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DETERMINATION OF ANTIBACTERIAL ACTIVITY OF GINGER EXTRACT AGAINST ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS

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ABSTRACT

The discovery of new antibiotics and semi-synthetic derivatives are the major global concern due to rise of antibiotic resistance to drug resistant pathogens that have acquired resistance mechanisms. Natural bioactive components from medicinal plants show antibacterial effect on bacteria. Rhizome of the Zingiber officinale (ginger) is on the most widely consumed natural product worldwide. The extracts of Zingiber officinale were obtained using maceration method using 50% ethanol and distilled water. The ginger extracts subjected to antibacterial activity against Escherichia coli and Staphylococcus aureus by disk diffusion method. The results showed significant antibacterial activity against Escherichia coli compared to Staphylococcus aureus with 50% ethanol extract whereas the distilled water extract greater inhibitory effect on Staphylococcus aureus than on Escherichia coli. This study indicated that the Zingiber officinale extracts have potential antibacterial effect against the two bacterial strains. Zingiber officinale could be studied for bioactive components and introduced as antibiotic medication for inhibiting bacterial development and as a potential replacement for synthetic bactericides

INTRODUCTION

Herbs and spices have been used in Asian countries for antibacterial characteristics that enhance the safety and shelf life of foods by reacting against foodborne pathogens and rotting bacteria in the environment from ancient times. In traditional Asian medicine, herbs and spices have been recognized as sources of natural antibacterial agents to treat various types of infectious diseases. As a result, herbal and spice extracts have received a lot of attention as potential antibacterial agents. In addition, in response to the global demand for preservative-free cosmetics, herbal and spice extracts with antibacterial properties have been applied in the cosmetic industry to decrease the chances of allergies associated with the presence of methylparabens (Nabavi et al., 2015). Bacteria are developing resistance to frequently used antibiotics as a result of higher drug use, causing a reduction in the efficiency of current medicines, demanding the discovery of novel antimicrobial agents (Amrita et al., 2009).

The antimicrobial activities of ginger were considered in this investigation. Herbs and spices, as well as their constituents, have antibacterial, antioxidant, anti-inflammatory, anti-diabetic, and anti-tumor characteristics, making them useful in the treatment of a variety of medical conditions. The rhizome of ginger (Zingiber officinale) is a popular dietary condiment that is deemed safe and can be used to treat a variety of conditions. According to several studies, ginger can help prevent cancer by neutralizing and stimulating various cellular pathways (Rahmani et al., 2014).

Ginger (Zingiber officinale) has a variety of active compounds, including terpenes and oleoresin, often known as ginger oil. Ginger additionally contains volatile oils in the range of 1% to 3%, as

well as non-volatile spicy compounds such as oleoresin. Sesquiterpene hydrocarbons and phenolic substances such as gingerol and shogaol, as well as lipophilic rhizome extracts, yielding potentially active gingerols which can be metabolized to shogaols, zingerone, and paradol, are the primary components found from terpene (Rahmani et al., 2014).

Ginger is quite beneficial in the prevention of diseases. However, the actual mode of action of ginger in the treatment of diseases remains unknown. Ginger plays a significant role in the treatment in illness management by modulating a variety of biological activities, as described below (Rahmani et al., 2014);

1. Ginger has antioxidant properties that protect macromolecules from damage induced by free radicals/oxidative stress (Rahmani et al., 2014).
2. Ginger has been shown to have anti-inflammatory properties (Rahmani et al., 2014).
3. Ginger also functions as an anticancer agent by modulating genetic pathways such as tumor suppressor gene activation, apoptosis modulation, and VEGF inhibition (Rahmani et al., 2014).
4. Antibacterial and other biological qualities of gingerol and paradol, as well as shogaols and zingerone The antibacterial activity of ethanolic ginger extract against a number of illnesses was discovered to be significant (Rahmani et al., 2014).

Drug resistance is on the rise all throughout the world, and it's being responsible for many treatment failures. Antibiotics are an efficient therapy for bacteria, but they can potentially have negative side effects. Ginger has been proven in studies to play an important role in preventing microbial development or acting as anti-microbial agents (Rahmani et al., 2014).

Scientific work on the antibacterial properties of ginger revealed that it exhibits antibacterial activities against Escherichia coli and Staphylococcus aureus. In addition, ethanolic ginger extract has the largest inhibitory zone towards Salmonella typhi. The antibacterial and antifungal properties of ginger rhizome are due to various components. Many studies have found that gingerol and shagelol are more active agents. Several researches have shown that ginger has broad antibacterial action, and that the ethanolic extract of ginger powder has strong inhibitory effects for Candida albicans (Rahmani et al., 2014). Essential oils can be extracted from a variety of plant components, including roots, barks, berries, seeds, rhizomes, leaves, and flowers, utilizing procedures like hydro distillation, steam distillation, solvent extraction, the Soxhlet method, and maceration. The maceration method was applied as the extraction tool in this research, and ginger was extracted using ethanol and distilled water.

Ginger extract was tested for antibacterial properties against Staphylococcus aureus and Escherichia coli. Staphylococcus aureus is a gram-positive bacterium that causes a wide range of clinical illnesses. Staphylococcus aureus infections are frequent in both community-acquired and hospital-acquired settings. Due to the advent of multi-drug resistance bacteria like MRSA (Methicillin-Resistant Staphylococcus aureus, treating remains difficult. Staphylococcus aureus does not usually lead to infections on healthy skin, but if it enters a human's internal tissues or bloodstream, it can cause a number of potentially deadly diseases (Taylor and Unakal, 2021). Escherichia coli is a facultative anaerobic bacterium that is gram-negative and rod-shaped. The most of Escherichia coli strains inhabit humans and animals' gastrointestinal tracts as natural flora. But certain strains have

developed into pathogenic Escherichia coli by the acquisition of virulence factors via plasmids, transposons, bacteriophages, and pathogenicity islands (Lim, 2010).

Antibiotics are medications that are used to treat bacterial infections. Antibiotics are available in many different forms. Each one is only effective against a specific bacterial strain. Antibiotic sensitivity test also known as antibiotic susceptibility test is a method of determining which antibiotic would be most effective for treating an infection and determining the antibacterial properties of various drugs (MedlinePlus, 2021). The ABST test is also used to determine a treatment for infections that are resistant to antibiotics. When regular antibiotics become less effective or ineffective against some bacteria, this is known as antibiotic resistance. Antibiotic resistance can make diseases that

Sample	Scientific name
Ginger	Zingiber officinale

were once easily curable into severe, even life-threatening conditions (MedlinePlus, 2021).

For this project, ABST was carried out for ginger extract with solvents such as ethanol and distilled water against Staphylococcus aureus and Escherichia coli using different positive controls such as ciprofloxacin and gentamicin. Inhibition zones were measured and recorded.

Objectives

General objective

- Determine the antibacterial activity of ginger (Zingiber officinale) against Escherichia coli and Staphylococcus aureus.

Specific objectives

- Extract ginger oil from ginger using mortar and pestle.
- Perform antibacterial sensitivity tests on Escherichia coli and Staphylococcus aureus.
- Compare the antibacterial activity of ginger.
- Observe the synergistic effect of the combination of ginger extract with antibiotics.

MATERIALS AND METHODOLOGY

Sample preparation

Table 1: Sample preparation

Sample	Scientific name
Ginger	Zingiber officinale

- Mortar and Pestle

Preparation of Media

Reagents	Consumables	Equipment	
		Name	Manufacturer
Mueller Hinton Agar/ Broth (Himedia)	Conical flasks	Analytical balance	
Nutrient Agar/ (Himedia)	Measuring cylinder	Autoclave	Meditry instruments

Nutrient Broth (Himedia)	Falcon tubes	
Distilled Water	Petri dishes	
Soyabean Casein Digest agar (Himedia)	Spatula	
	Parafilm	
	Foil squares	

Table 2: Materials used in media preparation

ABST TESTS

Reagents	Materials	Equipment	
		Name	Manufacturer
Distilled water	Forceps	Micropipette	Nichiryo
Conc. H_2SO_4 (Daytona)	Test tubes	Autoclave	Meditry instruments
$BaCl_2$ (Daytona)	Micropipette tips	Incubator	
Gentamycin discs	Cuvette	Spectrophotometer	
	Measuring cylinder	Refrigerator	Sisil
	Aluminum foils		
	Filter paper discs		
	Glass vials		
	Cotton swabs		
	Parafilm		
	Tissues		

Table 3: Materials used in ABST testing

METHODOLOGY

Sample preparation

Samples were collected from the home garden and cleaned of any visible impurities. 50g of each sample was weighed out using an analytical balance, and chopped using mortar and pestle. Each sample was prepared within 24 hours of extraction.

Ginger was mixed with solvents according to the table. And were kept in 50 ml of falcon tubes and allow to mix well in roller mixture for 48 hours. And then they were filtered using muslin clothes and stored in new labelled falcon tubes.

Sample	Solvent	Ginger
1	50% ethanol 30 mL	10 g
2	Distilled water 30 mL	10 g
3	-	50 g

Table 4: Ginger Samples

Further microbial tests were carried out to evaluate the suitability of the extracted samples.

Preparation of 0.5% McFarland standard

Initially 1% H2SO4 was prepared by mixing 0.1 mL H2SO4 with 9.9 mL of distilled water. 1% BaCl2 was prepared by mixing 0.1g of BaCl2 in 9.9 mL of distilled water. Then the 9.95 ml of 1% H2SO4 was mixed with 0.05 mL of 1% BaCl2. The accuracy of the prepared McFarland standard was checked by determining the absorbance at 625nm using a spectrophotometer.

Preparation of Nutrient broth

13g of Nutrient broth was dissolved in 1000 mL of distilled water. Then mixed well and placed in the autoclave for 30 min at 121 ℃.

Preparation of MHA

28g of Muller Hinton agar was dissolved in 1000 mL of distilled water. Then mixed well and placed in the autoclave for 30 min at 121 ℃.

Preparation of MH- Broth

28g of Muller Hinton broth was dissolved in 1000 mL of distilled water. Then mixed well and placed in the autoclave for 30 min at 121 ℃.

Preparation of Soyabean Casein Digest agar

40g of soyabean Casein Digest Agar was dissolved in 1000 mL of distilled water. Then mixed well and placed in the autoclave for 30 min at 121 ℃.

Preparation of overnight bacterial cultures

Nutrient broth was prepared according to the manufacturer's instructions (Appendix 1). Two tubes were labelled separately as E. coli and S. aureus. The broth was inoculated with primary cultures of bacterial strains using a sterile inoculation loop. Turbidity was checked against the freshly prepared 0.5 McFarland solution. The tubes were covered with parafilms and incubated for overnight at 37℃. Fresh subcultures were prepared every week as needed.

Preparation of discs

Filter paper discs were made by using a hole punch on Whatman filter paper no.1. The prepared discs were placed in a glass vial and autoclaved at 121°C for 30 minutes.

Disc diffusion method

Mueller-Hinton agar was prepared according to the manufacturer details (Appendix 3). Prepared agar plates were streaked by using a sterile cotton swab with the prepared subcultures of Escherichia coli and Staphylococcus aureus separately. Prepared discs were dipped in the samples and placed on the agar plates. Gentamycin disc was used as a positive control and distilled water was used as a negative control. All the plates were sealed with parafilms and incubated at 37°C for 24 hours. Then the minimum inhibitory zones were measured and recorded.

Minimum Inhibitory Concentration (MIC test), tube dilution method

Mueller-Hinton broth was prepared according to the manufacturer's instructions (Appendix 5). Both the bacterial samples of E. coli and S. aureus were prepared by checking the turbidity against 0.5 McFarland solution. Two sets were prepared separately for both bacterial strains. To each test tube 900µL of Mueller-Hinton broth was added and also 1000µL of sample was added to test tubes and did the dilution. Finally, 100µL of E. coli and S. aureus cultures were added to each set separately except for positive control and made the total volume up to 2.0 mL. All the tubes were capped with Al. foil and incubated for 24 hours at 37℃.

Minimum Bactericidal Concentration (MBC test)

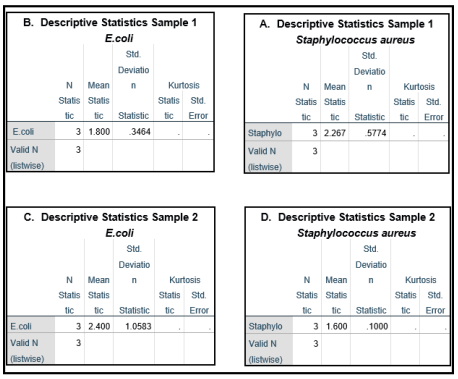
Soyabean casein digest agar was prepared according to the manufacturer's instructions (Appendix 5). 10µL of each sample from MIC test were added on to the agar plates according to the table using a micropipette. And plates were streaked using the glass spreader. All the plates were sealed with parafilms and incubated at 37°C for 24 hours. Results were observed.

Test tube number	Plate number
S3	S3
S4	S4
S5	S5
E3	E3
E4	E4
E5	E5

Table 5: MBC test tubes and plates

RESULTS

Figure 1: This gives the descriptive statistics of Escherichia coli and Staphylococcus aureus from both sample 1 and 2.



B. Descriptive Statistics Sample 1
E.coli

	N	Mean	Std. Deviation	Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Std. Error
E.coli	3	1.800	.3464	.	.
Valid N (listwise)	3				

A. Descriptive Statistics Sample 1
Staphylococcus aureus

	N	Mean	Std. Deviation	Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Std. Error
Staphylo	3	2.267	.5774	.	.
Valid N (listwise)	3				

C. Descriptive Statistics Sample 2
E.coli

	N	Mean	Std. Deviation	Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Std. Error
E.coli	3	2.400	1.0583	.	.
Valid N (listwise)	3				

D. Descriptive Statistics Sample 2
Staphylococcus aureus

	N	Mean	Std. Deviation	Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Std. Error
Staphylo	3	1.600	.1000	.	.
Valid N (listwise)	3				

	Zone of inhibitions in ABST									
Descriptive Statistics		Sample 1			Sample 2			Sample 3		
	Bacteria	ABST 1 (cm)	ABST 2 (cm)	ABST 3 (cm)	ABST 1 (cm)	ABST 2 (cm)	ABST 3 (cm)	ABST 1 (cm)	Positive Control (cm)	
	Escherichia coli	1.40	2.00	2.00	1.20	3.20	2.80	1.20	2.4 - 2.6	
Mean		1.80			2.40			-		
Standard deviation		0.35			1.06			-		
	Staphylococcus aureus	1.60	2.60	2.60	1.70	1.60	1.50	2.20	2.5 - 3.4	
Mean		2.27			1.60			-		
Standard deviation		0.58			0.10			-		

Table 5. Zone of inhibitions for ABST

DISCUSSION

As a result of growing public awareness about health issues, natural antimicrobials have recently been produced to control microbial infections. Medicinal plants and spices are one of the most common natural antibacterial agents in meals, and they have been utilized to treat common health problems by various cultures for thousands of years. The creation of antimicrobial drugs based on natural plant products has become increasingly significant, as newly discovered treatments are expected to be effective against multidrug-resistant bacteria (Karuppiah and Rajaram, 2012).

Herbal medicine has long been associated with ginger. Its gingerol-related components, in particular, have been shown to have antibacterial, antifungal, and medicinal effects (Park and Lee, 2008). In this research antibacterial activity of ginger (Zingiber officinale) was tested against Escherichia coli and Staphylococcus aureus. According to several researches, ginger rhizomes essential oil contains phenols, terpenes, and alkaloids, among other components (Rahmani et al., 2014). On the test organisms, ginger ethanol or methanol extracts had higher inhibitory results (19.23 mm 3.42) than other extracts. In a similar investigation, the maximum inhibitory effect (24 mm) of this ginger powder extract on S. aureus (ATCC 25923) was less than 30 mm for S. aureus utilizing fresh ginger rhizome ethanol extract (Ewnetu, Lemma and Birhane, 2014). The ethanolic extracts of ginger rhizomes were found to have antibacterial activity against five clinical isolates, with growth inhibition zones ranging from 4 to 16 mm. Bacillus sp. (16.55 mm) had the largest inhibitory zone, followed by E. coli (15.50 mm), and Pseudomonas aeruginosa (P.aeruginosa) (14.45 mm) (Karuppiah and Rajaram, 2012). In this research, Sample 1 was ginger extract with distilled water, sample 2 was ginger extract with ethanol and sample 3 was just the ginger extract. The zones of inhibition in disc diffusion were ranged between 1 to 2.6 cm in sample 1, 1.5 to 1.6 cm in sample 2 and 2.2 cm in sample 3 against Staphylococcus aureus. In Escherichia coli zones were ranged between 1.4 to 22 cm in sample 1, 1.2 to 3.2 cm in sample 2 and 1.2 cm in sample 3. These results show ginger extract is more effective against Escherichia coli and Staphylococcus aureus with distilled water is more effective than ginger with ethanol. In here, sample 1 and sample 2 of Escherichia coli shows some synergistic effect with gentamycin antibiotic discs. This shows evidence for synergistic effect of ginger with antibiotics.

The MBC test was used to measure the bactericidal activity of NPs from the aqueous extract against a number of harmful microbes. It's worth noting that MBC is the lowest medicine concentrations required to kill over 99 percent of the bacteria examined. The aqueous extract of ginger root proved efficient against S. aureus and S. aeruginosa in the MBC test, whereas the ethanolic extracts were efficient over E. coli (Farmoudeh, Shokoohi, and Ebrahimnejad, 2021). In this research MBC test did not give proper results.

The microorganisms Escherichia coli and Staphylococcus aureus have been shown to be inhibited by ginger ethanol

extract. Ginger extract and n-hexane methanol have antimicrobial properties. Gingerol and shogaol are phenolic chemicals that have antibacterial action. These chemicals cause permeability changes and the release of intracellular elements including ribose and Na-glutamate by damaging the membrane and cell wall. These chemicals also alter bacterial membrane functions such as electron transport, food intake, nucleic acid and protein synthesis, and enzyme activity. The harmful bacteria's activity is inhibited by both of these ways (Nadifah and Sari, 2016).

Immunomodulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic, and anti-emetic properties are the most commonly documented effects of ginger. Ginger is a potent antioxidant that can help to reduce or prevent free radical formation. It's thought to be a safe herbal treatment with only a few minor side effects. Gentamicin can produce harmful reactive oxygen species such as superoxide, hydrogen peroxide, and the hydroxyl radical, which is why it's commonly utilized to cause oxidative and necrotic damage. Gentamicin can cause sperm count to decrease. It has been established that gentamicin plays a role in the induction of apoptosis and oxidative stress. Ciprofloxacin, gentamicin, neomycin, streptomycin, and ofloxacin have all been shown to cause apoptosis in testes, according to recent studies (Zahedi et al., 2012). Other research found that ginger can help rats boost their caudaepididymal sperm stores by lowering apoptosis in the testis. In fact, combining ginger with gentamicin was able to counteract gentamicin's sperm-count-decreasing effects (Zahedi et al., 2012).

CONCLUSION

Overall, the findings of this research revealed that ginger extract could be used as a new antimicrobial agent for inhibiting bacterial growth and as a potential alternative to bactericides. And also, the findings reveal that ginger extracts show antibacterial properties against E. coli and S. aureus, as well as synergistic effects with antibiotics like gentamycin. Combinations of these pharmaceuticals can be used as an alternative use in therapy, lowering the required minimum dosages and allowing them to be utilized more effectively while saving money of patients. The results showed significant antibacterial activity against Escherichia coli compared to Staphylococcus aureus with 50% ethanol extract whereas the distilled water extract greater inhibitory effect on Staphylococcus aureus than on Escherichia coli. This study indicated that the Zingiber officinale extracts have potential antibacterial effect against the two bacterial strains. Zingiber officinale could be studied for bioactive components and introduced as antibiotic medication for inhibiting bacterial development and as a potential replacement for synthetic bactericides.

Future work

Because newly discovered treatments are expected to be helpful against multidrug resistant bacteria, drug discovery based on natural medicinal herbs has become extremely valuable (Karuppiah and Rajaram, 2012). Further research on ginger extract with various antibiotics can be carried out to determine which antibiotics have the best synergistic effect with ginger. Soxhlet extraction can be used to extract ginger essential oil, which can be applied to find antibiotics that have stronger synergistic effect against E. coli and S. aureus. Hopefully, this will allow the development of a more effective and efficient treatment. The minimum inhibitory concentration of ginger extract which is required to inhibit the visible growth of a microorganism can be determined by performing the MIC test,

and the minimum bactericidal concentration test can be done to determine the minimum ginger extract concentration that is required to kill 99.9% of the testing microorganisms such as E. coli can be determined using the Minimum bactericidal concentration test.

Ginger inhibits NFkB, COX2, and LOX, induces apoptosis, activates a tumor suppressor gene, and modifies a variety of biological functions. Ginger and its ingredients instill hope in a revolutionary therapeutic technique. Clinical studies should be the focus of future study to determine how effective it is and what role it plays in modulating biological pathways (Rahmani et al., 2014). However, the active ingredients of ginger must be isolated and their toxicity, adverse effects, and pharmacokinetic features must be determined (Karuppiah and Rajaram, 2012).

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