamomile, rosehip, sage, and echinacea tea were also prepared and stored as described above (Peng et al., 2010). All the teas were purchased from domestic markets and herbalists.

**Antibiotics and Antifungals**

Erythromycin, ciprofloxacin, linezolid, ampicillin, ampicillin–sulbactam, cefuroxime, amikacin, ceftazidime, doxycycline, and fluconazole were kindly provided by their manufacturers, and itraconazole and nystatine were purchased from Sigma (Sigma, St. Louis, MO, USA). The stock solutions from the dry powders were prepared at a concentration of 1280 mg/L for the antifungals and 5120 mg/L for the antibiotics. They were stored frozen at −80°C for up to six months.

**Media**

Mueller–Hinton broth (MHB; Difco Laboratories, Detroit, Mich., USA) and Roswell Park Memorial Institute 1640 medium (RPMI) supplemented with L-glutamine and buffered with morpholine propanesulfonic acid (Sigma, St. Louis, MO, USA) were used for all the experiments. The pour plates of Tryptic soy agar and Sabouraud dextrose agar (Difco Laboratories) were used for the colony counts.

**Antimicrobial Activity**

The antimicrobial activities of the teas were primarily scanned by using the Clinical and Laboratory Standards Institute (CLSI, 2014) disc diffusion method. The minimum inhibitory concentrations (MIC) of the teas that had an antimicrobial activity, which was observed from disc diffusion tests, were determined by using the microdilution technique, as described by CLSI (CLSI, 2006). Serial two-fold dilutions ranging from 128 to 0.06 mg/L for ampicillin; 64 to 0.03 mg/L for erythromycin, linezolid, ampicillin–sulbactam, cefuroxime, amikacin, ceftazidime, and
doxycycline; and 32 to 0.015 mg/L for ciprofloxacin and antifungals were prepared in MHB and RPMI respectively. Each well was inoculated with the overnight cultures of the bacteria and fungi that gave the final concentrations of $1 \times 10^6$ and $1 \times 10^3$ colony forming units/ml (cfu) respectively. The trays were covered and placed in plastic bags to prevent evaporation, and then incubated at 37°C for bacteria and yeast, 24 and 48 h respectively. The sterility and growth controls were also added. The MIC was defined as the lowest concentration of the antimicrobials to completely inhibit the visible growth, as described by CLSI. For antifungals, the lowest concentration inhibiting any visible growth at 48 h was used as the MIC for nystatine whereas the lowest concentration associated with a significant reduction in turbidity compared with the control well at 48 h was used as the MIC for fluconazole and itraconazole. Experiments were performed in duplicates.

**Determination of Fractional Inhibitory Concentration Index (FICI)**

The interactions between the teas and the antimicrobials were tested by using the microbroth checkerboard technique (Pillai, Moellering&Eliopoulos, 2005). Each microtiter well containing the mixture of teas and antimicrobials in different final concentrations ranging from 2× MIC to 1/8× MIC was inoculated with fresh cultures overnight. After incubation at 37°C for 18-20 h, the following formulas were used to calculate the FIC index: $\text{FIC}_A = (\text{MIC}_A \text{ in combination})/(\text{MIC}_A \text{ alone})$, $\text{FIC}_B = (\text{MIC}_B \text{ in combination})/(\text{MIC}_B \text{ alone})$, and the FIC index = $\text{FIC}_A + \text{FIC}_B$. The combination value was derived from the highest dilution of the antimicrobial combination that permitted no visible growth. With this method, a FICI of $\leq 0.5$ was considered synergistic, of $> 0.5–4$ was considered to be additive, and of $> 4.0$ was considered to be antagonistic (Odds, 2003). The experiments were performed in duplicates.

**Time Kill Assays**
The killing kinetics of the tea extracts, which were significantly synergistic or antagonistic with antibiotics, were determined by using the time-kill method according to the National Committee for Clinical Laboratory Standards (NCCLS, 1999). The time kill curves (TKC) were constructed by plotting the mean colony counts (log 10 cfu/ml) versus time. The bacterial suspensions of six different clinical isolates were incubated at 37°C with gentle shaking, and the viable bacterial counts were performed after 0, 2, 4, 7, and 24 h incubation. One milliliter of the bacterial suspension was withdrawn and serially diluted with a sterile saline solution. Fifty and 100 µl of each dilution were spotted on the agar plates, and the cfu was determined after the overnight incubation of the plates at 37°C. An antibiotic-free control was included for each strain. The lower limit of the detection for the time kill assays was 1 log 10 cfu/ml. The antibiotic carry-over was controlled by the inhibition of the colonial growth at the side of the initial streak according to the NCCLS guidelines. The results were interpreted by the effect of the combination in comparison with that of the most active agent alone. Synergy and antagonism were defined as a 2 log 10 decrease and increase respectively in the colony count at 24 h. The bactericidal activity was defined as a $\geq 3$ log 10 cfu/ml decrease from the initial inoculum.

**Results**

**Susceptibility**

Of the 31 teas (24 different herbs and seven bag teas), only 15 showed inhibition zones against one or more microorganisms in the disk diffusion assays (Table S.1). The MIC values of the teas that were active in the disk diffusion test, along with the antibiotic and antifungal activities against clinical and standard strains of the bacteria and fungi are summarized in Tables 1 and 2. According to these results, the clinical isolates are more sensitive to teas than the standard strains. Rosehip, rosehip bag, and pomegranate blossom were the most effective teas
against bacteria. Thyme, wormwood, mint, black, and green teas were highly effective against *S. aureus*. Moreover, echinacea bag and cinnamon teas were active against the clinical strains of *S. aureus* and *C. albicans* respectively.

**Checkerboard**

The results of the combination studies performed using the microbroth checkerboard technique against the clinical and standard strains are shown in Tables 3 and 4. With a FICI of $\leq 0.5$ as the borderline, synergistic interactions were observed between ampicillin or ampicillin–sulbactam, and various tea combinations against *S. aureus*, *E. coli*, or *A. baumannii*. Moreover, with a FICI of $> 4$ as the borderline, antagonistic effects were observed particularly between rosehip, pomegranate, or rosehip bag teas, and ciprofloxacin, erythromycin, cefuroxime, ampicillin–sulbactam, amikacin, or doxycycline against various microorganisms. There were no antagonist interactions between the teas and the antifungals.

**Time Kill Assays**

The results of the TKC analyses showed that with a 3 log 10 kill as the borderline, none of the herbal teas alone showed bactericidal activity at their indicated concentrations, whereas in the combinations with various antibiotics they were bactericidal against *P. aeruginosa* and *S. aureus*. The synergistic interactions of teas and antibiotics were observed especially rosehip bag tea and antibiotic combinations against *S. aureus* and *P. aeruginosa*. Besides this, we also observed synergistic combinations also between ampicillin and tea combinations against *S. aureus*. Antagonistic or early antagonistic (4–7 h) interactions especially observed between roshiep bag tea and antibiotics combinations against *E. coli*. Otherwise antagonistic or early antagonistic (4–7 h) interactions were rare and seen ciprofloxacin, amikacin and cefuroxime and rosehip, black tea and green tea bag teas against several bacteria. The results are shown in Fig. 1, 2 and 3.
Discussion

Traditionally, complementary and alternative medicines are widely used and are rapidly growing health systems, including Chinese medicine, Indian ayurveda, and Arabic medicine, which use plant material, animal parts, and/or minerals (WHO, 2002). Among them, the potential health-promoting effects of plants can be traced back to the earliest recorded history (Dubick, 1986). Even though other materials such as foods are used to promote health and treat diseases, none of them have received more attention than herbs. The use of herbs includes herbal materials, herbal preparations, and finished herbal products that contain active ingredients, the parts of plants, other plant materials, or their combinations (WHO, 2002).

Some of the most popular natural products, which are gaining more importance because of their increasing antibiotic resistance, are herbal teas. Herbal teas such black, green, peppermint, sage, and thyme, are widely used for the protection and treatment of human diseases worldwide. It is known that teas, especially those that contain catechin, have many health-promoting abilities such as antibacterial, antifungal, and antiviral (Bansal et al., 2013). The antimicrobial activities of this catechin containing black and green teas has been previously demonstrated against a variety of organisms, including multiresistant clinical isolates of gram-negative and -positive bacteria and also yeasts (Anand, Kaul&Sharma, 2006; Gordon&Wareham, 2010; Hu et al., 2002; Isogai et al., 2001; Osterburg et al., 2009; Park et al., 2011). In this study, we examined the antimicrobial activities of 31 different herbal teas, both alone and in combination with chemical antimicrobials. According to these experiments, rosehip, rosehip bag, pomegranate blossom, thyme, wormwood, mint, echinacea bag, cinnamon, black, and green teas were found to be effective against most of the studied microorganisms. In general, the studied teas showed a better antimicrobial activity against gram-positive bacteria compared with the others. We hypothesized that the differences in
the antimicrobial activities of the various teas would depend on either the type of microbial strain or the tea. Similar results have been obtained by other researchers (Hu et al., 2001; Novy et al., 2013). These results suggested that herbal teas could be a prophylactic or first base treatment agents for bacterial infections.

Combinations of two or more antimicrobial drugs are necessary to treat MDR or pan-resistant bacterial infections. Because mono therapy is no longer adequate, combination therapies seem to be the next logical choice; however, neither antibiotic–antibiotic combinations nor antibiotic plus non antibiotic adjuvant combinations have been successful in combating MDR infections (Tangden, 2014). Apparently, herbal teas are becoming a large part of alternative or complementary medicine, either as a single agent or as an adjuvant in antimicrobial chemotherapy (Hu et al., 2001; Cho, Oh&Oh, 2011). Antibiotic and herbal tea combinations may be recommended for severe infections in order to rapidly enhance bactericidal activity and help prevent or delay the emergence of resistance.

In this study, we examined the in vitro interactions between teas and antimicrobials by using one of the most simple and best known tests, namely the microbroth checkerboard technique. We have characterized all three of the expected effects, including synergistic, additive, and antagonistic interactions between the tea and antimicrobial combinations. Synergy was more frequently observed between ampicillin, ampicillin–sulbactam, or nystatine, and various tea combinations. Similarly, Hu et al., (2001) found that ampicillin–sulbactam and EGCG combinations were synergistic against MRSA strains. Lee et al., (2005) also showed that ciprofloxacin and catechin combinations were synergistic against E. coli in a chronic bacterial prostatitis rat model. Similar results were obtained by others, particularly between catechins and antibiotic combinations against gram-positive bacteria (Hu et al., 2001; Novy et al., 2013; Zhao
et al., 2002). Although ampicillin or nystatine combinations were synergistic, most of the
ciprofloxacin, erythromycin, cefuroxime, or amikacin and various tea combinations, especially
the rosehip, rosehip bag, and pomegranate blossom teas, were found to be antagonistic against all
of the studied bacteria. Similarly Hu et al., (2002) found that EGCG showed antagonistic
interactions with vancomycin, teicoplanin, or polymyxin B against MRSA.

The clinical usage of antibiotic combinations is common, especially in the treatment of
patients with serious illnesses, polymicrobial infections, and infections caused by MDR or pan-
resistant microorganisms. The most desirable targets for combination therapy are synergistic
drug interactions followed by the prevention of resistance and minimization of toxicity and cost.
When deciding the combined antimicrobial treatment, it is very important to know the possible
interactions between the antimicrobial agents for the success of the therapy. In contrast,
antagonism is the most disadvantageous outcome for clinicians because the effect of the
combination may be less than that of drug alone (Pillai, Moellering&Eliopoulos, 2005). In this
study, we found that some of the antibiotic–herbal tea combinations have an antagonistic
interactions. Thus, herbal teas, particularly rosehip and pomegranate blossom, should be either
consumed alone or avoided in the course of the antibiotic treatment.

Although MIC is still the gold standard for determining the antimicrobial activities of
agents, and the microbroth checkerboard is the most simple and widely used technique for the
assessment of combination effects, these techniques do not provide any information about the
time course of the antimicrobial activities. TKC studies can be used to overcome this limitation.
In this study, according to the TKC results, the synergistic interactions against S. aureus were
more frequent between ampicillin and tea combinations, just as those in the results of the
checkerboard technique. On the other hand, antagonistic interactions were not as frequent in the
checkerboard technique. There were only a few ciprofloxacin and tea combinations that had an
antagonistic or early antagonistic (within 4–7 h) effect. The difference in our combination results
between the TKC and checkerboard techniques may cause the bacteriostatic drug interactions
from the checkerboard technique, whereas the bactericidal interactions were obtained from the
TKC analyses. According to these results black tea, green tea and rosehip bag teas could be used
effectively and safely while ampicillin treatment as enhancer of antibacterial treatment.

Nevertheless black, green and rosehip bag teas should not be used during the antibiotic treatment
especially with ciprofloxacin due to their adverse effects.

Conclusion

When we examined the antimicrobial activities of various herbal teas, alone and in
combination with antibiotics, our findings showed that herbal teas have antimicrobial activities
against gram-positive and -negative bacteria and yeast when they were used alone. The
combinations of herbal teas with antibiotics showed synergistic, additive, or antagonistic effects,
depend on the antibiotic or kind of tea. Consequently, using herbal teas alone or with some
chemical antimicrobials could be an effective alternative treatment strategy against various
pathogenic microorganisms. Furthermore, herbal teas alone or in combination may help reduce
the severity of a disease; however, some combinations with antibiotics could reduce the efficacy
of the primary antibiotic and thus, should not be used together.

References

Akova M. 2016. Epidemiology of antimicrobial resistance in bloodstream infections. Virulence.
2016. 7(3):252-266. DOI:10.1080/21505594.2016.1159366
Anand PK, Kaul D, Sharma M. 2006. Green tea polyphenol inhibits Mycobacterium tuberculosis survival within human macrophages. The International Journal of Biochemistry & Cell Biology 38: 600–609. DOI 10.1016/j.biocel.2005.10.021

Bansal S, Choudhary S, Sharma M, Kumar SS, Lohan S, Bhardwaj V, Syan N, Jyoti S. 2013. Tea: A native source of antimicrobial agents. Food Research International 53: 568-584. DOI 10.1016/j.foodres.2013.01.032

Cho YS, Oh JJ, Oh KH. 2011. Synergistic anti-bacterial and proteomic effects of epigallocatechin gallate on clinical isolates of imipenem-resistant Klebsiella pneumoniae. Phytomedicine 18: 941-946. DOI: 10.1016/j.phymed.2011.03.012

Clinical and Laboratory Standards Institute (CLSI). 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 7. ed. Approved Standard M7-A7. Wayne, PA.

Clinical and Laboratory Standards Institute (CLSI). 2014. Performance and standards for antimicrobial susceptibility testing; twenty-second informational supplement M100-S24, Vol. 34 No. 1. Wayne, PA.

Dubick MA. 1986. Historical Perspectives on the Use of Herbal Preparations to Promote Health. The Journal of Nutrition 116:1348-1354.

Giamarellou H. 2010. Multidrug-resistant gram-negative bacteria: how to treat and for how long. International Journal of Antimicrobial Agents 36: S50–S54. DOI 10.1016/j.ijantimicag.2010.11.014.

Gordon NC. Wareham DW. 2010. Antimicrobial activity of the green tea polyphenol (-)-epigallocatechin-3-gallate (EGCG) against clinical isolates of Stenotrophomonas maltophilia.
Hu ZQ. Zhao WH. Hara Y. Shimamura T. 2001. Epigallocatechin gallate synergy with ampicillin/sulbactam against 28 clinical isolates of methicillin-resistant *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy* 48: 361-368. DOI 10.1093/jac/48.3.361

Hu ZQ. Zhao WH. Yoda Y. Asano N. Hara Y. Shimamura T. 2002. Additive, indifferent and antagonistic effects in combinations of epigallocatechin gallate with 12 non-beta-lactam antibiotics against methicillin-resistant *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy* 50: 1051–1054 DOI 10.1093/jac/dkf250

Isogai E. Isogai H. Hirose K. Hayashi S. Oguma K. 2001. In vivo synergy between green tea extract and levofloxacain against enterohemorrhagic *Escherichia coli* O157 infection. *Current Microbiology* 42: 248–251. DOI 10.1007/s002840110212

Khosravi AD, Mohammadian A. 2016. Efflux MexAB-mediated resistance in multidrug and pan-drug resistant strains of *Pseudomonas aeruginosa* isolated from patients with burn and wound infections. *Jundishapur J Nat Pharm Prod*. 11(1): e25352, DOI: 10.17795/jjnpp-25352.

Lee YS. Han CH. Kang SH. Lee SJ. Kim SW. Shin OR. Sim YC. Lee SJ. Cho YH. 2005. Synergistic effect between catechin and ciprofloxacin on chronic bacterial prostatitis rat model. *International Journal of Urology* 12: 383–389. DOI 10.1111/j.1442-2042.2005.01052.

Martis N. Leroy S. Blanc V. 2014. Colistin in multi-drug resistant *Pseudomonas aeruginosa* blood-stream infections a narrative review for the clinician. *Journal of Infection*, DOI 10.1016/j.jinf.2014.03.001.
McKay DL. Blumberg JB. 2006. A review of the bioactivity and potential health benefits of chamomile tea (*Matricaria recutita L*). *Phytotherapy Research* 20: 519-530. DOI 10.1002/ptr.1900

McKay DL. Blumberg JB. 2006. A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita L*). *Phytotherapy Research* 20: 619-633. DOI 10.1002/ptr.1936

Moderno PM. Carvalho M. Silva BM. 2009. Recent patents on *Camellia sinensis*: source of health promoting compounds. *Recent Patents on Food, Nutrition & Agriculture* 1:182-192. DOI 10.2174/2212798410901030182

National Committee for Clinical Laboratory Standards (NCCLS). 1999. Methods for determining bactericidal activity of antimicrobial agents. Approved Guideline. M26-A. Wayne, PA.

Novy P. Rondevaldova J. Kourimska L. Kokoska L. 2013. Synergistic interactions of epigallocatechin gallate and oxytetracycline against various drug resistant *Staphylococcus aureus* strains in vitro. *Phytomedicine* 20: 432-435. DOI 10.1016/j.phymed.2012.12.010.

Odds FC. 2003. Synergy, antagonism, and what the chequerboard puts between them. *Journal of Antimicrobial Chemotherapy* 52: 1. DOI 10.1093/jac/dkg301

Osterburg A. Gardner J. Hyon SH. Neely A. Babcock G. 2009. Highly antibiotic-resistant *Acinetobacter baumannii* clinical isolates are killed by the green tea polyphenol (-)-epigallocatechin-3-gallate (EGCG). *Clinical Microbiology and Infection* 15: 341-6. DOI 10.1111/j.1469-0691.2009.02710.x

Park BJ. Taguchi H. Kamei K. Matsuzawa T. Hyon SH. Park JC. 2011. In vitro antifungal activity of epigallocatechin 3-O-gallate against clinical isolates of dermatophytes. *Yonsei Medical Journal* 52: 535-538. DOI 10.3349/ymj.2011.52.3.535.
Patel SH. 2005. *Camellia sinensis*: historical perspectives and future prospects. *Journal of Agromedicine* 10: 57-64. DOI 10.1300/J096v10n02_08

Peng Q. Huang Y. Hou B. Hua D. Yao F. Qian Y. 2010. Green tea extract weakens the antibacterial effect of amoxicillin in methicillin-resistant *Staphylococcus aureus* infected mice. *Phytotherapy Research* 24: 141–145. DOI 10.1002/ptr.2952

Pillai SK. Moellering Jr RC. Eliopoulos GM. 2005. Antimicrobial combinations. In: Lorian, V, ed. *Antibiotics in Laboratory Medicine*. 5th ed. Lippincott Williams and Wilkins, Philadelphia (PA), 365-440

Shan B. Cai YZ. Brooks JD. Corke H. 2007. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. *International Journal of Food Microbiology* 117: 112–119. DOI 10.1016/j.ijfoodmicro.2007.03.003

Tangden T. 2014. Combination antibiotic therapy for multidrug-resistant Gram-negative bacteria. *Upsala Journal of Medical Sciences Early Online* 119: 149-153 DOI 10.3109/03009734.2014.899279.

World Health Organization (WHO). 2002. WHO Traditional medicine strategy 2002–2005.

World Health Organization Geneva

Zhao WH. Hu ZQ. Hara Y. Shimamura T. 2002. Inhibition of penicillinase by epigallocatechin gallate resulting in restoration of antibacterial activity of penicillin against penicillinase-producing *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* 46: 2266-2268. DOI 10.1128/AAC.46.7.2266-2268.2002.
Table 1 (on next page)

The MIC values of herbal teas against standard and clinical strains of microorganisms (%).

(-): Not determined
|                     | Thyme | Wormwood | Mint | Roship | Pomegranate blossom | Black tea | Green tea | Oregano | Cinnamon bag | Rosehip bag | Black bag | Green bag | Sage bag | Mint bag | Echinacea bag |
|---------------------|-------|----------|------|--------|---------------------|-----------|-----------|---------|--------------|-------------|-----------|-----------|----------|---------|----------------|
| **Standard strains**|       |          |      |        |                     |           |           |         |              |             |           |           |          |         |                |
| MRSA                | 0,31  | 0,62     | -    | 2,5    | 2,5                 | 0,31      | 0,07      | -       |              | 2,5         | -         | 0,31      | -        | -        | -                |
| MSSA                | -     | -        | -    | 2,5    | 2,5                 | 0,31      | 0,07      | -       |              | 2,5         | -         | 0,15      | -        | -        | -                |
| E.faecalis         | -     | -        | -    | 2,5    | 1,25                | -         | -         | -       |              | 2,5         | -         | -         | -        | -        | -                |
| E.coli             | -     | -        | -    | 2,5    | 1,25                | -         | -         | -       |              | 2,5         | -         | -         | -        | -        | -                |
| K.pneumoniae       | -     | -        | -    | 2,5    | 1,25                | -         | -         | -       |              | -           | -         | -         | -        | -        | -                |
| P.aeruginosa       | -     | -        | -    | 2,5    | 1,25                | -         | -         | -       |              | 2,5         | 1,25      | -         | -        | -        | -                |
| A.baumannii        | -     | -        | -    | 2,5    | 2,5                 | -         | -         | -       |              | 2,5         | 1,25      | -         | -        | -        | -                |
| C.albicans         | -     | -        | -    | -      | -                   | 0,15      | 0,07      | -       |              | -           | -         | -         | -        | -        | -                |
| **Clinical isolates**|       |          |      |        |                     |           |           |         |              |             |           |           |          |         |                |
| MRSA               | 0,62  | 1,25     | 0,62 | 1,25   | 1,25                | 0,62      | 0,15      | 0,31    |              | 2,5         | 0,31      | 0,15      | 0,62     | 0,62     | -                |
| MSSA               | 0,62  | 0,62     | 0,31 | 1,25   | 1,25                | 0,31      | 0,07      | 0,31    |              | 2,5         | 0,31      | 0,07      | 0,62     | 0,62     | 0,62             |
| E.faecalis        | -     | -        | -    | 1,25   | 0,62                | -         | -         | -       |              | 1,25        | -         | -         | -        | -        | -                |
| E.coli            | -     | -        | -    | 2,5    | 2,5                 | -         | -         | -       |              | 2,5         | -         | -         | -        | -        | -                |
| K.pneumoniae      | -     | -        | -    | 2,5    | 2,5                 | -         | -         | -       |              | 2,5         | -         | -         | -        | -        | -                |
| P.aeruginosa      | -     | -        | -    | 1,25   | 1,25                | -         | 1,25      | -       |              | 2,5         | 2,5       | -         | -        | -        | -                |
| A.baumannii       | -     | -        | -    | 1,25   | 1,25                | -         | 0,31      | -       |              | 1,25        | 0,62      | 0,62      | -        | -        | -                |
| C.albicans        | -     | -        | -    | -      | -                   | -         | -         | 2,5     |              | -           | -         | -         | -        | -        | -                |
Table 2 (on next page)

The MIC values of antibiotics and antifungals against standard and clinical strains of microorganisms (µg/ml)

ERY: erythromycin, CIP: ciprofloxacin, AMP: ampicillin, LZD: linezolid, SAM: ampicillin-sulbactam, CXM: cefuroxime, AMK: amikacin, CAZ: ceftazidime, DOX: doxycycline, FLU: fluconazole, ITRA: itraconazole, NYS: nystatine. (-): Not determined
### MIC (µg/ml)

| Microorganisms | ERY | CIP | AMP | LZD | SAM | CXM | AMK | CAZ | DOX | FLU | ITRA | NYS |
|----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|-----|
| **Standard**   |     |     |     |     |     |     |     |     |     |     |      |     |
| MRSA           | -   | 1   | -   | 2   | -   | -   | -   | -   | -   | -   | -    | -   |
| MSSA           | 0.25| 1   | 0.25| -   | -   | -   | -   | -   | -   | -   | -    | -   |
| E. faecalis    | -   | 1   | 2   | 2   | -   | -   | -   | -   | -   | -   | -    | -   |
| E. coli        | -   | 0.015| - | - | 4 | 2 | - | - | - | - | - | - |
| K. pneumoniae  | -   | 0.015| - | - | 1 | 0.25| - | - | - | - | - | - |
| P. aeruginosa  | -   | 0.25| - | - | - | - | 2 | 1 | - | - | - | - |
| A. baumanii    | -   | 1   | - | - | 2 | - | - | - | 0.0625 | - | - | - |
| C. albicans    | -   | -   | - | - | - | - | - | - | 1 | 0.25 | 2 | |
| **Clinical**   |     |     |     |     |     |     |     |     |     |     |     |     |
| MRSA           | -   | 32  | -   | 2   | -   | -   | -   | -   | -   | -   | -    | -   |
| MSSA           | 0.25| 0.5 | 128 | -   | -   | -   | -   | -   | -   | -   | -    | -   |
| E. faecalis    | -   | 4   | 4   | 4   | -   | -   | -   | -   | -   | -   | -    | -   |
| E. coli        | -   | 0.015| - | - | 16 | 0.5| - | - | - | - | - | - |
| K. pneumoniae  | -   | 0.03| - | - | 4 | 2 | - | - | - | - | - | - |
| P. aeruginosa  | -   | 0.25| - | - | - | - | 4 | 1 | - | - | - | - |
| A. baumanii    | -   | 16  | - | - | 64 | - | - | - | 8 | - | - | - |
| C. albicans    | -   | - | - | - | - | - | - | - | - | 0.25 | 0.25 | 2 |
Table 3

The FIC indexes of herbal tea and antibiotic combinations against Gram positive bacteria and *C. albicans*.

R: rosehip, PB: pomegranate blossom, BT: black tea, GT: green tea, R B: rosehip bag, GT B: green tea bag, T: thyme, W: wormwood, M: mint, S B: sage bag, G: ginger, E B: echinacea bag, BT B: black tea bag, O: orengo, C: cinnamon (-): Not determined:
| Herbal teas+ | MSSA | MRSA | E. faecalis | C. albicans |
|-------------|------|------|-------------|-------------|
|             | ERY  | CIP  | AMP | CIP | LZD | CIP | LZD | AMP | FLU | ITRA | NYS |
| **Clinical isolates** | | | | | | | | | | | |
| R           | 5    | 9    | 0.6 | 9   | 2   | 9   | 0.6 | 1.1 | -   | -   | -   |
| PB          | 5    | 9    | 0.6 | 9   | 2   | 5   | 1   | 0.5 | -   | -   | -   |
| BT          | 1    | 4    | 0.3 | 2   | 2   | -   | -   | -   | -   | -   | -   |
| GT          | 2    | 2    | 0.1 | 0.7 | 1   | -   | -   | -   | -   | -   | -   |
| R B         | ≥9   | 9    | 0.3 | 9   | 1   | 1.1 | 2   | 2   | -   | -   | -   |
| GT B        | 2    | 2    | 0.1 | 2   | 1   | -   | -   | -   | -   | -   | -   |
| T           | 1    | 0.6  | 0.5 | 1   | 0.7 | -   | -   | -   | -   | -   | -   |
| W           | 2    | 0.6  | 0.5 | 0.7 | 0.7 | -   | -   | -   | -   | -   | -   |
| M           | 0.6  | 2    | 0.7 | 1   | 1   | -   | -   | -   | -   | -   | -   |
| S B         | 1    | 0.6  | 0.7 | 2   | 1   | -   | -   | -   | -   | -   | -   |
| G           | 0.6  | 3    | 0.1 | 1   | 0.6 | -   | -   | -   | -   | -   | -   |
| E B         | 1    | 2.2  | 0.1 | -   | -   | -   | -   | -   | -   | -   | -   |
| BT B        | 3    | 2    | 0.5 | 0.7 | 0.6 | -   | -   | -   | -   | -   | -   |
| O           | 0.6  | 1.5  | 0.7 | 0.7 | 0.6 | -   | -   | -   | -   | -   | -   |
| C           | -    | -    | -   | -   | -   | -   | -   | -   | 0.7 | 0.7 | 0.7 |
| **Standard strains** | | | | | | | | | | | |
| R           | 5    | 5    | 1   | 8   | 0.6 | 5   | 2   | 0.7 | -   | -   | -   |
| PB          | 5    | 5    | 2   | 9   | 1   | 9   | 1   | 0.7 | -   | -   | -   |
| BT          | 0.7  | 5    | 0.7 | 2   | 0.7 | -   | -   | -   | 0.7 | 0.7 | 0.5 |
| GT          | 2    | 2    | 1   | 2   | 0.7 | -   | -   | -   | 0.7 | 0.6 | 0.3 |
| R B         | 5    | 5    | 2   | 9   | 2   | ≥9  | 2   | 0.7 | -   | -   | -   |
| GT B        | 0.75 | 2    | 1   | 3   | 0.7 | -   | -   | -   | -   | -   | -   |
| T           | -    | -    | -   | 2   | 0.7 | -   | -   | -   | -   | -   | -   |
| W           | -    | -    | -   | 3   | 0.7 | -   | -   | -   | -   | -   | -   |
| C           | -    | -    | -   | -   | -   | -   | -   | -   | 0.7 | 0.7 | 1.1 |
Table 4 (on next page)

The FIC indexes of herbal tea and antibiotic combinations against Gram negative bacteria

R: rosehip, PB: pomegranate blossom, GT: green tea, R B: rosehip bag, GT B: green tea bag, ,
BT B: black tea bag, BT: black tea (-): Not determined
| Herbal teas+ | **E. coli** | **P. aeruginosa** | **A. baumannii** | **K. pneumoniae** |
|-------------|-------------|-------------------|------------------|------------------|
|             | CXM SAM CIP | CIP AMK CAZ       | SAM CIP DOX      | CXM SAM CIP      |
| **Clinical isolates** |             |                   |                   |                   |
| R           | 9 2 ≥9      | 5 ≥5 2            | 0.7 9 0.7        | 5 9 9            |
| PB          | 3 0.7 ≥8    | 5 5 2             | 0.7 ≥8 0.7       | 9 3 9            |
| GT          | - - -       | ≥9 2 2            | 1 1 1            | - - -            |
| R B         | 1.5 0.3 ≥9  | 5 ≥5 1.5          | 0.5 ≥8 0.7       | ≥4 2 ≥8          |
| GT B        | - - -       | - - -             | 1 5 3            | - - -            |
| BT B        | - - -       | 9 ≥5 1.5          | 0.5 5 2          | - - -            |
| **Standard strains** |             |                   |                   |                   |
| R           | 5 0.7 5     | 5 5 2             | 0.7 9 5          | 5 3 5            |
| PB          | 5 1 5       | 9 9 1             | 2 9 3            | 5 3 5            |
| BT          | - - -       | - - -             | - - -            | - - -            |
| GT          | - - -       | 5 0.7 1           | 2 2 1.5          | - - -            |
| R B         | 5 1 5       | 5 9 2             | 2 9 5            | - - -            |
| BT B        | - - -       | 5 5 1             | 2 5 5            | - - -            |
Figure 1 (on next page)

Time kill curves of herbal tea + antibiotic combinations against *E. coli* and *K. pneumoniae*

**Fig 1.** Herbal teas + antibiotic combinations observed by time-kill determinations against standard and clinical strains of *E. coli* and *K. pneumonia* at 1× MIC. The X-axis represents time, and Y-axis represents the average of logarithmic standard and clinical bacteria survivals. Control: Bacteria without any antimicrobial treatment. RB: rosehip bag, SAM: ampicillin-sulbactam, CIP: ciprofloxacin, CXM: cefuroxime.
time kill curves of herbal tea + antibiotic combinations against *P. aeruginosa* and *A. baumannii*

**Fig 2.** Herbal teas + antibiotic combinations observed by time-kill determinations against standard and clinical strains of *P. aeruginosa* and *A. baumannii* at 1× MIC. The X-axis represents time, and Y-axis represents the average of logarithmic standard and clinical bacteria survivals. Control: Bacteria without any antimicrobial treatment. RB: rosehip bag, BTB: black tea bag, AMK: amikacin, CIP: ciprofloxacin, CAZ: ceftazidime, SAM: ampicillin-sulbactam, DOX: doxycycline.
Time kill curves of herbal tea + antibiotic combinations against *S. aureus* and *E. faecalis*.

**Fig 3.** Herbal teas + antibiotic combinations observed by time-kill determinations against standard and clinical strains of *S. aureus* and *E. faecalis* at 1× MIC. The X-axis represents time, and Y-axis represents the average of logarithmic standard and clinical bacteria survivals. Control: Bacteria without any antimicrobial treatment. BT: black tea, RB: rosehip bag, GT: green tea, R: rosehip, PB: pomegranate blossom, AMP: ampicillin, CIP: ciprofloxacin, ERY: erythromycin.
