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

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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.

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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 (Giamarellon 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). Other herbs such as

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 1×10^6 and 1×10^3 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: $FIC_A = (MIC_A \text{ in combination})/(MIC_A \text{ alone})$, $FIC_B = (MIC_B \text{ in combination})/(MIC_B \text{ alone})$, and the FIC index = $FIC_A + FIC_B$. The combination value was derived from the highest dilution of the antimicrobial combination that permitted no visible growth. With this method, a FICI of ≤ 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 ≤ 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 rosehip 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.

206 **Discussion**

207 Traditionally, complementary and alternative medicines are widely used and are rapidly
208 growing health systems, including Chinese medicine, Indian ayurveda, and Arabic medicine,
209 which use plant material, animal parts, and/or minerals (WHO, 2002). Among them, the potential
210 health-promoting effects of plants can be traced back to the earliest recorded history (Dubick,
211 1986). Even though other materials such as foods are used to promote health and treat diseases,
212 none of them have received more attention than herbs. The use of herbs includes herbal
213 materials, herbal preparations, and finished herbal products that contain active ingredients, the
214 parts of plants, other plant materials, or their combinations (WHO, 2002).

215 Some of the most popular natural products, which are gaining more importance because of
216 their increasing antibiotic resistance, are herbal teas. Herbal teas such black, green, peppermint,
217 sage, and thyme, are widely used for the protection and treatment of human diseases worldwide.
218 It is known that teas, especially those that contain catechin, have many health-promoting abilities
219 such as antibacterial, antifungal, and antiviral (Bansal et al., 2013). The antimicrobial activities of
220 this catechin containing black and green teas has been previously demonstrated against a variety
221 of organisms, including multiresistant clinical isolates of gram-negative and -positive bacteria and
222 also yeasts (Anand, Kaul&Sharma, 2006; Gordon&Wareham, 2010; Hu et al., 2002; Isogai et al.,
223 2001; Osterburg et al., 2009; Park et al., 2011). In this study, we examined the antimicrobial
224 activities of 31 different herbal teas, both alone and in combination with chemical antimicrobials.
225 According to these experiments, rosehip, rosehip bag, pomegranate blossom, thyme, wormwood,
226 mint, echinacea bag, cinnamon, black, and green teas were found to be effective against most of
227 the studied microorganisms. In general, the studied teas showed a better antimicrobial activity
228 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.

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Table 1(on next page)

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

(-): Not determined

1

	Thyme	Wormwood	Mint	Rosehip	Pomegranate blossom	Black tea	Green tea	Oregano	Cinnamon	Rosehip bag	Black bag	Green bag	Sage bag	Mint bag	Echinac 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.baumanii	-	-	-	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

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Microorganisms	MIC (μg/ml)											
	ERY	CIP	AMP	LZD	SAM	CXM	AMK	CAZ	DOX	FLU	ITRA	NYS
Standard strains												
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 isolates												
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

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Table 3(on next page)

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 :

1

	MSSA			MRSA		*E. faecalis*			*C. albicans*		
Herbal teas+	**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

2

3

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

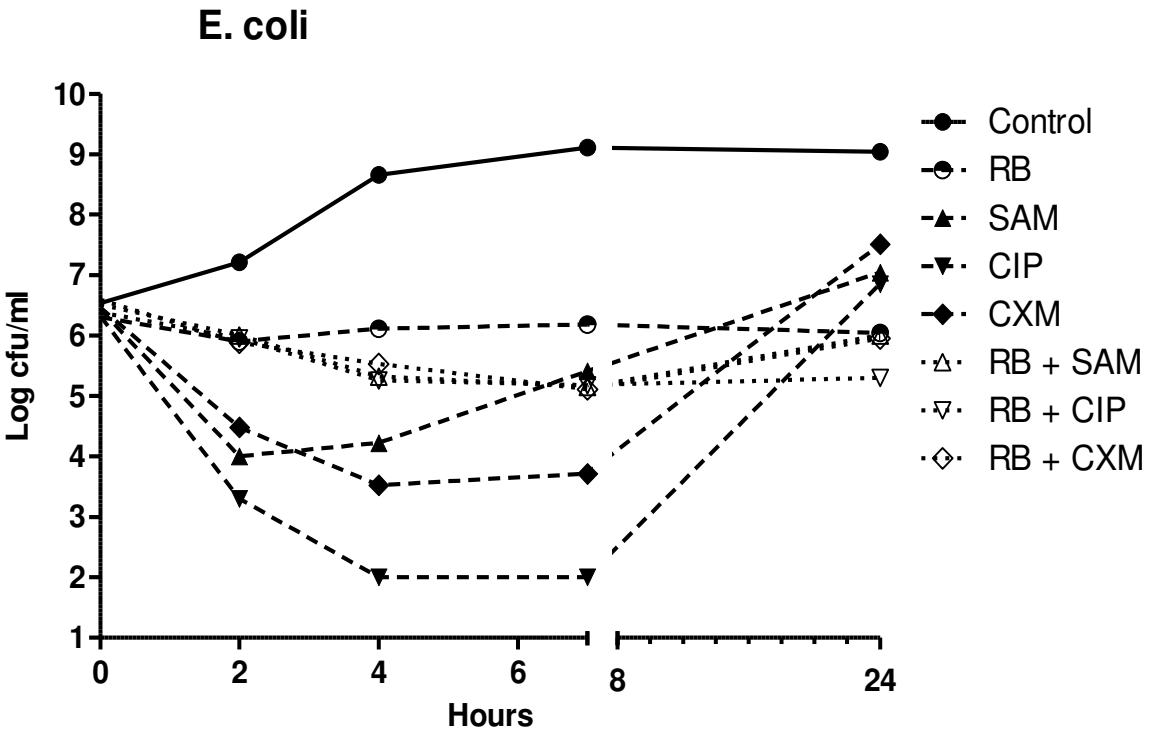
	E. coli			*P. aeruginosa*			*A. baumannii*			*K. pneumoniae*		
Herbal teas+	**CXM**	**SAM**	**CIP**	**CIP**	**AMK**	**CAZ**	**SAM**	**CIP**	**DOX**	**CXM**	**SAM**	**CİP**
Clinical isolates												
R	9	2	≥9	5	≥5	2	0.7	9	0.7	5	9	9
PB	3	0.7	≥8	5	5	2	0.7	≥8	0.7	9	3	9
GT	-	-	-	≥9	2	2	1	1	1	-	-	-
R B	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	-	-	-

1

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.



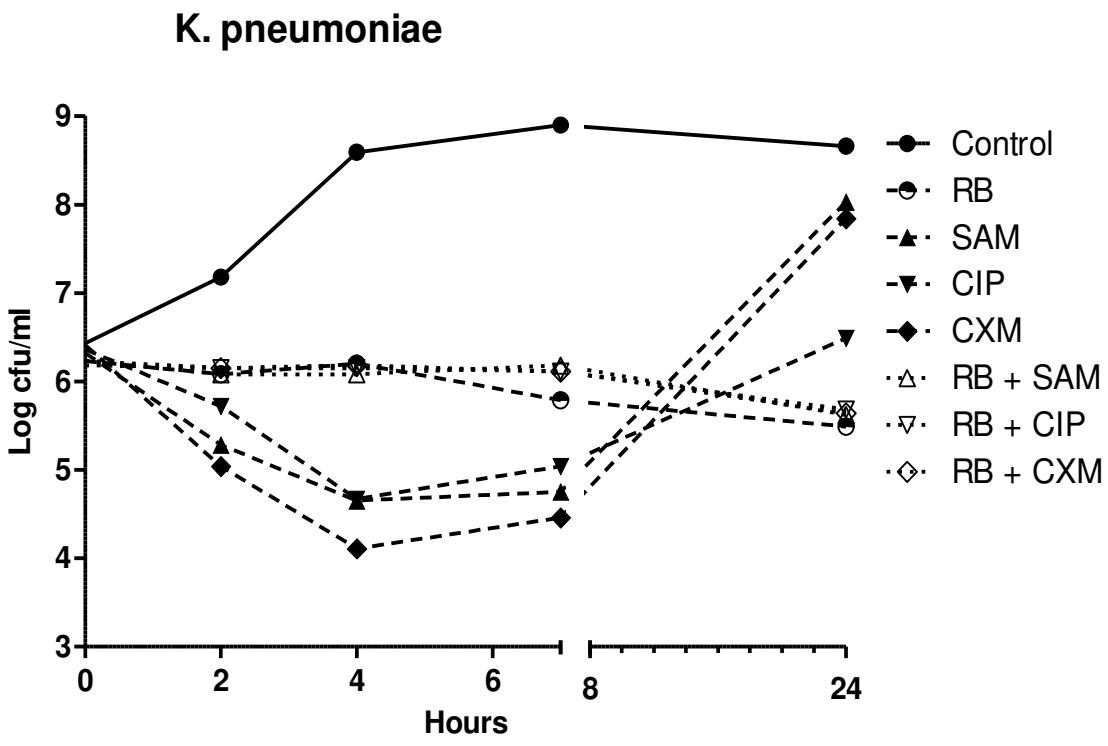
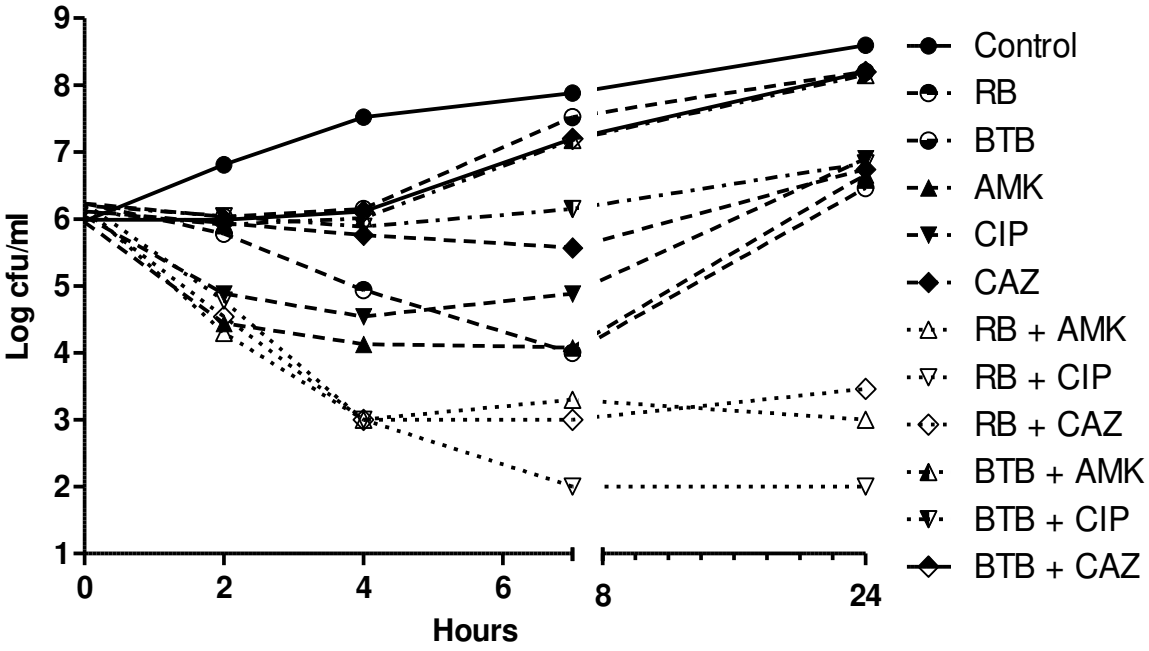


Figure 2(on next page)

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.

P. aeruginosa



A. baumanii

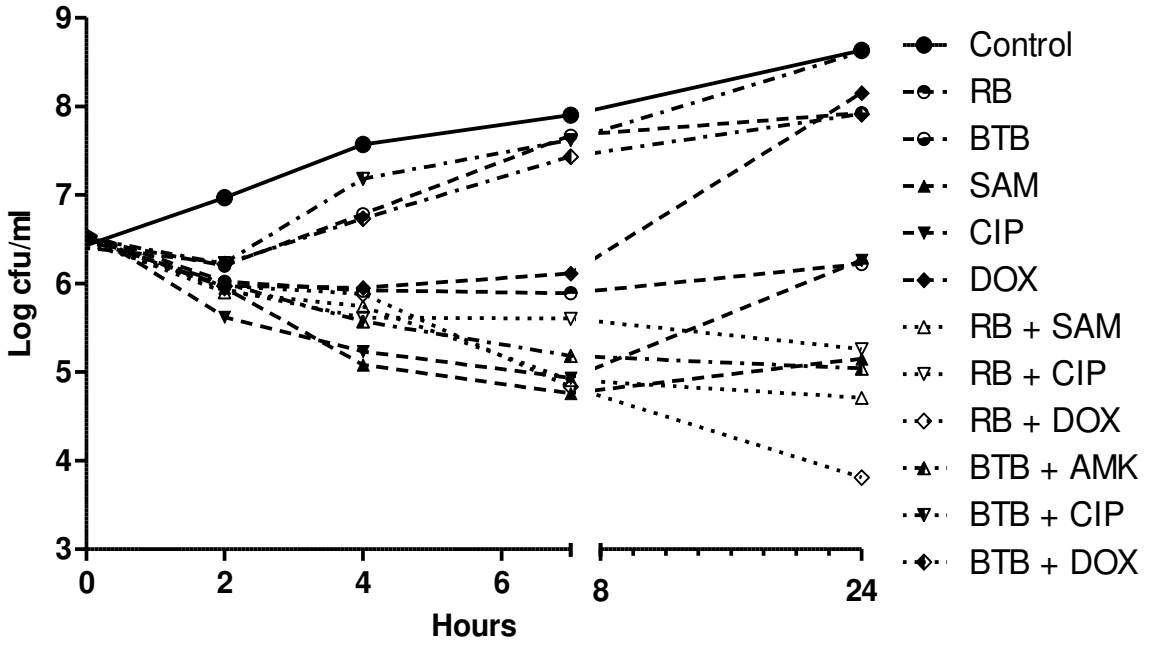


Figure 3(on next page)

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.