RESEARCH ARTICLE

Effect of *Zingiber officinale* Ethanol Extract on *Staphylococcus aureus* in Culture Media

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ABSTRACT

Plant-derived products have been used for medicinal purposes for centuries. This study aimed to investigate the antimicrobial activity of ethanolic extracts of ginger (*Zingiber officinale*) against *Staphylococcus aureus*, which prepared in many concentrations of ethanol ginger extract (100, 200, 300 & 400mg/mL) and study the activity of the antibiotic Ciprofloxacin (5µg), Ampicillin (30µg) & Amoxicillin (25µg) by Measurement of the inhibition zone around the colony on Muller-Hinton. The antibacterial screening of the different extracts of *Z. officinale* and antibiotics showed different inhibition values on the agar-based on the type of used plant, extract concentration, and type of bacteria. The highest antibacterial potential was observed for the ethanolic (*Z. officinale*) extract, whereas other (*Z. officinale*) extracts show closed results in general. Also, our study has found the diameter of inhibition zones increased when increasing the extract concentration significantly ($p < 0.05$). Minimum inhibition of ethanolic extracts of ginger was (0.312–0.642) mg/mL against used bacteria, while the minimum bactericidal concentration (MBC) was 1.248mg/mL.

Keywords: *Ethanolic extracts*, Inhibition zones, *Zingiber officinale*.

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INTRODUCTION

Ginger, called a ginger rhizome, is the plant's roots (Zingiber officinale) be used for treatment. Its genus is *Z. officinale,* and its family is *(*Zingiberaceae). As well, its plant family has others several other types such as cardamom, galangal, and turmeric.[1] *Z. officinale* has many groups of flower sprouts (white and pink), which changed to yellow flowers. Therefore it has a beautiful shape and grows in hot regions.[2]

The plant has several effects, including anti-oxidant, antibacterial, anti-fungal, antiprotozoal, anti-rhinoviral, anti-emetic, anti-insecticidal, and anti-inflammatory.[3] Also, several studies confirm that the pharmacological effect of ginger is analgesic, the anti-hypersensitive effect.[4]

The ginger can remove the free radical from the body and work as an anti-oxidant; therefore, it doesn't allow lipid oxidation, so it is used as stomach protective.[5] Also, ginger has a radio-preventive effect. Therefore, it is used for preventing gamma radiation which produces from radiation exposure.[6] Also, ginger is used as antineoplastic activity and chemical preventive; therefore, it could use in the treatment of tumours.[7] The ginger has anti-glycating activity. Therefore it is used for the treatment of cataracts that formed in Patients with diabetes mellitus.[8] Composition of ginger is fat 0.9%, protein 2.3%, water 80.9%, fiber 2.4%, carbohydrates 12.3%, and minerals 1.2%. Moreover, the ginger contains Fe, Ca, and P and contains riboflavin, thiamine, vitamin C, and niacin.[9]

Fresh ginger contains several active compounds, such as gingerol. Also, the ginger powder composed of volatile oil (2–3)%, carbohydrates (60–70)%, protein (9)%, fatty oil (3–6)%, water (9–12)%, ash (8)%, crude and fiber (3–8)%.[10]

The shogaol is the main compound in ginger, is composed of Oleoresin (3–5)%, shogaol, gingerol, paradol, and zingerone (4–7.5)%.[11] The ginger in some reported showed high activity against colon bacteria,[12], and inhibits the growth of some bacteria as *Proteus sp, E. coli, Salmonella spp, Strep. spp* and *Staph. Spp.*[13,14]

Ginger has a potential antimicrobial effect and anti-fungal effect.[15] The ginger can prevent the growth of *Aspergillus.*[16] The watery ginger liquid reveals inhibition activity against *Lacto-bacillus acidophilus, Sacharomyces cerevisiae, Mycoderma spp,* and *Aspergillus niger,* at (14–10–12–4)% respectively.[17]

The gingerols substance increases the activity of the gut canal and has several other medical effects analgesic such as antibacterial and sedative.[18] Many studies found the ginger could use in the treatment of nausea, morning sickness.[19] Also, there are several reports founded the ginger has high activity

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in the treatment of cold, rheumatism, diabetes, inflammation, and heat cramps.[20,21] Its activity is attributed to absorbent, carminative, and aromatic compounds.[22] The gingerol is the primary antimicrobial substance that can destroy the cell wall, leading to cytoplasmic leakage.[23]

MATERIAL AND METHODS

Plant Materials

Z. officinale were taken from the local markets in Al-Diwaniya province, Iraq. Department of Clinical Laboratory Science, collage of pharmacy has recognized the premeditated plant.

Preparation and Plant Extracts

Seeds were kept at 45°C under lower light, then mashed using the mechanical blender a gruff powder. A weighed quantity (100 gm) of the powdered plant then went through continuous hot extraction in the soxhlet apparatus with ethanol. The extracts were purring by filter paper and intensified to dryness at evaporator in (25°C) till completely solvent had been removed to give pure Concentration of *Z. officinale* extract with a yield of 100–200–300–400 mg/mL respectively with the dried starting plant material.[24]

Test Microorganisms

The organisms of *S. aureus were* recieved from the Microbiology laboratory of the Veterinary Medicine /Al-Qadisiyah University. They were subcultured on Nutrient agar slants and used throughout the studies; It was kept at 40°C for the next steps.[25]

The Technique of Antibacterial Activity

The diffusion method on agar is used for the extraction of *Z. officinale* to evaluate antibacterial activities. It was placed 1.0mL of broth cultures of the bacteria ($1.0x10^7$ CFU/mL) in a Petri dish with 15mL of molten nutrient agar, and by inserting the broth cultures into sterile Petri dishes, combining the molten agar, quietly circular motion to ensure spreading of the microorganisms. After that, it was allowed to harden on a flat surface. On the center of growth, three wells (6mm) as diameters using a sterile pasture pipette and complete with 100µL of each concentration of plant extracts. The distribution of the material extract for one hour and then put in an incubator at 37°C for overnight. At the end of the incubation period, the plates were collected and examined for any zones inhibition.[26] Negative Control plates (diluted solvent) and standard reference antibiotics plates (Ciprofloxacin (5 µg), Ampicillin (30 µg), Amoxicillin (25 µg), and ready for testing antibacterial effecting. Each experiment repeated three times and calculate the diameter of zone inhibition.

Determination of MIC and MBC Values

Minimum inhibitory concentration (MIC) is dependent on the micro-dilution method by applying several diluted tubes of the plant extracts based on NCCLS.[7] The concentration of dilution was done to be lie between 6.25 and 0.781mg/mL. Bacteria were contained about (10^5) CFU/mL. The test plates were incubated for 18 hours at 37¼°C. The most negligible concentration of MIC was taken, which was inhabited by bacteria. Then sub-culturing the test dilutions onto a fresh solid medium and incubated at 37°C (24) hours. The Highest dilution was chosen the MBC.[27]

Statistical Analysis

The reading is done by using the mean ± S.E.M. and was done comparing between the groups. (ANOVA) the test is used in the SPSS software program (V18) and submitted to (LSD) test P-value ≤ 0.05.

RESULTS

The study was done to determine the antibacterial influence of Ginger extracts compared with some antibiotics against *S. aureus* growth in culture media.

The antibacterial results showed that different extracts prepared from *Ginger* compared with standard positive control were done by agar well diffusion and serial microdilution against *S. aureus*.

The antibacterial activity of *Z. officinale* ethanolic extract is demonstrated in Table 1, and the results of the activity of Positive control are shown in Table 2. The sensitivity of the bacteria is increased with increasing extract concentration. The inhibition zone was 20 ± 1.2 mm for 100mg/mL concentration, and (24.5 ± 1) mm for 200 mg/mL, (27.8 ± 0.9) mm for 100 mg/mL, and 35.2 ± 1.2 for 400 mg/mL.

The antibacterial activity of extract at concentration 300 mg/mL caused a zone of inhibition more than concentration 100, 200 mg/mL, and more than Amoxicillin. However, the most effective concentration was 400mg/mL of *Z. officinale* extract; it was more than other concentrations and ethanol

Table 1: The antibacterial effects of different extracts of *Ginger* compared with some antibiotics against *S. aureus* growth in culture media.

Concentration of Ginger extract (mg/mL)	*The inhibition zone of S. aureus growth in the plate (mm)*
100	$20 \pm 1.2^{(f)}$
200	$24.5 \pm 1^{(e)}$
300	$27.8 \pm 0.9^{(d)}$
400	$35.2 \pm 1.2^{(a)}$

Each value represent means ± standard error, each well contains volume 100µL the values of inhibition zone measured by mm in the diameter where same letters (no significant difference) and vice versa at ($p < 0.05$).

Table 2: Illustrate the zone of inhibition to *S. aureus* caused Positive control (reference antibiotics)

Positive control	*The inhibition zone of S. aureus growth in the plate (mm)*
Ciprofloxacin	$32 \pm 1.4^{(b)}$
Ampicillin	$30 \pm 1^{(c)}$
Amoxicillin	$16.2 \pm 2.2^{(g)}$
Negative control	
Ethanol 40%	$8.2 \pm 0.8^{(h)}$

Each value represents means ± standard error, each well contains the volume of 100µL, the values of inhibition zone measured by mm in the diameter with letters (no significant difference) and vice versa at ($p < 0.05$).

Table 3: MIC and MBC values of many of *Ginger extract* against *S. aureus*.

The concentration of ginger extract												
0.039	0.078	0.156	0.312	0.624	1.248	2.5	5	10	20	40	MIC	MBC
-	-	-	+	+	++	++	++	++	++	++	0.312	1.248

- Represent no effect
+ Represent the value of MIC
++ Represent the value of MBC

and antibiotics, even ciprofloxacin. The value of MIC of *Z. officinale* ethanolic extract was 0.312 mg/mL, while the MBC was 0.156 mg/mL, as Table 3.

DISCUSSION

The study was aimed at determining the effects of Ginger on *S. aureus* pathogen. Our experiments showed that *S. aureus* showed many degrees of sensitivities when the ginger was used. The sensitivity of bacteria was high with an increase of concentrated extract of ginger.

The antibacterial activity of ginger is attributed to its chemical composition. Zingiberene and sesquiterpenoids are the main compounds in ginger. Moreover, there are other substances such as β-sesquiphellandrene, farnesene, and bisabolene.[28] Phenols and phenolic compounds were found widely in food products. Their significant anti-oxidant activity has been shown in many studies. Phenolic compounds of herbs are known as one of the best sources of natural anti-oxidant.[29]

Gram (+) bacteria have no outer membrane, so it is more susceptible to the phenols.[30] Giriraju and Yunus study showed inhibitory effects of 10% ginger extract against Candida albicans, *Enterococcus faecalis* and *Streptococcus mutants*, Ethanolic extract of ginger (*Z. officinale*) with 20mg/mL had a more substantial effect on *Pseudomonas aeruginosa* than Escherichia coli, Unlike, in the study, water extract of ginger at the high dose (500mg) had no antimicrobial effect on Escherichia coli, *Salmonella*, *Shigella*, and *Bacillus cereus.*[31]

It is considered medically powerful because it inhibits prostaglandin and leukotriene formation, which are products that influence blood flow and inflammation.[32]

The ginger ethanolic solution has high effectiveness against *S. aureus* at MIC at 100µg/mL, but it does not have against *P. aeruginosa* and *E. coli.*[33] The ethanolic extract solution of ginger demonstrated the potential effect on E. coli and *P. aeruginosa,*[34,35] and found that sensitivity of *E. coli* is low when he uses ethanolic, aqueous, hexane, ethyl acetate, and methanolic.

In the present study, the MIC values of the plant extract components were taken in this study were lower than the MBC values. It is believed that *Z. officinale* extracts became bactericidal in higher concentrations and bacteriostatic in lower concentrations.

CONCLUSION

Z. officinale has antimicrobial activity against the bacteria used in our study, may be present non-enough amount of active gradient compound in the crude extracts to show complete activity with the dose levels employed. The conclusion is advised to use *Z. officinale* to treat infectious diseases.

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