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Original Article

Evaluation of the Antibacterial Effect of Garlic and Ginger on ESBL and KPC-Producing *Pseudomonas aeruginosa* Strains

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Abstract

Background and Aim: Due to the problems caused by the formation of biofilms in industry and medicine as well as the development of drug resistance, new methods are required to inhibit resistant microorganisms, particularly in the biofilm form. The aim of the present research was to determine the effect of garlic and ginger on the biofilm formation of *Pseudomonas aeruginosa*.

Materials and Methods: In this study, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of garlic and ginger extracts against *Pseudomonas aeruginosa* were assessed by microtiter plate method. Biofilm formation was investigated by the use of microtiter plate method and staining with crystal violets.

Results: The minimum inhibitory concentrations of garlic and ginger extracts against ATCC 27853 standard strain *Pseudomonas aeruginosa* were 40 and 2.5 mg / ml respectively. Moreover, it was 80 and 20 mg / ml for ESBL, KPC and ESBL + KPC generating strains respectively. The mean percentages of the biofilm inhibition of *Pseudomonas aeruginosa* by garlic and ginger extracts were 61.98% and 66.81% respectively.

Conclusion: Garlic and ginger extract might be used in different compounds to inhibit *Pseudomonas aeruginosa* and its biofilm formation.

Keywords: Biofilm, Garlic, Ginger, *Pseudomonas aeruginosa*, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC)

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Introduction

As an opportunistic pathogen, pseudomonas aeruginosa (P. aeruginosa) is one of the major causes of death in in hospital burn patients and patients with cystic fibrosis. *P. aeruginosa* is capable of forming biofilms in different environments (1, 2). Biofilms contain microorganisms attached to living and non-living surfaces that are molded into a hydrous polymeric matrix (3). The most prominent feature of the biofilm is its resistance to antimicrobial agents (4). In fact, biofilm is the biggest failure factor for biocides and antibiotics in the treatment of infections (5). Biofilms pose many problems in medical, industrial

and environmental fields (6). High doses of antibiotics are needed to eliminate biofilms, and one of the goals of microbiologists today is to discover new ways to combat biofilms and reduce the effective dose of antimicrobial agents (7). Antibiotic resistance in bacteria, particularly in Gram-negative bacteria, has been recently increasing worldwide (8). This phenomenon makes researchers consider the use of effective antimicrobial agents with less side effects instead of less effective antimicrobial agents with more unwanted side effects (9, 10).

Medicinal herbs are of particular value and significance in the health and well-being of communities both in terms of treatment and prevention of diseases. Investigators' attempts to introduce compounds with anti-biofilm properties have resulted in the identification of plant compounds that naturally use plants to defend themselves against bacterial establishment (11, 12). These compounds include are alkaloids, terpenoids, flavonoid and coumarins, peptides, glycosides, nucleosides and polyphenols (13, 14).

Ginger plant, scientifically known as Zingiber officinale, is an edible herb that has long been used in the treatment of various diseases (15). Ginger that contains gingerol, vitamins, minerals and antioxidants promotes high levels of health. As a heart conditioner, ginger has anti-nausea, anti-clotting, antibacterial, antioxidant, anti-cough, anti-liver toxins, diuretic and anti-flatulence properties. It could increase intestinal and gastric secretions and lower cholesterol level. Moreover, it is a digestive stimulant, and is used as an analgesic for toothache. Finally, it is used in the treatment of colds and flu (16, 17). Traditional medicine uses ginger to treat rheumatoid arthritis and gastric ulcer. The androgenic antioxidant activity of ginger has been known worldwide to treat diseases (18). Ginger consists of a family of tropical plants which is especially abundant in India and Malaysia. This plant has more than 1200 species and 53 genera. The genus zingiber comprises nearly 85 species of aromatic plants ranging from East Asia to the tropical regions of Australia. Zingiber is a Sanskrit word for ginger rhizome which means "horn-shaped"(19, 20).

Garlic, with the scientific name Allium sativum, is one of the foods that has long been considered for nutritional values and therapeutic effects (21, 22). It has many health benefits such as being an immune enhancer. Moreover, it has anti-allergic, anticonvulsant, and anti-cancer properties. It is also used as a cholesterol-lowering agent. Furthermore, it is used in the treatment of throat infection, influenza, colds, hepatitis, constipation, and in the controlling of bacterial infections, fungi and yeasts (23). Researchers have indicated that garlic is able to prevent the growth of Gram-positive, Gram-negative and toxic acid-producing bacteria (22). The higher antibacterial properties of garlic are due to functional differences of thiol-disulfide between sulfur compounds and thiol group in bacterial enzymes such as RNA and DNA polymerase, thioredoxin reductase and trypsin which can cause this disorder (24). The aim of the present study was to investigate the impact of garlic and ginger on the ability of biofilm formation in the clinical isolates of *P. aeruginosa* ATCC27853, ESBL-producing, and KPC-producing *P. aeruginosa* isolates.

Materials and Methods

Bacterial Strains

In this descriptive in vitro study, strains of ESBL and KPC – producing *P. aeruginosa* were prepared from Pasteur Institute of Iran, and the lyophilized *P. aeruginosa* ATCC 27853 strain was prepared from Iranian Scientific and Industrial Research Center.

Sample Collection and Preparation of Extracts

Ginger (Zingiber officinale) and garlic (Allium sativum) used in this research were purchased from the local market of Khorramabad, Iran. Soaking the extracts was done by the maceration method. After crushing the garlic and grating the ginger root, they were dried at room temperature and shade, and then weighed with digital scales. 20 g of dry ginger was mixed with 200 ml of absolute ethanol, and 50 g of dry garlic was mixed with 500 ml of absolute ethanol, and was subsequently placed on a shaker for 48 hours at ambient temperature. It was then mixed with Whitman filter paper. No. 1 that was filtered and dried at 40°C in the oven. Finally, various concentrations were prepared and stored at 4°C (25).

Phenotypic Screening and Confirmatory Tests for ESBL and KPC–Producing Strains

Screening of ESBL and KPC–producing strains was

performed based on the disc diffusion method. ESBL-producing strains were identified using ceftazidime (30µg), cefotaxime (30µg), ceftriaxone (30 µg), Cefoxitin (30 µg), alone and combined with clavulanic acid (Rosco, Denmark) according to the criteria proposed by Clinical Laboratory Standards Institute (CLSI) (26). Strains were tested for KPC carbapenemases production on disks containing imipenem and imipenem with 400 µg of boronic acid on Muller Hinton medium. When the diameter of the growth inhibitory zone around the imipenem with boronic acid was ≥ 5 mm larger than that around imipenem disc alone, it was regarded positive for the identification of KPC production (27).

Antimicrobial Assay Using Disc Diffusion Method

100 µl of the microbial suspension was poured onto the Mueller-Hinton agar culture medium and was spread on the culture medium. Subsequently, a blank paper disk (prepared by Antibody Tab) was placed on the culture medium, and 15 µl of the extract was extracted on the disk. It was dumped. Plates were incubated at 37°C for 24 hours, and then the diameter of the inhibition zone was measured by a ruler. Finally, the diameter of the auras was compared with positive control (gentamicin antibiotic) and negative control (solvent) (28).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC of different ginger and garlic extracts were determined by dilution method in 96-well microplates. Thus, after culturing the bacteria on Muller-Hinton agar medium and holding it at 37°C for 24 hours, it was adjusted to a final density of 8 log CFU/mL. The dilution of the extracts was carried out ranging from 100 mg/ml to 0.01 mg/ml for MIC against *P. aeruginosa* in 96-well microliter plates. Hence, the plates contained the serial dilutions of the ginger and garlic extracts on MHB. The extracts were diluted ranging from 100 mg/ml to 0.01 mg/ml for MIC against *P. aeruginosa*. In this method, the bacterial culture and broth without the plant extract were used as positive control. The incubation of the contents of each well was carried out at 37°C for 24 h. Subsequently, the visual indication of bacterial growth was fulfilled using

2, 3, 5-triphenyltetrazolium chloride. The MIC of the essential oils was considered as the lowest concentration that indicated zero growth. Each test was performed in triplicate. We identified the last well that lacked turbidity due to microbial growth as MIC. To determine the MBC of the wells that we considered to be MICs, we planted two wells on the neutron agar medium and placed them in the incubator at the appropriate temperature and time. We considered MBC as the concentration of wells at which the germs had not grown (29).

The Effect of the Extracts on the Biofilm Formation of *P. aeruginosa* Isolates

Microtiter plate staining and 0.1% crystal violet staining were used to examine the effect of the ethanolic extracts of garlic and ginger on the biofilm formation of Pseudomonas aeruginosa. First, each well was plated in every well of 96-well 100 µl Müller Hinton broth, and in all wells of the first column except 100 µl negative control wells of the extract prepared at the initial concentration of 80 mg / ml. Liter (in triplicate) was added. Similar to MIC, the extract was transferred to subsequent wells. However, the final volume of wells in the MIC assay was 100 µl, but the final volume of each well was 200 µl for biofilms. Subsequently, 100 µl of diluted bacterial suspension was added to all wells (except the negative control wells) with the dilution of 2×10^6 CFU / ML. Finally, the bacterial concentration of each well was equivalent to 10^6CFU / ML. The 96-well plate was incubated at 37 ° C for 24 hours. The wells were then emptied and washed three times with sterile PBS buffer. The microtiter plate was then dried at room temperature and 200 µL of ethanol was added to each well to stabilize the biofilm population. After 15 minutes, the ethanol wells were discarded and the 96-well plate was dried at room temperature. Then, to each well, 200 µl 0.1% crystal violet was added for 5 min. Subsequently, the additional dyes were washed with water and the 96-well plate was dried at room temperature. In the next step, 200 µL of 33% glacial acetic acid was added to each well, and the dyes attached to the wells were dissolved in acid. The light absorption at 630 nm was then measured by ELISA reader (30-32).

Percentage reduction of the biofilm production of *Pseudomonas aeruginosa* was calculated based on the following formula (33):

((ODC-ODB) - (ODT-ODB)) / (ODC-ODB)} × 100% = percent reduction in biofilm production (ODC: Optical absorption of positive control wells, ODB: Optical absorption of Blanc wells (Negative control) and ODT: Optical absorption of wells treated with extract).

Statistical Analysis

Data were analyzed using SPSS software, version 23, and analysis of variance, as wells as Duncan test at the significance level less than 0.05.

Results and Discussion

Antimicrobial Effect of the Disk Diffusion Method

Garlic and ginger extracts at the concentration of 40 mg / ml caused 12 and 16 mm growth zone around ATCC 27853 standard strain. However, no growth zone was observed at lower concentrations. On the other hand, as the extract concentration increased above 40 mg / ml, the growth zone around the well also increased. The solvent showed no growth halo while the gentamicin halo diameter was 24 mm for ATCC 27853 strain which was higher than both extracts in all the three dilutions (Table 1).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MBC) of the Ethanol Extracts of Garlic and Ginger

The antibacterial effect of garlic extract at different concentrations showed that despite the relative resistance of most strains to the concentrations used, the highest sensitivity was at 80 mg / ml and the minimum inhibitory concentration was 80 mg / ml of garlic extract in which three beta-lactamase generating strains were inhibited at this concentration. However, MIC which is the standard strain of ATCC27853 at 40 mg/ml of the garlic extract completely disappeared at 80 mg / ml. The antibacterial effect of ginger extract at different concentrations also indicated that despite the relative resistance of most strains to the concentrations used, the highest sensitivity was observed at concentrations of 40 mg/ml and 80 mg / ml. The highest MIC was 20 mg/ml of the ginger extract inhibited by beta-lactamase producing strains (Table 3). While the highest MBC was in the 40 mg / ml concentration of the garlic extract, 100% of the bacteria were killed and the strain ATCC27853 was completely destroyed at 5 mg/ml (Table 2).

Biofilm Formation in the Presence of the Extract

The results of the biofilm formation of *P. aeruginosa* strains showed that beta-lactamase generating strains and ATCC27853 strain were able to form biofilms by microtiter plate staining and crystal violet staining. The investigation of the effect of these extracts on the biofilm formation of *P. aeruginosa* indicated that the in vitro ethanol extract of garlic and ginger was also effective on biofilm formation. The results of the

Table 1: Diameter of inhibition zone of *P. aeruginosa* ATCC27853 (mm) after treatment with different concentrations of ethanol extract of garlic, ginger and antibiotic.

	Garlic			Ginger			
Strains	**50mg/ml**	**40mg/ml**	**30 mg/ml**	**50mg/ml**	**40mg/ml**	**30 mg/ml**	**Gentamicin**
ESBL+	-	-	-	14 mm	mm13	-	-
KPC+	-	-	-	14 mm	13mm	-	-
ESBL+KPC	-	-	-	14 mm	13 mm	-	-
ATCC27853	15 mm	12mm	-	17 mm	16 mm	-	24mm

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MBC) of garlic and ginger ethanolic extracts against *P. Aeruginosa* ATCC27853 (mg / ml).

	Garlic		Ginger	
Strains	**MBC**	**MIC**	**MIC**	**MBC**
ESBL+	-	80	20	40
KPC+	-	80	20	40
ESBL+KPC	-	80	20	40
ATCC27853	80	40	2.5	5

Table 3: Inhibitory pattern of *P. aeruginosa* strains at different concentrations of ethanol extract of garlic and ginger (mg/ml).

	Different concentrations of ethanol extract of garlic (mg/ml)								**Different concentrations of ethanol extract of ginger (mg/ml)**							
Strains	80	40	20	10	5	2.5	1.25	0.625	80	40	20	10	5	2.5	1.25	0.625
ESBL+	1	2	2	2	2	2	2	2	0	0	1	2	2	2	2	2
KPC+	1	2	2	2	2	2	2	2	0	0	1	2	2	2	2	2
ESBL+KPC	1	2	2	2	2	2	2	2	0	0	1	2	2	2	2	2
ATCC27853	0	1	2	2	2	2	2	2	0	0	0	0	0	1	2	2

2: Indicates the enormous growth of microorganisms
1: Indicates low growth of microorganisms
0: Indicates the lack of growth of microorganisms

Table 4: Percentage inhibition of biofilm formation of *P. aeruginosa* strains by different concentrations of garlic and ginger.

	Different concentrations of ethanol extract of (mg/ml) ginger								**Different concentrations of ethanol extract (mg/ml) of garlic**							
Strains	80	40	20	10	5	2.5	1.25	0.625	80	40	20	10	5	2.5	1.25	0.625
ESBL+	95.5	95.5	92.6	87.8	72.2	68.8	35.3	28.9	89.1	84.2	82	75.6	75.6	55.5	40.6	28.9
KPC+	89.6	88.7	70.6	66.3	57.7	55.1	50	40.6	95.7	92.2	92.2	85.3	72.2	66.3	35.3	22.5
ESBL+KPC	71.9	66.2	57.3	53.9	50.5	48.3	47.1	35.3	88.7	86.5	84.1	72.5	68.1	53.9	41.7	23.9
ATCC27853	65.1	65.1	61.7	0	0	0	0	0	100	100	95.5	85.6	78.5	22.9	22.9	20
mean	80.52	78.87	70.55	69.33	60.13	57.4	44.13	34.93	93.37	90.72	88.45	79.75	73.6	49.65	35.12	23.82

effect of distinct concentrations of these extracts on the biofilm formation of *P. aeruginosa* have been presented in Table 4. The effect of the ethanol extract of garlic on the biofilm formation of *P. aeruginosa* showed that it was effective on biofilm formation. Moreover, the effect of ginger ethanolic extract on the formation of *P. aeruginosa* biofilm showed that the extract had a greater ability to inhibit biofilms, and on average, 66.81% inhibited the biofilm formation. *P. aeruginosa* was concentration-dependent by the ethanol extract of garlic and ginger. The highest rate of the inhibition of biofilm formation at 80 mg/ml concentration and the lowest rate of the inhibition of biofilm formation at the concentration of 0.625 mg/ml were determined.

The statistical analysis of the effect of garlic and ginger extracts on biofilm formation indicated that ginger extract could not exhibit a significant difference with garlic extract ($P < 0.05$). Regarding the impact of the ethanolic extract of ginger on the biofilm formation of beta-lactamase-producing strains and standard strain ATCC27853, no significant difference was observed between the beta-lactamase-producing strains and standard strain ATCC27853 ($p=0.56$). The effect of the ethanol extract of garlic on the biofilm formation of beta-lactamase producing strains and standard strain of *P. aeruginosa* was significant ($p=0.001$).

P. aeruginosa is considered as an opportunistic pathogen. The increasing bacterial resistance that has become multiple resistance has hindered the curing of infections caused by this bacterium (34, 35). Given the significance of biofilms in the pathogenicity and antibiotic resistance of *P. aeruginosa*, an attempt to find antimicrobial compounds that can kill biofilm-producing bacteria at lower densities seems necessary (36). Therefore, The aim of the present study was to evaluate the effect of the ethanol extracts of garlic and ginger on the biofilm formation of beta-lactamase producing strains and ATCC27853 *P. aeruginosa*.

The results showed that the effect of ginger extract on *P. aeruginosa* for the concentration of 40 mg/ml diameter of the growth zone for ATCC 27853 strain were 16 and 13 mm on average for ESBL and KPC producing strains respectively. Increasing the extract concentration increased the inhibitory zone diameter

by 50 mg / ml to the standard strain ATCC27853, and this was the case with ESBL and KPC producing strains. However, lower growth zone concentrations were not observed. According to the study conducted by Cimanga *et al.*, if the growth zone diameter is 15 mm or above this level, the activity will be very high, the growth zone diameter between 15 and 10 mm indicates moderate activity and the growth zone diameter is less than 10 mm. M indicates the inactivation of the extract (37). Gull *et al.* reported in a study of the antibacterial effect of the ethanolic extract of this plant on *P. aeruginosa* that the average diameter of the inhibition zone formed against the bacterium was about 14 mm. However, in this study a ginger extract against *P. aeruginosa* inhibited 16 mm growth zone indicating the stronger antibacterial effect of ginger extract against this bacterium in the present study (15).

The results of the effect of garlic extract on *P. aeruginosa* for 40 mg / ml non-growth zone diameter for standard strain ATCC27853 equals 12 mm. The ESBL and KPC-producing strains were not observed at the concentrations studied. In a study by Molana *et al.*, the comparison of the effect of garlic extract on standard and clinical strains showed that garlic extract had a inhibitory effect on standard strains but had no effect on clinical strains which is not consistent with the present study (38). In the research carried out by Rostami-Rad *et al.*, garlic extract had a greater effect on *P. aeruginosa* ATCC27853 strain. However, it did not show a significant effect on the ESBL strain of garlic extract which is consistent with the present study (39). Dankert *et al.* investigated the effect of the growth inhibition of garlic extract by Agar diffusion test on a number of bacteria including *P. aeruginosa* (41). According to this study, all organisms were inhibited by garlic extract but a high concentration of garlic extract had a bactericidal effect on *P. aeruginosa* which is consistent with our study (40).

The remarkable results of the present study include the confirmation of the inhibitory and lethal effects of ginger extract on *P. aeruginosa* ATCC27853 and beta-lactamase producing strains with MIC values of 2.5 and 20 mg/ml, respectively. MBC values were also 5 mg/ml for standard strain ATCC27853 and 40 mg/ml for beta-lactamase producing strains, confirming the good inhibitory and lethal ability of ethanolic extract against ginger *P. aeruginosa*. In other studies, the inhibitory and lethal effects of this extract were investigated. Yahya *et al.* investigated the inhibitory effect of ginger ethanolic extract on *P. aeruginosa* and a MIC of 12.5 mg/ml (41).

Differences in the MIC rates reported by different researchers are consistent with the present study. One of the main reasons is the percentage of different compounds of antimicrobial agents in plant extracts that depends on the geographical area, plant variety, plant age, drying method and extraction method (42).

In our study, the ethanol extract of garlic had inhibitory effect on *P. aeruginosa* ATCC27853 and beta-lactamase producing strains. However, this effect was observed only at high concentrations. The highest MIC was at 80 mg/ml of garlic extract which inhibited three beta-lactamase producing strains. MIC is the standard strain of ATCC27853 at a concentration of 40 mg/ml of garlic extract and is completely eliminated at a concentration of 80 mg/ml, which is consistent with the study conducted by Dankert *et al* (40). They were inhibited by garlic extract but the high concentration of garlic extract had bactericidal effects on *P. aeruginosa* (40). The results of the research carried out by Sabahi *et al.* revealed that clinical isolates of *P. aeruginosa* are resistant to garlic extract (43). In the study conducted by Molana *et al.*, Chloroform extract of 40 mg / ml had a lethal effect on the standard strain of *P. aeruginosa* and 35 mg / ml inhibitory effect on standard and clinical strains (38) which is consistent with the present study.

According to the results of studies in this field, treatment of infections caused by biofilm-producing strains is still one of the major human problems that need further research. It is generally difficult to eliminate biofilms after formation (44, 45). Hence, further studies are required to evaluate the various constituents in the extract of medicinal plants and to compare different components of their constituents in indigenous areas and to identify superior breeds.

Conclusion

The results of the present study indicated that apart from having antibacterial effect, in vitro garlic and ginger extracts have antibiotic effect against *P. aeruginosa* and the biofilm formation of beta-

lactamase producing strains and ATCC27853 strain of *P. aeruginosa*. As antibiotic resistance is progressively increasing and biofilm formation by *P. aeruginosa* increases antibiotic resistance, further studies on these plants are required. These plants were used as effective antimicrobial agents.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Kirisits MJ, Prost L, Starkey M, Parsek MR. Characterization of colony morphology variants isolated from Pseudomonas aeruginosa biofilms. Appl Environ Microbiol. 2005;71(8):4809-21.
2. Sauer K, Cullen M, Rickard A, Zeef L, Davies DG, Gilbert P. Characterization of nutrient-induced dispersion in Pseudomonas aeruginosa PAO1 biofilm. Journal of bacteriology. 2004;186(21):7312-26.
3. Mulcahy LR, Isabella VM, Lewis K. Pseudomonas aeruginosa biofilms in disease. Microbial ecology. 2014;68(1):1-12.
4. Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. International journal of antimicrobial agents. 2010;35(4):322-32.
5. Stewart PS. Mechanisms of antibiotic resistance in bacterial biofilms. International Journal of Medical Microbiology. 2002;292(2):107-13.
6. Morikawa M. Beneficial biofilm formation by industrial bacteria Bacillus subtilis and related species. Journal of bioscience and bioengineering. 2006;101(1):1-8.
7. Huq A, Whitehouse CA, Grim CJ, Alam M, Colwell RR. Biofilms in water, its role and impact in human disease transmission. Current Opinion in Biotechnology. 2008;19(3):244-7.
8. Woodford N, Turton JF, Livermore DM. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS microbiology reviews. 2011;35(5):736-55.
9. Palaniappan K, Holley RA. Use of natural antimicrobials to increase antibiotic susceptibility of drug resistant bacteria. International journal of food microbiology. 2010;140(2-3):164-8.
10. Pavithra P, Janani V, Charumathi K, Indumathy R, Potala S, Verma RS. Antibacterial activity of plants used in Indian herbal medicine. International Journal of Green Pharmacy (IJGP). 2010;4(1).
11. Nair R, Chanda S. Activity of some medicinal plants against certain pathogenic bacterial strains. Indian Journal of Pharmacology. 2006;38(2):142.
12. Sasidharan S, Chen Y, Saravanan D, Sundram K, Latha LY. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African Journal of Traditional, Complementary and Alternative Medicines. 2011;8(1).
13. Berdy J. Bioactive microbial metabolites. The Journal of antibiotics. 2005;58(1):1.
14. Davies J, Ryan KS. Introducing the parvome: bioactive compounds in the microbial world. ACS chemical biology. 2011;7(2):252-9.
15. Gull I, Saeed M, Shaukat H, Aslam SM, Samra ZQ, Athar AM. Inhibitory effect of Allium sativum and Zingiber officinale extracts on clinically important drug resistant pathogenic bacteria. Annals of clinical microbiology and antimicrobials. 2012;11(1):8.
16. Kumar G, Karthik L, Rao KB. A review on pharmacological and phytochemical properties of Zingiber officinale Roscoe (Zingiberaceae). Journal of Pharmacy Research. 2011;4(9):2963-6.
17. Sharifi-Rad M, Varoni E, Salehi B, Sharifi-Rad J, Matthews K, Ayatollahi S, et al. Plants of the genus Zingiber as a source of bioactive phytochemicals: From tradition to pharmacy. Molecules. 2017;22(12):2145.
18. Afzal M, Al-Hadidi D, Menon M, Pesek J, Dhami M. Ginger: an ethnomedical, chemical and pharmacological review. Drug metabolism and drug interactions. 2001;18(3-4):159-90.
19. Grzanna R, Lindmark L, Frondoza CG. Ginger—an herbal medicinal product with broad anti-inflammatory actions. Journal of medicinal food. 2005;8(2):125-32.
20. Banerjee S, Mullick H, Banerjee J, Ghosh A. Zingiber officinale:'a natural gold'. Int J Pharmaceutical Bio-Sci. 2011;2:283-94.
21. Amagase H. Clarifying the real bioactive constituents of garlic. The Journal of nutrition. 2006;136(3):716S-25S.
22. Roy J, Shakleya DM, Callery PS, Thomas JG. Chemical constituents and antimicrobial activity of a traditional herbal medicine containing garlic and black cumin. African Journal of Traditional, Complementary and Alternative Medicines. 2006;3(2):1-7.
23. Goncagul G, Ayaz E. Antimicrobial effect of garlic (Allium sativum) and traditional medicine. J Anim Vet Adv. 2010;9(1):1-4.
24. Tam VH, Chang K-T, Abdelraouf K, Brioso CG, Ameka M, McCaskey LA, et al. Prevalence, resistance mechanisms, and susceptibility of multidrug-resistant bloodstream isolates of Pseudomonas aeruginosa. Antimicrobial agents and chemotherapy. 2010;54(3):1160-4.

25. Ajayi AM, Dunde W, Abba S, Dare S, Okpanachi O, Tanayen J, et al. Phytochemical, acute toxicity and anti-inflammatory studies on aqueous extract of Hallea rubrostipulata stem bark. Internatinal Journal of Pharmacy and Biomedical Science. 2012;3(4):203-6.
26. Hsueh P-R, Ko W-C, Wu J-J, Lu J-J, Wang F-D, Wu H-Y, et al. Consensus statement on the adherence to Clinical and Laboratory Standards Institute (CLSI) Antimicrobial Susceptibility Testing Guidelines (CLSI-2010 and CLSI-2010-update) for Enterobacteriaceae in clinical microbiology laboratories in Taiwan. Journal of Microbiology, Immunology and Infection. 2010;43(5):452-5.
27. Giani T, Tascini C, Arena F, Ciullo I, Conte V, Leonildi A, et al. Rapid detection of intestinal carriage of Klebsiella pneumoniae producing KPC carbapenemase during an outbreak. Journal of Hospital Infection. 2012;81(2):119-22.
28. Kamazeri TSAT, Samah OA, Taher M, Susanti D, Qaralleh H. Antimicrobial activity and essential oils of Curcuma aeruginosa, Curcuma mangga, and Zingiber cassumunar from Malaysia. Asian Pacific journal of tropical medicine. 2012;5(3):202-9.
29. Alade P, Irobi O. Antimicrobial activities of crude leaf extracts of Acalypha wilkesiana. Journal of Ethnopharmacology. 1993;39(3):171-4.
30. Habibipour R, Moradi Haghgou L. Study on hydro-alcoholic extract effect of pomegranate peel on pseudomonas aeruginosa biofilm formation. Avicenna Journal of Clinical Medicine. 2015;22(3):195-202.
31. O'Toole GA, Gibbs KA, Hager PW, Phibbs PV, Kolter R. The global carbon metabolism regulator Crc is a component of a signal transduction pathway required for biofilm development by Pseudomonas aeruginosa. Journal of bacteriology. 2000;182(2):425-31.
32. Agarwal R, Singh S, Bhilegaonkar K, Singh V. Optimization of microtitre plate assay for the testing of biofilm formation ability in different Salmonella serotypes. International Food Research Journal. 2011;18(4):1493.
33. Nyenje ME, Green E, Ndip RN. Biofilm formation and adherence characteristics of Listeria ivanovii strains isolated from ready-to-eat foods in Alice, South Africa. The Scientific World Journal. 2012;2012.
34. Farrell DJ, Flamm RK, Sader HS, Jones RN. Antimicrobial activity of ceftolozane-tazobactam tested against Enterobacteriaceae and Pseudomonas aeruginosa with various resistance patterns isolated in US hospitals (2011-2012). Antimicrobial agents and chemotherapy. 2013;57(12):6305-10.
35. Magiorakos AP, Srinivasan A, Carey R, Carmeli Y, Falagas M, Giske C, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection. 2012;18(3):268-81.
36. Banin E, Brady KM, Greenberg EP. Chelator-induced dispersal and killing of Pseudomonas aeruginosa cells in a biofilm. Appl Environ Microbiol. 2006;72(3):2064-9.
37. Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, et al. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. Journal of ethnopharmacology. 2002;79(2):213-20.
38. Molana Z, Shahandeh Z. Effect of garlic (allium sativum) and garlic extract on growth inhibition of pseudomonas aeroginosa. 2003.
39. Rostamirad M, Javadi A, Akbari T, Heidarpour A, Jamali M, Mahdavi R, Et Al. The Antimicrobial Effect of Methanolic Extracts of Achillea Wilhelmsii, Myrtus Communis, and Allium Sativum on Pseudomonas Aeruginosa. 2017.
40. Dankert J, Tromp T, Klasen H. Antimicrobial activity of crude juices of Allium ascalonicum, Allium cepa and Allium sativum. Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene Erste Abteilung Originale Reihe A: Medizinische Mikrobiologie und Parasitologie. 1979;245(1-2):229-39.
41. Yahya M, Saifuddin N, Hamid U. Zingiber officinale ethanolic extract inhibits formation of Pseudomonas aeruginosa biofilm. Int J Pharm Bio Sci. 2013;3(46):e54.
42. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. International journal of food microbiology. 2004;94(3):223-53.
43. Sabahi M, esmaeili r, dastan D, Alikhani My. Antibacterial Activity of Aqueous and Alcoholic Extracts of Garlic and Aloe Vera Against Clinical Isolates of Staphylococcus aureus, Pseudomonas aeruginosa and E.coli. Iranian Journal of Medical Microbiology. 2018;12(4):288-93. doi: 10.30699/ijmm.12.4.288.
44. Mah T-F, Pitts B, Pellock B, Walker GC, Stewart PS, O'Toole GA. A genetic basis for Pseudomonas aeruginosa biofilm antibiotic resistance. Nature. 2003;426(6964):306.
45. Loo C-Y, Rohanizadeh R, Young PM, Traini D, Cavaliere R, Whitchurch CB, et al. Combination of silver nanoparticles and curcumin nanoparticles for enhanced anti-biofilm activities. Journal of agricultural and food chemistry. 2015;64(12):2513-22.

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