Exploring Zingerone as a Natural Antibacterial Agent: Potential Applications in Food Safety

By

MENGYU HU

MASTER OF BIOTECHNOLOGY

College of Science and Engineering, Flinders University of South Australia, Bedford Park, SA 5042, Australia

Supervisor: Professor. Harriet Whiley

Institute: Flinders University

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DECLARATION

I certify that, to the best of my knowledge and belief, this thesis does not contain any material that has previously been published or written by another person, with the exception of instances where appropriate citation is made within the text. It also does not incorporate, without acknowledgement, any material that has previously been submitted for a degree or diploma at any university.

Mengyu Hu

15th June 2024

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The candidate understands that other writers who added intellectual value to the project may be included as co-authors of any publication that comes from this effort, with decisions about authorship made in consultation with the supervisors.

Mengyu Hu

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Mengyu Hu, Professor. Harriet Whiley

Email contact:

Hu0548@flinders.edu.au

harriet.whiley@flinders.edu.au

**Corresponding Author*: Mengyu Hu

Email: Hu0548@flinders.edu.au

College of Science and Engineering, Flinders University of South Australia, GPO Box 2100, Bedford Park, SA, 5042, Australia

Highlights

To extract ginger by using ethanol and methanol and water in rotary vacuum distillation.

To test disinfection efficiency of ginger ethanol extraction, methanol extraction, ginger oil and zingerone against *Escherichia coli (E. coli)* using the minimum inhibitor concentration (MIC) broth dilution method.

To compare the disinfection efficacy of zingerone against chemical disinfectant (10% Bleach).

Abstract

Most of the traditional food-grade disinfectants are made of chemicals. There are several drawbacks and issues with traditional food-grade disinfectants, including toxicity, corrosiveness, instability, and irritation, all of which the safety and quality of humans and the environment. Research into zingerone, one of ginger (Zingiber officinale) 's active chemicals that has strong antibacterial properties, is still in its early stages. From the perspective of food-grade disinfectants, existing studies have not found that zingerone has adverse effects on human health and does not have the corrosive effects of traditional disinfectants. Scientists plan to extract multiple extractions from ginger to compare with zingerone and use the minimum inhibitor concentration broth dilution method with *Escherichia coli* as the test organism to evaluate their disinfection efficiency. Throughout the testing process, they will adhere to appropriate controls, repeat tests and follow safety procedures. And evaluate the disinfection efficiency compared with traditional disinfectant(10% bleach). The final experimental results confirmed that zingerone has good antibacterial properties and stability, and zingerone has great potential as a revolutionary natural disinfectant.

Keywords: zingerone, natural disinfectant, *Escherichia coli,* minimal inhibitor concentration broth dilution method.

1.1 INTRODUCTION

Foodborne illnesses, which encompass a broad spectrum of ailments, are attributed to viruses found in food and are seen as an increasing public health concern. Consuming food tainted with foodborne pathogens or harmful substances produced from these pathogens is the cause of several ailments. Bacterial cross-contamination between food and food surface materials, as well as poor hygiene measures in the food processing industry, are common ways that these infections spread (Park et al., 2020). A promising source of new broad-spectrum antimicrobials that are environmentally safe, reasonably priced, and generally accessible and have low levels of skin cytotoxicity, corrosion, and environmental toxicity is phytochemicals (Gomes et al., 2016). A large number of studies have confirmed that ginger extract and ginger oil have strong antibacterial capabilities (Agrawal et al.2012, Ahmed et al, 2022, Kumar et al, 2013). While shogaols and gingerols are recognised for their antibacterial effects, Zingerone research is still in its infancy. As one of the bioactive substances in ginger, zingerone has no research showing its antibacterial ability against *E. coli*. Zingerone inhibits biofilm formation and enhances the antibiofilm efficacy of ciprofloxacin against *salmonella* (Kumar et al, 2013). The study compared the antibacterial abilities of zingerone with ginger extract and ginger oil against *E. coli* to evaluate the potential of zingerone as a natural antibacterial substance. And compare the disinfection ability of zingerone with other chemist disinfectant to provide commercial value for the development of new disinfectants.

Escherichia coli

A pathogenic serotype of *E. coli* that produces verotoxins is called *Escherichia coli* (Mueller, 2023). The Enterobacteriaceae family includes this Gramme negative motile rod, which is the main cause of hemorrhagic colitis in humans. *E. coli* can survive in unpasteurized milk and processed meat for long periods of time (Mueller, 2023). Previous studies have shown that *E. coli* poses food safety risks primarily through contamination of processing equipment, packaging materials and utensils. Currently, the most common method of eliminating *E. coli* from food exposure environments remains the use of chemical disinfectants (Taormina & Beuchat, 1999). Nevertheless, the bactericidal impact of chemical disinfectants is weakened by the potent and effective biofilm forming skills of *E. coli* strains. Meanwhile, some *E. Coli* strains that are resistant to chemical disinfectants have been found as a result of the prolonged and unscientific use of chemical disinfectants (Taormina & Beuchat, 1999). Disinfectants can cause bacteria, including *E. Coli*, to grow resistant over time. The establishment of resistant strains can reduce the efficacy of a disinfectant when it is used regularly or for an extended period of time (Mueller, 2023). Furthermore, customers are reluctant to embrace chemical disinfectants due to their potential toxicity and lack of environmental friendliness (Curran et al., 2019). Thus, there is an immediate need for a natural disinfectant with strong antibacterial properties.

Zingiber officinale

The Zingiberaceae family includes herbaceous flowering plants like the ginger plant. Zingiber officinale Roscoe, the perennial plant's scientific name, is made up of a pseudo-stem, yellow flowers, and tuberous rhizomes, popularly known as ginger root or simply "ginger" (Mahomoodally et al., 2021). Due to their aromatic scent and strong flavour, the rhizomes of ginger plants are the most sought-after part.

Therefore, ginger is a crucial element in cooking. Originating in Asia, ginger was traditionally used as a flavouring ingredient in a variety of forms, including fresh, dried, pickled, powdered, and preserved (Mahomoodally et al., 2021). Perhaps more intriguingly, though, because of its many health benefits, ginger was also employed as a tonic root to cure a wide range of ailments. 97% of the essential oils extracted from the Zingiber officinale rhizome are composed of 69 volatile compounds. The chemicals with the highest concentrations include Zingiberene (28,62%), Camphene (9,32%), Ar-curcumene (9,09%), Phellandrene (7,97%), E-Farnesene (5,52%), Bisabolene (5,40%), and Pinene (2,57%). Their biological properties have been extensively studied, and they have been shown to have antibacterial, antioxidant, cytotoxic, insecticidal, and anti-inflammatory properties. They are also used to preserve food characteristics. Non-volatile compounds called oleoresins are the main source of bioactive components found in the rhizome of Zingiber officinale (Arcusa et al., 2022). As of right now, 34 oleoresins—or 88.6% of the total composition—have been found. The gingerols (1-(4-hydroxy-3-methoxyphenyl)-5-hydroxyalcan-3-one), shogaols (1-(4-hydroxy-3-methoxyphenyl)-4-decen-3-one), and paradols are the most important subgroups of these (Arcusa et al., 2022). Compared to gingerols, which are mostly present in fresh ginger rhizomes, shogogols are more common in dried ginger rhizomes (Kumar et al., 2014). Gingerol analogs, which are thermally labile, undergo dehydration processes to produce the equivalent shogaols, which are more stable and have larger pharmacological effects than their antecedents and are in charge of the characteristically intense flavor of dried ginger. This chemical change occurs when the rhizomes are thermally dried and stored for an extended period of time. By means of bacterial metabolism, 6-shogaol is changed into 6-paradol. Ginger also contains other phenolic compounds as

quercetin, zingerone, gingerenone-A, and 6-dehydrogingerdione (Arcusa et al., 2022).

Antimicrobial activity of ginger compounds

Most of the ginger-added disinfectants on the market use ginger as the volatile aroma part. Studies using ginger oil and ginger extract are very effective as additives for water disinfection and achieve high disinfection rates (Mane et al., 2021). The antimicrobial activity of ginger essential oil, extracts, and oleoresins mainly depends on their chemical composition, the extraction solvent, the methodology used to obtain it, and the process to which the ginger was submitted (Kumar et al., 2023). The quality of the ginger that is recovered is also impacted by the solvent choice (Kumar et al., 2023). The solvents can be divided into green solvents [water, ethanol, glycerol, fatty acids/oils, acetic acid, ionic liquids, carbon dioxide (CO2), deep eutectic solvents and natural deep eutectic solvents (NADES), etc.] and other solvents such as acetone, chloroform, butanol, methanol, ethyl acetate, methyl acetate, benzene, hexane, cyclohexane, etc (Beristain-Bauza et al., 2019). Phytochemicals from ginger crop must be extracted in a way that preserves their natural structure and qualities in order to provide high-quality goods. Therefore, choosing a proper phytochemical extraction technique is crucial (Hafeez et al., 2021). The novel methods include pressurised liquid extraction (PLE), high hydrostatic pressure extraction (HHP), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), pulsed electric field extraction (PEF), vibro-cavitation extraction, extraction under vacuum-oscillating boiling conditions, extractions in mills, and extraction in rota (Kumar et al., 2023). Maceration,

percolation, decoction, reflux extraction, and Soxhlet extraction are some of the commonly used conventional methods (Redfern et al., 2014).

Most of these compounds are insoluble in water; thus, aqueous extracts exhibit lower antimicrobial activity than essential oil, oleoresins, and organic extracts (Beristain-Bauza et al., 2019). In this sense, ginger essential oil showed a high fungicide and antibacterial effect due to its high eugenol concentration (Singh et al) . The antimicrobial activity of volatile constituents of ginger essential oil decreases in the following order: phenolic compounds > alcohols > aldehydes > ketones > ethers > hydrocarbons (El-Baroty et al). It was concluded that the optimum drying condition was oven drying at 70°C in which maximum gingerol preserve. The quantity of 6-gingerol, 8-gingerol, 10-gingerol were 2.61mg/g, 2.27mg/g, 2.44mg/g (mg/g Dry weight DW) respectively at opti- mum drying condition (Hafeez et al., 2021). The results obtained using ginger extract as an antibacterial agent differ from those obtained using ginger essential oil. For example, Agrawal et al noted that methanol extract inhibited microorganisms more effectively than extracts obtained with any other solvent. Methanol extract had significant inhibitory effects on both Gram-positive and Gram-negative bacteria, which may be attributed to the polarity of methanol (Beristain-Bauza et al., 2019).

A large number of studies have confirmed that ginger extract and ginger oil have strong antibacterial capabilities (Agrawal et al.2012, Ahmed et al, 2022, Kumar et al, 2013). While shogaols and gingerols are recognised for their antibacterial effects, Zingerone research is still in its infancy. As one of the bioactive substances in ginger, zingerone has no research showing its antibacterial ability against *E. coli*. Zingerone inhibits biofilm formation and enhances the antibiofilm efficacy of ciprofloxacin

against salmonella (Kumar et al, 2013). The study compared the antibacterial abilities of zingerone with ginger extract and ginger oil to evaluate the potential of zingerone as a natural antibacterial substance. And compare the disinfection ability of zingerone with other plant-based disinfectants to provide commercial value for the development of new disinfectants.

Minimum inhibitory concentration (MIC)

In microbiology, the minimum inhibitory concentration (MIC) is a crucial metric for assessing how well antimicrobial drugs work against microorganisms. The lowest dose of an antimicrobial agent (such as an antibiotic) that prevents a microbe from growing visibly after it has been cultured in a certain medium for the entire night is known as the minimum inhibitory concentration (Wiegand et al., 2008). MICs are usually ascertained by laboratory testing techniques like agar or broth dilution.

MIC agar dilution: Different amounts of antimicrobial are added to a nutrient agar medium and a fixed number of cells are then plated on the surface of the plate (Wiegand et al., 2008). Agar dilution is a static analysis of MIC.

MIC broth dilution: (usually performed using a 96-well microtiter plate) involves placing bacteria into a liquid growth medium in the presence of various doses of antimicrobial (Wiegand et al., 2008). After a predetermined 24-hour incubation period, growth is assessed, and the MIC value is determined. The broth dilution method allows for the observation of bacterial growth curves over a 24-hour period.

In clinical microbiology and the treatment of infectious diseases, minimum inhibitory concentration (MIC) values play a crucial role in determining the efficacy of antibiotics against particular microorganisms. MIC testing yields valuable information that helps direct antibiotic therapy and track developments in antibiotic resistance.

Hypothesis and specific aims

Hypothesis
The disinfection efficiency of zingerone will higher than ginger extraction and standard disinfectant.

Specific aims
Aim 1: To extract ginger by using ethanol and methanol and Ginger oil in rotary vacuum distillation.

Aim 2: To test disinfection efficiency of ginger ethanol, extraction methanol extraction, ginger oil and zingerone with *E. coli* using the minimum inhibitor concentration broth dilution method.

Aim 3: To compare the disinfection efficacy of zingerone against chemical disinfectant (10% bleach).

2.1 MATERIALS AND METHODS

2.1.1 Materials

Australian ginger, ginger powder, Nutrient Broth (OXOID CM0001), Nutrient Agar (OXOID CM0003), ethanol, methanol, Phosphate buffered saline tablet (PBS) (SIGMA), 99.8% purity zingerone (sigma-aldrich), *Escherichia coli* (ATCC C-3000).

2.1.2 Methods

i. Methanol/ethanol extraction method (fresh ginger)

First, the ginger must be peeled and then sliced. The obtained ginger slices are dried for 27 hours at 50 to 60 °C in a cabinet dryer before grind into powder. Following preparation, separate conical flasks containing 25 grams of each crushed ginger are placed inside. To begin the extraction procedure, 100 ml of methanol or ethanol solvent is added to each conical flask. Following that, the conical flasks are set up on a mechanical shaker and shaken for 20 minutes at 300 rpm to guarantee complete mixing and phytochemical extraction from the ginger. Then, solid residues are eliminated by filtering the solvent-spice mixture with Whatman No. 1 filter paper. At 85 °C, rotary evaporation is used to concentrate the filtrate that is produced. In order to prepare it for further analytical processes, the concentrated extract is lastly kept chilled in sealed vials at 4 ± 1 °C.

ii. Ginger Oil extraction Method

Similar to the above procedure, however ultrasonic waves help throughout the entire operation. First, in a scientific setting, 25 g of ginger powder and 100 ml of water solvent were added to a centrifuge tube, and the mixture was extracted with water for 30 minutes. Following the completion of the reaction, the mixture in the flask was

allowed to come to room temperature before being centrifuged, filtered, and vacuum-dried in a rotary evaporator. After that, it was put in a drying oven set to 105 degrees for an hour.

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Figure 1: A water bath heating mechanism, a reflux condenser, and a round-bottom flask make up the main body of the extraction apparatus (picture from Wkielab).

iii. Preparation of Broth and Bacterial Culture

Nutrient agar medium was used to cultivate *E. coli* colonies in accordance with accepted practices. Following colony formation, three to five colonies with comparable morphology were selected using an inoculation loop, injected into ten milliliters of broth, and cultured for eighteen to twenty-four hours at 37°C. Following incubation, remove the supernatant from the bacterial solution by centrifuging it for 15 minutes at 3000 rpm using a centrifuge. Utilizing the pass-through dilution procedure, resuspend the centrifuged *E. coli* in PBS and dilute the bacterial solution

to an optical density of 0.1 at 600 nm (concentration: $1x10^9$). Place the bacterial solution into an agar plate after repeated dilution with PBS to reach a concentration of $1x10^4$. Examine the colonies on the last plate after 24 hours of incubation at 37°C for the Petri dishes. The solution might be processed further if the count was between 30 and 300 CFU.

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Figure 2: It displays the operational process schematic diagram (picture from Peirong Reng).

iv. **MIC measurement using the broth dilution method**

Zingerone should be weighed properly, completely dissolved in 100% water, and then stored in a solution for subsequent use. The stock solution has a 30 mg/ml concentration.

In well A1, 20μL of the test solution with the highest concentration to be measured was added. Next, 10μL of PBS was added to wells A2 through A10. Finally, 10μL was sucked out of well A1 and put to hole A2, mixed thoroughly, and then 10μL was sucked out of hole A2 and added to hole A3, and so on. Dilute the gradient to well A10 in an analogous manner, discarding the final 10μL of the diluted liquid. 180μL of

nutritional broth (NB) was added to wells A1–A10. For the holes B1~B10, C1~C10, D1~D10, E1~E10, and F1~F10, repeat the procedures. A1~A10 was then given 10μL water and B1~B10 was then given 10μL PBS as the standard groups, while C1~C10, D1~D10, E1~E10, and F1~F10 were given 10μL bacterial solution as the test group. At the moment, A1 through A10 have the following chemical concentrations: 50μg/ml, 25μg/ml, 12.5μg/ml, 6.3μg/ml, 3.2μg/ml, 1.6μg/ml, 0.8μg/ml, 0.4μg/ml, 0.2μg/ml, and 0.1μg/ml.

As a positive control group, add 10μl water, 180μl Nutrient broth (NB), and 10μl *E. coli* to each well in rows G and H. As a negative control group, add 200μl of NB. Lastly, cover the microplate, put it in a plate reader, adjust the temperature to 37 °C and OD600, and let it sit for 24 hours.

The overall operation procedure was repeated to determine the MIC of ginger ethanol extract, ginger methanol extract and ginger oil against Escherichia coli. The precise microplate configuration displayed in Table1.

v. **Comparative Test**

Zingerone's minimum inhibitory concentration (MIC) was compared with the MIC of food-grade disinfectants, which were ascertained by the use of the broth microdilution method. The benefits and drawbacks of zingerone as an antibacterial agent derived from natural sources were determined by contrasting its various attributes with those of food-grade disinfectants. Bleach was chosen as the comparison object in this investigation. The entire procedure was the same as for testing zingerone's minimum inhibitory concentration (MIC) against *Escherichia coli*.

Table 1: shows the specific configuration of the 96-well microplate.

CONCENTATION		50	25	12.5	6.3	3.2	1.6	0.8	0.4	0.2	0.1	Negative control	
		1	2	3	4	5	6	7	8	9	10	11	12
Standard (water)	A	ZINGERONE 1000μg/ml 10μL WATER 10μL BROTH 180μL	ZINGERONE 500μg/ml 10μL WATER 10μL BROTH 180μL	ZINGERONE 250μg/ml 10μL WATER 10μL BROTH 180μL	ZINGERONE 125μg/ml 10μL WATER 10μL BROTH 180μL	ZINGERONE 63 μg/ml 10μL WATER 10μL BROTH 180μL	ZINGERONE 32 μg/ml 10μL WATER 10μL BROTH 180μL	ZINGERONE 16 μg/ml 10μL WATER 10μL BROTH 180μL	ZINGERONE 8μ g/ml 10μL WATER 10μL BROTH 180μL	ZINGERONE 4μ g/ml 10μL WATER 10μL BROTH 180μL	ZINGERONE 2μ g/ml 10μL WATER 10μL BROTH 180μL	NB 200uL	NB 200uL
Standard (PBS)	B	ZINGERONE 1000μg/ml 10μL PBS 10μL BROTH 180μL	ZINGERONE 500μg/ml 10μL PBS 10μL BROTH 180μL	ZINGERONE 250μg/ml 10μL PBS 10μL BROTH 180μL	ZINGERONE 125μg/ml 10μL PBS 10μL BROTH 180μL	ZINGERONE 63 μg/ml 10μL PBS 10μL BROTH 180μL	ZINGERONE 32 μg/ml 10μL PBS 10μL BROTH 180μL	ZINGERONE 16 μg/ml 10μL PBS 10μL BROTH 180μL	ZINGERONE 8μ g/ml 10μL PBS 10μL BROTH 180μL	ZINGERONE 4μ g/ml 10μL PBS 10μL BROTH 180μL	ZINGERONE 2μ g/ml 10μL PBS 10μL BROTH 180μL	NB 200uL	NB 200uL
Test	C	ZINGERONE 1000μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 500μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 250μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 125μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 63μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 32μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 16μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 8 μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 4 μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 2 μg/ml 10μL *E.COLI* 10μL BROTH 180μL	NB 200uL	NB 200uL
	D	ZINGERONE 1000μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 500μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 250μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 125μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 63μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 32μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 16μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 8 μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 4 μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 2 μg/ml 10μL *E.COLI* 10μL BROTH 180μL	NB 200uL	NB 200uL
	E	ZINGERONE 1000μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 500μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 250μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 125μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 63μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 32μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 16μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 8 μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 4 μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 2 μg/ml 10μL *E.COLI* 10μL BROTH 180μL	NB 200uL	NB 200uL
	F	ZINGERONE 1000μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 500μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 250μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 125μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 63μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 32μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 16μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 8 μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 4 μg/ml 10μL *E.COLI* 10μL BROTH 180μL	ZINGERONE 2 μg/ml 10μL *E.COLI* 10μL BROTH 180μL	NB 200uL	NB 200uL
Positive control	G	PBS 10μL *E.COLI* 10μL BROTH 180μL	PBS 10μL *E.COLI* 10μL BROTH 180μL	PBS 10μL *E.COLI* 10μL BROTH 180μL	PBS 10μL *E.COLI* 10μL BROTH 180μL	PBS 10μL *E.COLI* 10μL BROTH 180μL	PBS 10μL *E.COLI* 10μL BROTH 180μL	PBS 10μL *E.COLI* 10μL BROTH 180μL	PBS 10μL *E.COLI* 10μL BROTH 180μL	PBS 10μL *E.COLI* 10μL BROTH 180μL	PBS 10μL *E.COLI* 10μL BROTH 180μL	NB 200uL	NB 200uL
	H	PBS 10μL *E.COLI* 10μL BROTH 180μL	PBS 10μL *E.COLI* 10μL BROTH 180μL	PBS 10μL *E.COLI* 10μL BROTH 180μL	PBS 10μL *E.COLI* 10μL BROTH 180μL	PBS 10μL *E.COLI* 10μL BROTH 180μL	PBS 10μL *E.COLI* 10μL BROTH 180μL	PBS 10μL *E.COLI* 10μL BROTH 180μL	PBS 10μL *E.COLI* 10μL BROTH 180μL	PBS 10μL *E.COLI* 10μL BROTH 180μL	PBS 10μL *E.COLI* 10μL BROTH 180μL	NB 200uL	NB 200uL

1.1 RESULT

3.14 The extraction rate of ginger extraction

The experimental results show that the yield of ginger extract obtained by single solvent extraction is extremely low. The original ginger powder is added as 25g. The amounts of methanol ginger extract, ethanol ginger extract and ginger oil obtained are only 8.73g, 5.43g and 2.83g(Figure 3). Therefore, the yields of methanol ginger extract, ethanol ginger extract and ginger oil are 34.9%, 21.7% and 11.3% respectively.

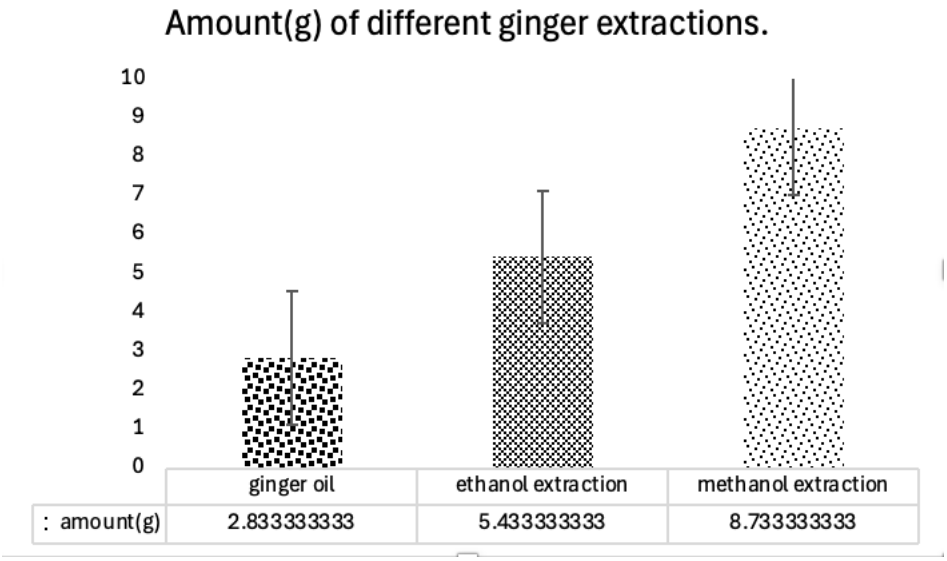

Figure 3: Amounts of methanol ginger extract, ethanol ginger extract, and ginger oil. Data are the means of three replicates (n = 3).

3.15 Antibacterial activity

The antibacterial properties of ginger solvent extract, 10% bleach and zingerone were evaluated against *Escherichia coli*. The results showed that ethanol extract, bleach and zingerone showed antibacterial activity, while methanol and ginger oil

extract had no effect. Ethanol extract was the most effective of all extracts in inhibiting microbial growth. Zingerone and bleach showed incomplete inhibition.

Table 2: It shows the antibacterial activity of ginger ethanol extract, ginger methanol extract, ginger oil, zingerone, 10% bleach against *E. coli*.

	Ethanol extract	Methanol Extract	Ginger oil	Zingerone	10% Bleach
Antibacterial activity	Complete inhibition in 24 h MIC=12.5 ug/ml	---	---	Incomplete inhibition in 24h MIC=50 ug/ml	Incomplete inhibition in 24h

3.16 Minimum inhibitory concentrations.

The MIC of the most potent ethanol extract of ginger was used by 96 plates, and the concentration-dependent effects of this extract are shown in Figure 1. The minimun inhibition concentation of ethanol extract is 12.5 ug/ml. The MIC was confirmed using readings of OD600 values. The results showed that ginger ethanol extract had potential antibacterial effects on *Escherichia coli* (Figures 4).

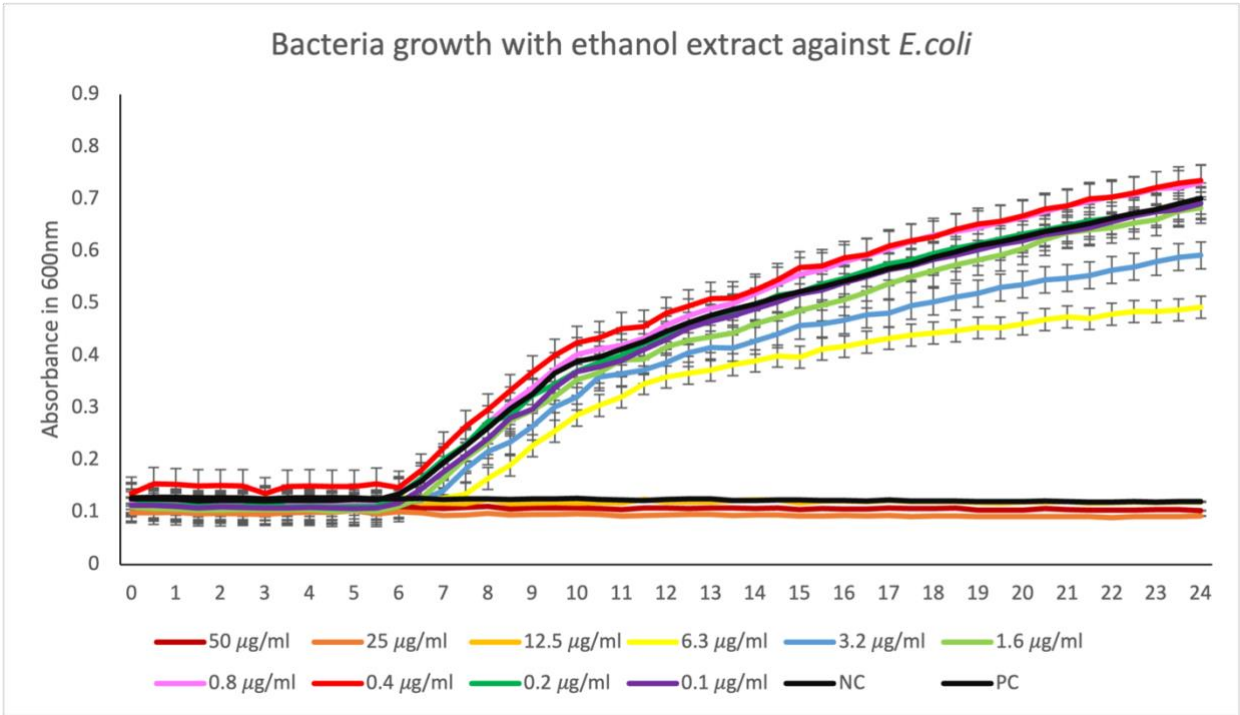

Figure 4: Determination of minimum inhibitory concentration based on growth curve analysis of ginger ethanol extract. Data are the means of four replicates (n = 4).

No significant antibacterial activity was found in ginger oil (Figure 5). In the first six hours, the bacteria were completely inhibited, and after six hours, the bacteria began to grow. Methanol ginger extract had the same results.

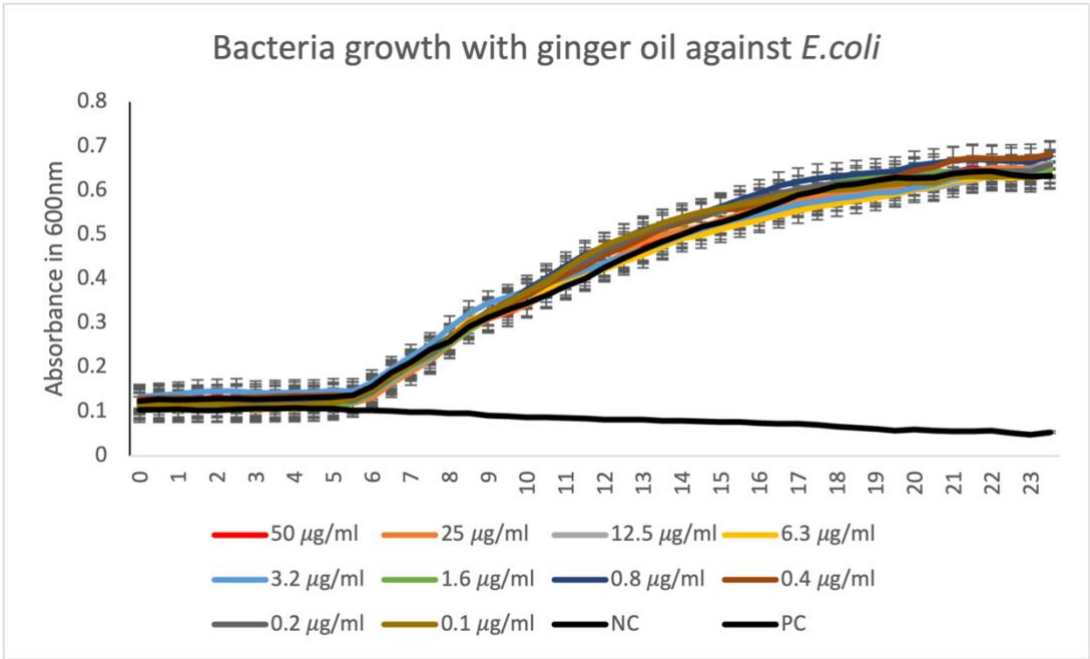

Figure 5: Determination of minimum inhibitory concentration based on growth curve analysis of ginger oil. Data are the means of four replicates (n = 4).

The MIC of zingerone in Figure 6 should be between 50ug/mL. Zingerone is available in powder form. Dissolve in water according to solubility to obtain a saturated solution. Solutions of different concentrations are obtained by dilution. The bacteria were completely inhibited in the first eight hours, and then showed a growth trend. At a concentration of 50, the *E. coli* hardly grew within 24 hours.

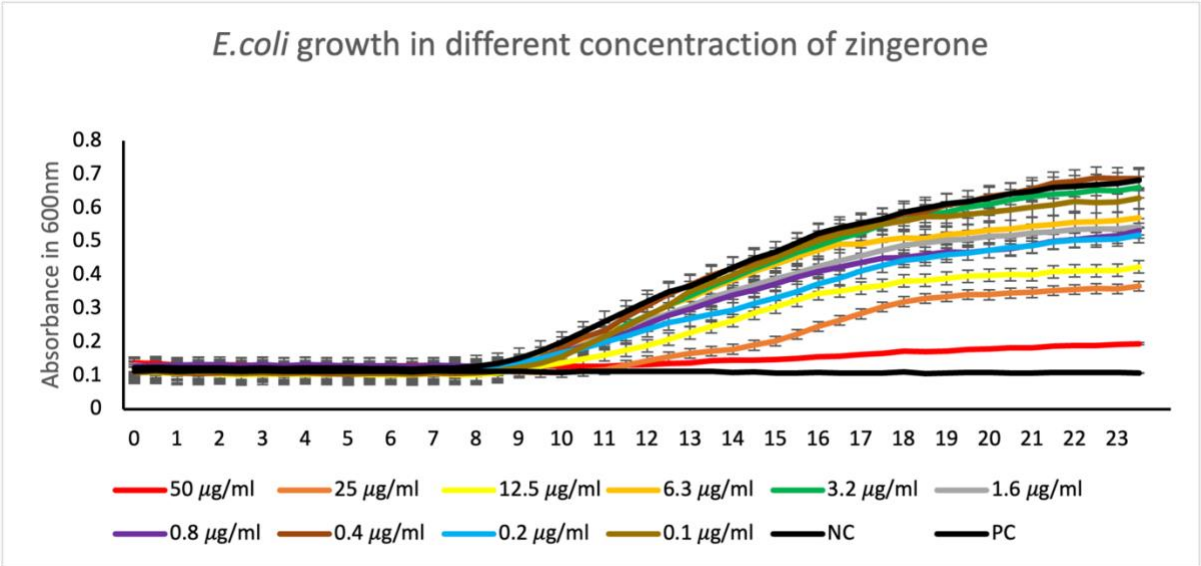

Figure 6: Determination of minimum inhibitory concentration based on growth curve analysis of zingerone. Data are the means of four replicates (n = 4).

In the comparative test of zingerone and bleach, bacterial growth occurred in both cases. 10% concentration of bleach is a common disinfectant concentration in laboratories and food processing plants. However, complete inhibition of bacteria only occurred within the first six hours, after which the bacteria slowly grew. However, compared with 10% bleach (figure 7), the disinfection effect of beach is significant, and the disinfection efficiency of 50 ug/mL zingerone is similar to that of bleach.

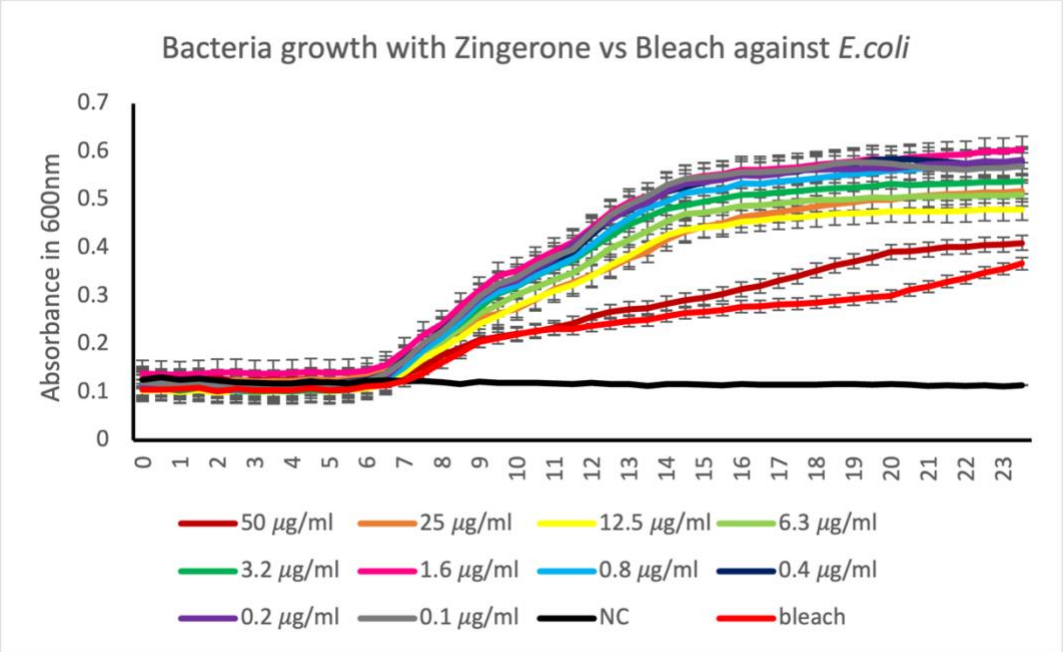

Figure 7: Determination of minimum inhibitory concentration based on growth curve analysis of zingerone and bleach (10%). Data are the means of four replicates (n = 4).

4.1 DISCUSSION

The composition of ginger methanol and ethanol extracts is unknown, and the active ingredients cannot be determined. The results show that it is very difficult to extract ginger oil in the laboratory, and even with ultrasound assistance, the yield is still very low. And the active ingredients need to be tested later.

The study found that, in contrast to the noteworthy results on zingerone, ginger oil did not exhibit substantial antibacterial activity. But even with zingerone's possible antibacterial qualities, the study found that, especially at 50% concentration, its disinfecting power was on par with a traditional disinfectant like bleach. Notably, even at a lesser concentration (10%), bleach's disinfecting power remained noticeably greater than zingerone's. These results imply that, although zingerone may have some potential as a natural disinfectant, in some situations its potency may not be greater than that of traditional disinfectants like bleach.

When determining if *E. coli* is susceptible to zingerone, the MIC test is a suitable method, particularly since previous research has indicated that zingerone has antibacterial qualities. To guarantee the validity of the data, appropriate controls were included, such as positive control(*E.coli* with broth) and negative control(without bacteria). It is important to put quality control procedures in place to guarantee the experiment's accuracy and repeatability. This entails keeping constant incubation conditions and employing defined techniques for creating zingerone solutions, dilutions, and bacterial cultures. Technical replicates are experiments that are repeated several times in order to measure variability and guarantee consistency in the outcomes.

To increase the disinfection effectiveness of formulations based on zingerone and investigate possible synergistic effects with other chemicals, more investigation and optimization may be required. Furthermore, comparative research with a wider variety of pathogens and in a range of environmental settings might offer more profound understanding of the real-world uses and restrictions of zingerone as a disinfectant.

Ultimately, even though zingerone offers a fascinating option for natural disinfection, further research and improvement are necessary before zingerone is widely used due to its low efficacy, especially in comparison to other disinfectants.

1.1 CONCLUSIONS

In summary, the study discussed in this article shows how new developments in the field of natural disinfectants, especially those based on, have the potential to provide an effective defense against bacterial infections like *E. coli.*

Although research on zingerone is still in its early stages, the fact that it can be found in many herbal spices and that it shares structural similarities with zidovudine, a well-known antibacterial molecule, point to the possibility of zingerone's use as a therapeutic agent. Furthermore, zingerone has no negative health consequences, making it a viable option for food-grade disinfectants.

Zingerone's potential as a ground-breaking natural disinfectant can be better understood by doing a comparative analysis with conventional disinfectants. Overall, the results open the door for more study and advancement in this exciting area by highlighting the considerable potential of zingerone, which is generated from ginger, as a reliable and secure substitute for disinfection in a variety of applications.

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