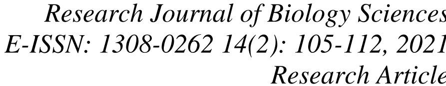

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Research Article

ANALYSIS OF ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY OF ZINGERONE

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ABSTRACT. Zingeron is a sharp non-volatile compound found in ginger. It has many bioactivities such as antioxidant, anti-inflammatory and antimicrobial properties originating from many bioactive components similar to zingerone found in the ginger structure. There are no large-scale antimicrobial and antibiofilm investigations of zingeron on various types of bacteria and fungi in the literature. In this study; The antimicrobial activity of zingerone on 14 bacteria and 2 fungal species was determined by Agar Well Diffusion, Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests. As a result of Agar Well Difusion test the inhibition concentration of zingeron was determined as 1 M. As a result of MIC and MBC tests, the bactericidal and bacteriostatic concentrations of zingeron vary between bacterial cultures. In addition, Antibiofilm activity of zingerone on the same microorganisms was determined. *Klebsiella pneumoniae* bacteria gave the best result at 0.0025 M zingeron concentration. No inhibitory properties of zingerone were observed on the fungi species used in the study.

Keywords: *zingeron, antimicrobial, antibiofilm, in vitro*

INTRODUCTION

Ginger (*Z. officinale* Roscoe), monocotyledonous, belonging to the Zingiberaceae family from the Zingiberales order, is a perennial herb reaching a height of about 0.6 to 1.2 meters, erect, rhizomes in the form of thick tubers. It is a plant that has been the preferred in various parts of the world to treat digestive disorders in humans, nausea and vomiting during pregnancy since ancient times. Due to these healing properties, it has taken its place among the medicinal plants that are widely used in Chinese and Japanese medicine [1, 2, 3].

As a result of chemical analyzes of ginger root in powder form, the presence of important components such as protein, carbohydrates, fat, fiber, iron, calcium, vitamin C and carotene has been revealed [4]. Ginger contains many non-volatile pungent compounds such as zingeron and many bioactive phenolic substances in its structure. Due to these bioactive components, it has many bioactivities such as antimicrobial, antioxidant and anti-inflammatory properties [5, 6, 7].

Antimicrobial substances are substances that, by their nature, have some properties that prevent or kill bacterial and fungal microorganisms [8, 9]. Antimicrobial products are widely used in medicine, food products, textile products, agriculture and livestock in the fight against harmful microorganisms [10, 11]. From past to present, many antimicrobial products have been discovered and synthesized for diseases and other

damages caused by bacteria and fungi. Although the lethal effect of many diseases on humans has been reduced with the discovery of antibiotics, the resistance of disease-causing microorganisms to these drugs poses a threat to humans again as a result of the unconscious and excessive use of antibiotics over time [12, 13, 14].

Microorganisms that generate the biofilm can occur from a single species, or they can be found as a community in which more than one species is nested. Microorganisms can choose living or non-living surfaces to form biofilms[15, 16, 17]. Most of the microorganisms in nature maintain their vitality as biofilms. Harsh environmental conditions and some stress factors are among the reasons why microbial organisms form biofilms [18]. The formation of a biofilm, especially in some microorganisms, is an important event for them to gain resistance to antimicrobial agents. This increasing resistance of biofilms can pose serious threats to human health. Biofilm formation is one of the main factors of infections caused by medical equipment in hospitals and spoilage of food. Therefore, it is very important to identify new antibiofilm products that can effectively prevent biofilm formation [19].

The aim of this study is to examine the antimicrobial and antibiofilm activities of zingerone that have not been extensively studied before on 16 microorganisms.

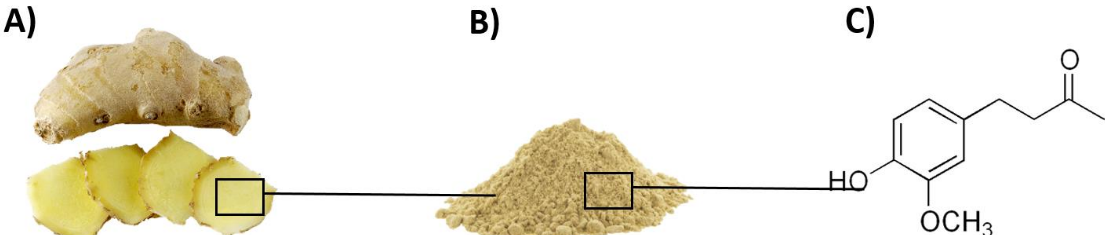


Fig. 1. *A) Ginger rhizome, B) Dried ginger powder, C) Structure of Zingerone*

MATERIALS AND METHODS

Microorganism Examples

To evaluate the antimicrobial and antibiofilm activities of zingerone, *Escherichia coli* ATCC 25922, *Bacillus subtilis* DSMZ 1971, *Enterobacter aerogenes* ATCC 13048, *Salmonella infantis*, *Klebsiella pneumoniae, Pseudomonas aeruginosa* DSMZ 50071, *Salmonella kentucky*, *Enterococcus faecalis* ATCC 29212, *Listeria innocua*, *Salmonella enteritidis* ATCC 13075, *Enterococcus durans*, *Salmonella typhimurium*, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* DSMZ 20044 bacterial species and *Candida parapsilosis, Candida albicans* fungus fungi species that were obtained from culture collections in Kahramanmaras Sutcu Imam University, Faculty of Science And Literature Microbiology laboratory were used.

Zingerone

The product catalog number W312401 was purchased from Sigma-Aldrich (Germany). Stock of this ≥ 96% pure material was stored at -20 °C. All working solutions were prepared fresh on the day of the study.

Agar Well Diffusion Method

For this test, first of all, Luria-Bertani (LB) medium was used to resuscitate fungi and bacteria from stock. Mueller Hinton Agar (MHA) medium was used in bacteria while Potato Dextrose Agar (PDA) medium was used in fungi for the agar well diffusion method.Bacterial and fungal solutions of 0.5 McFarland value were inoculated into agar petri dishes. Wells of desired diameter were opened using an agar drill. Zingeron, which was dissolved with 10% ethanol, was diluted with distilled water (dH20) as a result of the necessary calculations, and concentrations of 1 M, 0.5 M, 0.25 M, 0.1 Mand 0.01 M were prepared and 20 µl zingeron concentrations were put into the opened wells. At the end of the 16 hour incubation period, the diameters of the inhibition zones formed around the wells were measured and their antimicrobial activities were investigated. Each sample was tested in 3 replicates. Penicillin was used as the positive control, while solvent concentrations of the zingeron were used as the negative control. The diameter of the well is included in the zone diameters (mm).

Minimum Inhibition Concentration (MIC)

Minimum inhibition concentration values of zingerone were determined using sterile 96-well microplates. Luria-Bertani (LB) was placed in each well. Zingeron solution was added to the first well and the zingerone concentration in the wells was serially diluted to be 2.5 M, 0.25 M, 0.025 M, 0.0025 M. Then, 5 µl of microorganisms were added to the corresponding wells. Negative and positive control were also used. After incubation of the microplates at 37 °C for 18 hours, the absorbance values of the samples were measured at 600 nm wavelength in a spectrophotometer device to determine the lowest concentration at which zingerone inhibited microorganisms.

Minimum Bactericidal Concentration (MBC)

As a result of the MIC test, it was determined whether there was bacterial growth at all four concentrations of zingerone. For the MBC test, firstly, samples were taken from the four concentrations whose MIC test was completed with the help of sterile loop and inoculated into Mueller Hilton Agar (MHA) solid medium. It was incubated at 37 °C for 18-24 hours. Then, the concentration that kills all microorganisms was accepted as the minimum bactericidal concentration, that is, the MBC value. As a result of the MBC test, the growth of microorganisms and MIC values were compared.

Antibiofilm Activity

Microorganisms and 2.5 M, 0.25 M, 0.025 M, 0.0025 M zingeron concentrations were incubated in their respective wells in a 96-well plate at 37°C for 48 hours. After incubation, the wells were emptied and washed with distilled water (dH20). 96-well plates were allowed to dry at room temperature. 130 µl of 95% methanol was added to the wells and after 15 minutes of incubation for fixation, the methanol was removed and the plates were left to dry at ambient temperature. 125 µL of 0.1% crystal violet solution was added to the wells and kept at room conditions for 10 minutes. Then, until the crystal violet was removed from the wells, the plates were washed with distilled water (dH2O) and left to dry. 100 µL of 33% glacial acetic acid solution was added to the wells containing gram-positive bacteria attached to the well walls, and 100 µL of 95% ethanol solution to the wells containing gram-negative bacteria was added and left for 15 minutes at room conditions. Then, the measurement of the samples was carried out at 600 nm in the spectrophotometer device. The same measurement procedure was performed for both

positive and negative controls. Biofilm inhibition of zingerone was calculated with the following formula (Eqn. 1).

$$\% \text{ Reduction} = ((\text{Positive control} - \text{Test wells}) / \text{Positive}) \times 100$$

Eqn. 1

RESULTS AND DISCUSSION

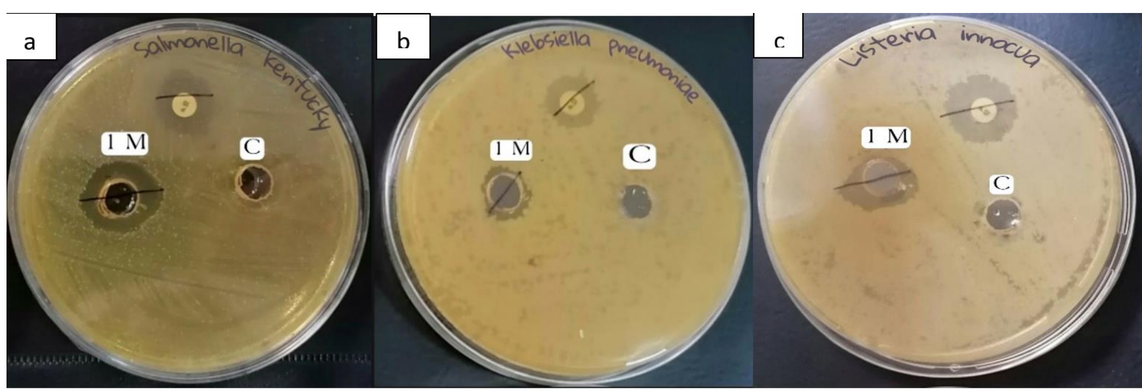
Agar Well Diffusion Results

Table 1, shows the analysis of the zone diameters (mm) of various zingeron concentrations (1, 0.5, 0.25, 0.1 and 0.01 M) on microorganisms over 16 hours by the Agar well diffusion test. It was determined that zingerone had activity on all bacteria at 1M concentration (Fig. 2).

***Table 1**. Agar Well Diffusion Test Result*

Microorganisms	0,01 M	0,1 M	0,25 M	0,5 M	1 M	Etanol	P 10
Escherichia coli	-	-	-	-	17	-	15
Bacillus subtilis	-	-	-	-	15	-	13
Enterobacter aerogenes	-	-	-	-	18	-	15
Salmonella infantis	-	-	-	-	12	-	14
Klebsiella pneumoniae	-	-	-	-	13	-	12
Pseudomonas aeruginosa	-	-	-	-	15	-	11
Salmonella kentucky	-	-	-	-	21	-	15
Enterococcus faecalis	-	-	-	-	16	-	15
Listeria innocua	-	-	-	-	17	-	17
Salmonella enteritidis	-	-	-	-	15	-	16
Enterococcus durans	-	-	-	-	15	-	17
Salmonella typhimurium	-	-	-	-	17	-	18
Staphylococcus aureus	-	-	-	-	14	-	13
Staphylococcus epidermidis	-	-	-	-	10	-	10
Candida parapsilosis	-	-	-	-	-	-	14
Candida albicans	-	-	-	-	-	-	14

(-): There is no zone of inhibition. (P 10): Penicillin 10 mg/ml. Ethanol (10%)



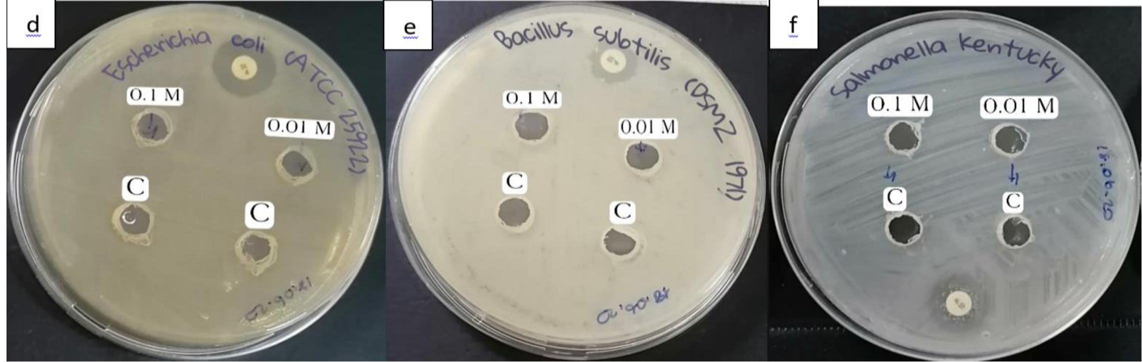


Fig. 2. *Visuals of some bacterial species that do not show an inhibition zone diameter at 1, 0.1, 0.01 M concentrations of zingerone as a result of the agar well diffusion test. ((+) Control: Penicillin (P 10), (-) Control (C): (zingeron solvent) a) Salmonella kentucky b) Klebsiella pneumoniae c) Listeria innocua) d) Escherichia coli ATCC 25922 e) Bacillus subtilis DSMZ 1971 f) Salmonella Kentucky*

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Results

In **Table 2** and **Fig. 3**, Shown, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) test results depending on the zingerone concentrations, While the minimum inhibitory concentrations of *Enterobacter aerogenes* ATCC 13048, Salmonella infantis, Pseudomonas aeruginosa DSMZ 50071, Salmonella Kentucky, Listeria innocua bacteria show a bacteriostatic effect at 0.025 M, they show a bactericidal effect at 0.25 M. In Candida parapsilosis and Candida albicans fungus species, no effect was observed on MIC and MBC results, consistent with the results of the agar well diffusion method.

Table 2*. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Results*

Microorganisms	MİK	MBC	UNIT
Escherichia coli **ATCC 25922**	0,025	2,5	M
Bacillus subtilis **DSMZ 1971**	0,25	2,5	M
Enterobacter aerogenes **ATCC 13048**	0,025	0,25	M
Salmonella infantis	0,025	0,25	M
Klebsiella pneumoniae	0,25	2,5	M
Pseudomonas aeruginosa **DSMZ 50071**	0,025	0,25	M
Salmonella kentucky	0,025	0,25	M
Enterococcus faecalis **ATCC 29212**	0,25	2,5	M
Listeria innocua	0,025	0,25	M
Salmonella enteritidis **ATCC 13075**	0,025	2,5	M
Enterococcus durans	0,25	2,5	M
Salmonella typhimurium	0,25	2,5	M
Staphylococcus aureus **ATCC 25923**	0,25	2,5	M
Staphylococcus epidermidis **DSMZ 20044**	0,25	2,5	M
Candida parapsilosis	-	-	M
Candida albicans	-	-	M

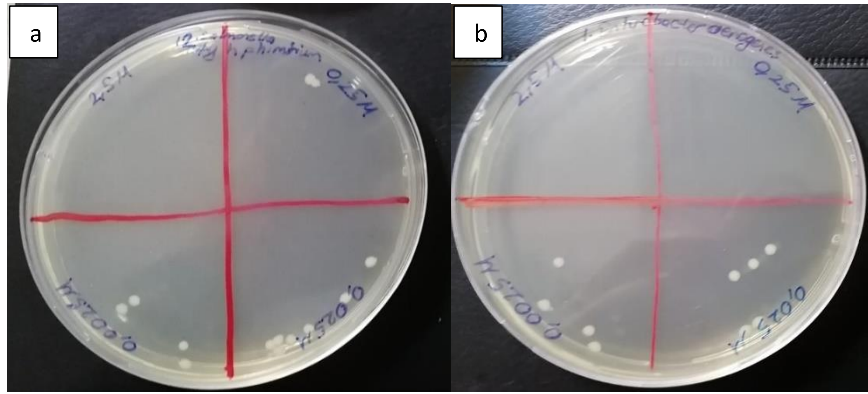


Fig. 3. Visual of a) Salmonella typhimurium, b) Enterobacter aerogenes as a result of MBC test

Antibiofilm Activity Results

Table 3 shows the percent values of the antibiofilm formation inhibitory effects of different concentrations of Zingerone against the test microorganisms measured at 600 nm. It was determined that the best Klebsiella pneumoniae strain inhibited biofilm formation at the lowest concentration of zingerone (0.0025 M). In addition, it was determined that 14 bacterial species at different concentrations inhibited biofilm formation. It has been shown that they do not inhibit biofilm formation in Candida parapsilosis, Candida albicans fungi species.

***Table 3**. Antibiofilm capacity of zingerone (%)*

Microorganisms	2,5 M	0,25 M	0,025 M	0,0025 M
***Escherichia coli* ATCC 25922**	87	81	76	70
***Bacillus subtilis* DSMZ 1971**	84	79	71	67
***Enterobacter aerogenes* ATCC 13048**	91	90	85	75
Salmonella infantis	88	86	81	70
Klebsiella pneumoniae	90	87	84	77
***Pseudomonas aeruginosa* DSMZ 50071**	85	82	71	66
Salmonella kentucky	88	86	72	58
***Enterococcus faecalis* ATCC 29212**	86	79	55	37
Listeria innocua	81	79	73	59
***Salmonella enteritidis* ATCC 13075**	90	82	78	63
Enterococcus durans	91	89	75	54
Salmonella typhimurium	63	59	55	35
***Staphylococcus aureus* ATCC 25923**	88	84	82	68
***Staphylococcus epidermidis* DSMZ 20044**	89	86	77	57
Candida parapsilosis	-	-	-	-
Candida albicans	-	-	-	-

CONCLUSION

In this study, firstly, Agar Well Diffusion test was performed to observe the antimicrobial effects of zingerone, which we think may have a natural antimicrobial effect on 14 bacteria and 2 fungi species. Then, Minimum Inhibitory Concentration (MIC) and

Minimum Bactericidal Concentration (MBC) tests were performed and the results were compared.

As a result of the literature search, no antimicrobial study of zingerone was found. Therefore, in our study, the antimicrobial effect of zingerone was demonstrated for the first time on 14 bacterial and 2 fungal species. In addition to the originality of our study within the scope of antimicrobial activity of zingeron, we think that the results we have obtained will shed light on the studies that can be done by researchers related to zingeron and will guide the researchers in terms of the development and advancement of scientific studies.

In our antibiofilm study, the percent values of the antibiofilm formation inhibitory effects of zingerone against 14 bacterial species and 2 fungi species at 4 different concentrations were examined and it was determined that the best Klebsiella pneumoniae strain inhibited biofilm formation at the lowest concentration of zingerone (0.0025 M). In addition, it was determined that 14 bacterial species at different concentrations inhibited biofilm formation. It was observed that they did not inhibit biofilm formation in Candida parapsilosis, Candida albicans fungi species. [20] observed that zingerone inhibited biofilm formation ($p< 0.05$) on the commonly used Pseudomonas aeruginosa strain, which also supports our results in their biofilm study.

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