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ANTIBACTERIAL ACTIVITY OF *ZINGIBER OFFICINALE* AGAINST FOODBORNE PATHOGENS

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ABSTRACT- *Zingeber officinale or ginger, used as traditional medicine for over 2,500 years. It has been found to possess a number of pharmachological properties including antimicrobial. The following study was conducted to investigate the antibacterial properties of ginger on foodborne pathogens which are S. aureus, B. cereus, E. coli and S. typhimurium. Screening of antibacterial activity of ethanolic extract of ginger was tested by disc diffusion method using impregnated filter paper with 1000mg/ml concentration of ethanolic extract of ginger on inoculated Mueller Hinton Agar plate. The preliminary screening showed inhibition activity against all tested bacteria. Based on zone sizes, the antibacterial activity was more against gram positive than gram negative bacteria. The Minimum Inhibitory Concentration (MIC) was performed using micro broth dilution test. The MIC of the extract vary between 31.25 and 125 mg/ml and Minimum Bactericidal Concentration (MBC) ranged between 62.5 and 250 mg/ml. S. aureus and B. cereus were more sensitive to extract of ginger, while E. coli and S. typhimurium were not very effective to ginger extract. B. cereus showed the greatest antibacterial effect with MIC value 31.25 mg/ml and MBC value 62.5 mg/ml. Result of this study showed that Z. officinale ethanolic extract had antimicrobial activities against S. aureus, B. cereus, E. coli and S. typhimurium, thus confirming their potentially used as microbial growth inhibitors in the foods and an application as natural antibacterial agent.*

Keywords: *Zingeber officinale, Foodborne, Antimicrobial Sensitivity Testing (AST), Minimal Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC)*

INTRODUCTION

Foodborne disease is still a crucial hurdle of the world, even in well-develop countries (Salgado et al., 2009). In Malaysia, high cases of foodborne infections have been recorded due to the proper environment and temperature that enhance the growth of most microorganisms (Abdul-Mutalib et al., 2015). In recent years, antibiotic resistance has been reported in many studies and resistance has been developed by the bacteria against various antibacterial drugs (Tacconelli et al., 2018). This has renewed an interest of using medicinal plants extract to treat foodborne infections. *Zingeber officinale (Z. officinale)* commonly known as ginger, is including in ginger family, Zingiberaceae. Ginger widely used around the world as spice or food additive and medicine (Chandarana *et al.,* 2004). *Z. officinale* most heavily consumed dietary substances in the world. In India ginger was used as the remedy for cough and asthma. Ginger also useful in India's traditional medicine system known as Ayurveda for the remedy of migraine, whereas, in European traditions, they use ginger tea for digestive disruption. Beristain-Bauza *et al.* (2019) have reported in his review that ginger has analgesic, antipyretic, antiviral, antidiabetic, anti-inflammatory, anthelmintic, anticancer, and antioxidant properties and is recommended against different gastrointestinal and respiratory disorders.

Recently, studies had been done and reported the positive reaction of ginger extracts toward the foodborne bacteria. Other studies also found that ginger extract potentially has antimicrobial effect against a wide range of bacterial, fungal and

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parasitic organism. A study done by Ekwenye and Elegalam, (2005) found that ethanolic extract of ginger were able to inhibit the growth of *Escherichia coli* (*E. coli*). Norajit *et al.,* (2007), also found that ginger extract can give antibacterial effect against *Staphylococcus aureus* (*S. aureus*) and *Listeria monocytogenes.* In addition, several reports have been published also concerning about the antimicrobial actions of ginger. Jagetia *et al.,* (2003) has investigated that ginger extract had a dose-dependent anti-bacterial activity against *Pseudomonas aeruginosa, Salmonelle typhimurium* (*S. typhimurium*)*, E. coli* and antifungal activity against *Candida albicans*. Fieker *et al.,* (2003) also found that ginger extract has the broadest range of anti-fungal activity measured either by the fungi inhibited or as the average diameter of the zones of inhibition.

The bacteria are the most common factors that cause foodborne infection. According to Kamath *et al.,* (2008) the most common bacteria causing foodborne diseases are *Staphylococcus*, *Shigella*, *Salmonella*, *Bacillus*, *Escherichia coli* (*E. coli*), *Clostridium* and *Pseudomonas*. *S. aureus* is known as one of the major causes of foodborne infections in humans in both community and hospital (Kadariya *et al.,* 2014). Food poisoning is caused by the toxins produced by the bacteria. Meanwhile, *Bacillus cereus* is a spore forming foodborne pathogen commonly associated with food products such as meat, vegetables, soup, rice and other dairy product (Marrollo, 2016). Salmonella are recognized as one of the leading causes of foodborne diseases. These pathogen bacteria have been focused in the food safety industry. *E. coli* are among the common foodborne microorganism that cause infection and intoxication. Food poisoning caused by *E. coli* is usually associated with eating unwashed vegetables and meat contaminated post-slaughter (Yang *et al.,* 2017). Therefore, the objective of this study is to determine antibacterial activity of *Z. officinale* ethanol extraction against foodborne bacteria pathogens and the minimal bactericidal concentration (MBC) of *Z. officinale*.

MATERIALS AND METHODS

Chemical and Reagents: 80% Ethanol (R&M Chemicals, United Kingdom); dimethyl sulphoxide (DMSO) (Fisher Scientific, United Kingdom; distilled water (Alam Medic Sdn.Bhd); dehydrate barium chloride (Hamburd Chemical Ltd); concentrated sulphuric acid (HmbG Chemicals, Germany).

Media culture: Mueller Hinton Agar (Merck); Mueller Hinton Broth (Merck); Tryptic Soy Agar (Merck); Tryptic Soy Broth (Merck); Nutrient Agar (Merck).

Bacteria strains: In this study, four different type of foodborne bacteria were used in this study including two species of gram positive and two species of gram negative bacteria : *S. aureus* (ATCC 25923) and *B. cereus* (ATCC 11778), *E. coli* (ATCC 25922) and *S. typhimurium* (ATCC 14028). These bacteria strains were obtained from the Department of Medical Technology, University Technology Mara, Jalan Othman, Petaling Jaya. Bacteria cultures were maintained on nutrient agar (NA) slopes, subcultured monthly and subsequently stored at 4^{o}C.

Antibiotics for positive controls: Tetracycline (30 µg), Streptomycin (25 µg) and Kanamycin (30 µg), (Oxoid Ltd, England).

Preparation of *Z. officinale* extract

Plant materials: A fresh rhizomes of ginger (*Z. officinale*) were purchased at Malaysian Agriculture Research and Development Institute (MARDI), University Putra Malaysia, Serdang. According to Ekwenye and Elegalam (2005), the fresh mature rhizomes of *Z. officinale* were washed with clean water and allowed to air dry. The outer covering of ginger manually peeled off and the materials will be sliced into cutlets. The materials were placed in a hot air oven for drying. At the temperature of 65^{o}C, ginger dried within 48 h (2days). The mixer grinder was used to pulverized the ginger cutlets into powder.

Preparation of extract: This method of extract preparation was following Ekwenye and Elegalam (2005) with some modification. The powder was weighed using electronic weighing balance. The solvent used for extraction is ethanol (80%). The 1 liter of 80% ethanol were prepared by adding 802 mL of 99.7% ethanol to 198 mL distilled water. Hundred gram (100g) of the ginger powder were weighed into 2000 mL round bottom flask and 1 liter of ethanol were used to dissolve it. By using heating mantle, the mixtures were heating at 78^{o}C under reflux condition for 3 hours. Later, the

mixture been filtered using vacuum sucking (GAST Model DOA-P504-BN) and Whatman No.1 filter paper. The filtrates were collected for evaporation. The solvent was removed with a rotaty vacuum evaporator (BUCHI Rotavapor R-200) at 60ºC (25mmHg). After evaporation, the extracts were put into sterile petri dish and put into oven at 60ºC until dryness. Finally, the dry extracts were stored in refrigerator at 0ºC for further use of antibacterial sensitivity testing

Preparation of pure concentration of *Z. officinale* extract: 1000 mg/mL solution of the extract were prepared by dissolving 1.0 g (1000mg) of the extracts in 1 ml of the suitable solvents. The ethanolic extracts will be reconstituted in DMSO (Ekwenye and Elegalam, 2005).

Preparation of different concentration *Z. officinale* extract: The modification of concentration of *Z. officinale* extract was prepared by dissolving 1 g of *Z. officinale* extract in 0.1 ml dimethyl sulphoxide (DMSO) and 0.9 ml dH_2O to get the concentration of 1000 mg/ml for the screening test against S. *aureus, B. cereus, E. coli* and *S. thypimurium.* Then, the MIC with the final concentrations of the extract were 500, 250, 125, 62.5, 31.25, 15.63, 7.82, 3.9 and 2.0 mg/ml been prepared.

Antibacterial Susceptibility Testing (AST) using Disc Diffusion Technique: Filter paper discs with diameter 6 mm were autoclave before being used. Then, the different concentrations of the *Z. officinale* extraction were impregnated into sterilized paper discs. By using micropipette, 20 µl of each concentration were pipette onto that sterile paper Whatman AA paper disks. The discs were dried in oven for two hours before been use in AST. The positive and negative controls must be done to confirm the efficiency of the AST. The negative controls were made by impregnated 20 µl DMSO onto the sterile filter paper (Nair and Chanda, 2007). A parallel analysis with commercial antimicrobial agents was conducted in order to compare their antimicrobial efficiency with the plant extracts. Tetracycline were used as a positive control for *S. aureus* and *S. typhimurium*. Meanwhile, 30µg Kanamycin and 30µg Tetracycline were used as a positive control for *E. coli*.

Inoculation of Bacteria: Bacteria stock cultures were maintained on Nutrient Agar slant. They were sub cultured monthly and subsequently stored at 4 ^{0}C. The bacteria were cultured on Nutrient Agar for 24 hours at 37 ^{0}C in order to get a single colony that were used to perform AST. For experimental purposes, the organisms were inoculated into 5 ml Tryptic Soy Broth (TSB) and incubated at 37 ^{0}C for 2 to 3 hours. The grow of the four bacteria culture in tryptic soy broth (TSB) were adjusted using distilled water to yield approximately 1.0 x 10^8 CFU/ml by compared with 0.5 McFarland standard. (NCCLS, 2000). With the use of a moist sterile swab the suspensions were spread on plates of Mueller Hinton agar (MHA).

AST Using Disc Diffusion Method: Paper discs that had been impregnated with 1000 mg/ml concentration of *Z. officinale* extract were placed on the surface of agar with flamed forceps and gently pressed down to ensure contact. After that, positive and negative control were placed. Plates were left to get dry for about 15 minutes and were incubated at 37 ^{0}C overnight. Each test was performed in duplicate and the antibacterial activity were reported as the mean of bacteria inhibition in diameters (mm) that be measured by using ruler. A reading of more than 6 mm indicated there were bacteria growth inhibition.

Determination of Minimum Inhibitory Concentration (MIC) using Disc Diffusion Method: The bacteria inoculums were prepared only for bacteria that were showing the sensitivity against *Z. officinale* extract in the AST screening process. There are four different concentrations of *Z. officinale* were used in this method. Then the antimicrobial discs were applied onto agar. The zones of inhibition were measured, and all tests were done in duplicate to ensure the reliability.

Determination of Minimal Bactericidal Concentration (MBC)

50µl from each well that shown no visible growth (clear) were subculture onto Tryptic Soy Agar (TSA) plate and incubated at 37ºC for 18-24 hours. The MBC was the lowest antibiotic concentration that allowed no survival of microorganism or the highest dilution in which no growth existed (NCCLS, 2000).

RESULTS AND DISCUSSION

The usage of plant as remedies has been practiced long time ago. The WHO has catalogued more than 20,000

plants species with medicinal properties providing treatments for diseases (Goncalves *et al.,* 2008). *Z. officinale* is medicinal plants used in tropical and subtropical countries to treat many disorders such as diarrhoea, cough and gastrointestinal disorder (Beristain-Bauza et al., 2019).

Antimicrobial Sensitivity Test (AST) is used to determine the efficacy of potential antimicrobials from *Z. officinale* extracts against several different microbial species that cause foodborne diseases. Agar disc diffusion method was chosen since this technique has been widely used to assay plant extracts for antimicrobial activity in the previous study (Chah *et al.,* 2006).

Before bacteria are inoculated in MHA media, the turbidity of inoculums bacteria must be compared with 0.5 McFarland standards. It has been recommended by NCCLS, (2000) that the turbidity of inoculums used for antimicrobial sensitivity testing should be adjusted to the concentration of a 0.5 McFarland standard. It is important because when we overlooked during adjustment of the inoculums, it may lead to false susceptible and false resistant results. If the inoculums are too light it may cause false susceptible of bacteria results and if too heavy, it may cause false resistant of bacteria results.

Ncube *et al.,* (2008) noted that successful of biologically active compounds from plant material is depend on the type of solvent used in the extraction process. The choice of solvent used will give effect on type of the bioactive compounds that we are interested to isolate. According to Parekh *et al* (2005), the most commonly used solvents for investigations of antimicrobial activity in plants are organic compounds (methanol and ethanol) and aqueous (water), but among this two, organic solvents have been found to give more consistent antimicrobial activity compared to water extracts. In this study, ethanol had been used for extraction solvent. Many previous studies had shown that ethanol extraction of *Z. officinale* have potential to give antimicrobial effect (Karuppiah, P. *et al.,* 2012; Lucky, E. et al., 2017, Naji, T. and Jassemi, M., 2010). Mahady *et al.,* (2003) provide evidence that the active constituents of ginger (gingerols) are effective *in vitro* against *Helicobacter pylori*, the bacteria that caused peptic ulcer disease and developmentm of gastric and colon cancer. They found that gingerol inhibited the growth of *H.pylori* $CagA^+$ strains *in vitro.*

For the screening of *Z. officinale* extract at the concentration 1000 mg/ml, the result showed that there were antibacterial activities of the extracts against both gram positive and gram negative bacteria (Table 1). *S. aureus* that are common foodborne causative agent had to be the most susceptible of all organism being studied (zone of inhibition is 18 mm). On another hand, the antibacterial activity of ginger extract was weaker against in gram negative bacteria. These result in parallel have also reported by few studies where garlic have shown to have antimicrobial activity against *S. aureus, E. coli, S. typhi, Shigella flexineri, Proteus mirabilis* and *Vibrio parahaemolyticus* (Shobana et al., 2009 and Vuddhakul et al., 2007).

Most of the plant extracts were more active against gram-positive bacteria than gram-negative bacteria, which are supported with previous reports that plant extracts are more active against gram positive bacteria than gram negative. This situation occurred might be due to the difference in bacterial cell wall constituents. Gram positive bacteria have a simpler cell wall, which consists primarily of multiple layers of peptidoglycan with teichoic acid polymers dispersed throughout. Gram negative bacteria have a cell wall that is thinner than gram positive plus these bacteria have extra membrane, so called outer membrane, composed of a lipid bilayer, some proteins and lipopolysaccharide, lies above the peptidoglycan layer. This membrane excludes certain drugs (antibiotics) from penetrating the cell. So that, gram negative bacteria which are generally more resistant to antibacterial agents than gram positive bacteria (Los *et al.,* 2007).

The MIC studies showed that ethanolic extract of *Z. officinale* was found to be active against all the organisms tested (*S. aureus*, *B. cereus*, *E. coli* and *S. typhimurium*). In this study, the microdilution broth susceptibility test was used. Its advantages include increased sensitivity for small quantities of extract which is important if the antimicrobial is scarce as is the case for much natural product ability to distinguish between bacteriostatic and bactericidal effects and quantitative determination of the MIC (Langfield *et al.,* 2004). In addition, this method can also be used for the wide variety of microorganisms, it is not expensive, and it presents reproducible results (Ncube *et al.,* 2008). In this present study, well with concentration of *Z. officinale* ethanolic extract 62.5 mg/ml was determined as the MIC for *S. aureus* that inhibited growth of *S. aureus*. Meanwhile, the MIC for *B. cereus* was determined at well with concentration of *Z. officinale* ethanolic extract 31.25 mg/ml. At the dilution of 62.50 mg/ml and 125.00 mg/ml of ginger ethanolic extract, the MIC was observed for gram negative bacteria; *E. coli* and *S. typhimurium* respectively.

The MBC is determined by subculturing the preparations that would have shown no evidence of growth in the MIC determination assay (Ncube *et al.*, 2008). MBC is the lowest concentration that kills the bacteria. Result obtained showed that MBC values for all tested bacteria were higher than their MIC values. The result showed that *B. cereus* is the most sensitive tested bacteria with the lowest value of MBC at the concentration 62.50 mg/ml. Besides that, the plates with concentration of 250 mg/ml showed no colony growth for *S. aureus, E. coli and S. typhimurium* (Table 2). Absence of growth indicates that the tested drug is bactericidal (bacterial-killing) while growth indicates that the drug is bacteriostatic (bacterial-inhibiting) at a particular dilution.

CONCLUSIONS

In this study, antibacterial effects of *Z. officinale* (ginger) on selected foodborne bacteria were investigated. Based on the results obtained, ethanolic extract of *Z. officinale* showed antibacterial activity against *S. aureus*, *B. cereus*, *E. coli* and *S. typhimurium*. This is concluded based on inhibitory zone that was obtained in this study. Ethanolic extract of *Z. officinale* showed more sensitive against gram positive bacteria (*S. aureus* and *B. cereus*) and not very effective against gram negative bacteria (*E. coli* and *S. typhimurium*). The results of this study agreed with the findings of previous workers (Norajit *et al.,* 2007; and Ekwenye and Elegalam, 2005).

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Table 1: Antibacterial sensitivity testing (AST) of *Z. officinale* extract against foodborne pathogens.

No	Organisms	Diameters zone of inhibition in millimeters (Mean)		
		Control Positive	Control Negative	Extract 1000 mg/ml
1	*S. aureus* ATCC 25923	27	-	18.0
2	*B. cereus* ATCC 11778	22.5	-	11.0
3	*E. coli* ATCC 25922	23 (TE30) 20 (K30)	-	8.5
4	*S. typhimurium* ATCC 14028	22.5	-	7.0

Table 2: Determination minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of *Z. officinale* against foodborne pathogens.

No	Organisms	MIC (mg/ml)	MBC (mg/ml)
1	*S. aureus* ATCC 25923	62.50	250
2	*B. cereus* ATCC 11778	31.25	62.5
3	*E. coli* ATCC 25922	62.50	250
4	*S. typhimurium* ATCC 14028	125.00	250

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