

An Evaluation of Antimicrobial Activity of Common *Zingiber officinale* Cultivars Grown in Sri Lanka

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ABSTRACT

Ginger (*Zingiber officinale*) has long been a therapeutic agent in traditional medicine systems worldwide and is a leading drug candidate in the pharmaceutical industry. In Sri Lanka, many forms of ginger are often used in the food industry, mainly as a spice; it also plays a vital role in the Ayurvedic medicine system. This study evaluated the antimicrobial activity of the two most cultivated cultivars of *Zingiber officinale* in Sri Lanka, the Sri Lankan (TG) and Chinese (CG) cultivars. Two types of extracts (ethanol and aqueous) were obtained from the rhizomes of each cultivar. The potential antimicrobial activity of the extracts was tested against three types of pathogenic test organisms, *Staphylococcus aureus* ATCC 25923, *Salmonella typhi* DSM 17058, and *Escherichia coli* ATCC 25922, using the agar well diffusion method along with positive control, chloramphenicol, and negative controls, 95% ethanol or distilled water. In conclusion, both *Zingiber officinale* cultivars exhibit varying antimicrobial potential, with ethanol extracts showing stronger activity than aqueous ones. None of the extracts was effective against *Escherichia coli*. The agar dilution method determined the minimum inhibitory concentration (MIC) of each ginger extract. Among the extracts, ethanol extracts showed higher effectivity than aqueous extracts, where all test organisms showed inhibition at a concentration of 20 mg/mL. The antimicrobial activity of the Chinese cultivar outperforms the Sri Lankan cultivar against target organisms, *Salmonella typhi*, and *Staphylococcus aureus*.

Keywords: Antimicrobial activity, Chinese cultivar, Sri Lankan Cultivar, *Zingiber officinale*.

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1. INTRODUCTION

The Ginger plant, *Zingiber officinale*, is a member of the Zingiberaceae family, including, but not limited to, cardamom and turmeric [1], [2]. It is an aromatic herb known for its use as a spice and a medicine, having an international reputation. Although Asia is the original home, it can also thrive in many tropical and subtropical climates [3]. Ginger's rhizome, which is fragrant and flaky, and bears, is its most valuable commercial component. Ginger is one of the most famous traditional herbal medicinal plants with numerous known nutritional properties, making it an excellent target for drug discovery [4]. Ginger plays an irreplaceable role in numerous treatments in the traditional medical systems of Ayurveda, Siddha, China, Arabia, Africa, and the Caribbean. However, it is often used as an herbal medicinal remedy and

household remedy to treat asthma, cough, and diarrheal conditions [5], [6] related to infections due to its proclaiming antimicrobial activity. Many pathogenic organisms, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella* spp., *Salmonella* spp., and *Proteus* spp., are susceptible to ginger's antibacterial properties [3], [7]. Due to this, ginger components are often used as a treatment for infectious diseases.

Ginger consists of sixty or more active elements, broadly categorized into two groups: non-volatile compounds, which include gingerols, paradols, shogaols, and zengenols, and volatile compounds, which consist of components with hydrocarbon backbones like monoterpenoids and sesquiterpenoids [8]. Dry ginger, in contrast, shows its pungency due to Shogaols [9], [10]. Depending on the area of cultivation and whether the product is fresh, dried, or



processed, the chemical composition of the ginger rhizome can vary greatly.

The antimicrobial properties of ginger and its extracts are primarily due to the phenolic compounds such as eugenols, gingerols, shogaols and zengenols and their symbiotic interaction with other compounds like β -sesquiphellandrene, cis-caryophyllene, zingiberene, α -farnesene, α - and β -bisabolene. Phenolic chemicals act as protein denaturants, altering the permeability of microbial cells and causing swelling and rupture. Additionally, the majority of phenolic compounds are metal chelators that bind to the active sites of proteins or metabolic enzymes, decreasing their activity and, as a result, the activity of the cell and cell division also decreases [11], [12].

The level of antimicrobial activity of ginger extracts, their essential oils, and oleoresin depends mainly on the chemical composition of the ginger, the type of solvent used for the extraction process, the technique used for the process, and the process it undergoes [9]. The majority of the components providing antimicrobial activities are insoluble in water. Hence, aqueous extracts' antibacterial activity was reportedly lower than that of essential oils, oleoresins, and organic extracts [13].

In Sri Lanka, Ginger is successfully grown at elevations up to 1500 m above mean sea level, with a minimum ideal annual rainfall of 1500 mm. The districts that lead in the growth of ginger are Kandy, Kurunegala, Gampaha, Kegalle, and Colombo [14]. The three main ginger cultivars cultivated in the country are Sri Lankan ginger or Traditional ginger (TG) (also known as Beheth Inguru) consists of small rhizomes with white colour fibrous flesh, Chinese ginger (CG) with large rhizomes with pale yellow colour flesh and watery consistency and Rangoon ginger with rhizomes are medium-sized and have multiple fingering. These three ginger cultivars are similar morphologically, with the only real difference being the size of the rhizomes [14], [15]. Although several cultivars of ginger are present in Sri Lanka, among those two cultivars are commonly cultivated: The traditional and the Chinese [16]. Among these cultivars, Chinese ginger has become more popular among cultivators due to its high-yielding capacities and among consumers for more abundance within the markets when compared to traditional ginger. Usually, in Ayurveda medicine and household medicinal purposes, traditional ginger is preferred to the Chinese cultivar due to its higher notes of pungency flavour and aroma than other cultivars. According to an antibacterial activity study carried out using methanolic extracts of ginger cultivars commonly grown in Sri Lanka against *Escherichia coli* and *Staphylococcus aureus*, Chinese ginger cultivars have outperformed the local ginger (Sri Lankan) cultivar [17]. However, limited studies have been conducted to assess these ginger cultivars' antimicrobial properties. The present study was carried out with the objectives of determining and comparing the level of antimicrobial activity of aqueous and ethanol extracts obtained from Sri Lankan and Chinese *Zingiber officinale* cultivars using the agar well diffusion method and to determine the minimum inhibitory concentration (MIC) of all *Zingiber officinale* extracts using the agar dilution method against three types of test organisms *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus*

aureus, which are common foodborne pathogens which have increasing levels of antibiotic resistance. Ethanol and water were used for the extraction process instead of methanolic extracts, which may have a certain level of toxicity to human and animal cells.

2. MATERIALS AND METHOD

2.1. *Zingiber officinale* Collection and Preparation

The fresh forms of *Zingiber officinale* (ginger rhizomes, Chinese cultivar [CG], and Sri Lankan cultivar [TG]) purchased from the local market were thoroughly washed using running tap water. Then these rhizomes were sliced into small pieces using a slicer to increase the surface area to elevate the drying rate. Then, these pieces were dried in a drying cabinet (55 °C for 6 hours). Dried materials were grounded to a fine powder using an electric grinder and were stored in an airtight dark bottle for further use.

2.2. Preparation of Extracts of *Zingiber officinale*

2.2.1. Aqueous Extract

Fine powder of each *Zingiber officinale* (20.0 g) was measured and dispensed into a conical flask with Distilled water (80.0 mL) and was soaked for 72 hours. The solution was carefully filtered with muslin cloth into a sterilized conical flask, and the obtained filtrates were sealed and stored in the refrigerator (4 °C).

2.2.2. Ethanol Extract

Fine powder of each *Zingiber officinale* (20.0 g) was measured and dispensed into a conical flask with Ethanol (95%, 80.0 mL). It was soaked for 72 hours after the solution was carefully filtered with muslin cloth into a sterilized conical flask, and the obtained filtrates were sealed and stored in the refrigerator (4 °C).

2.3. Preparation of Test Organisms

Bacterium isolates of *Escherichia coli* ATCC 25922, *Salmonella typhi* DSM 17058, and *Staphylococcus aureus* ATCC 25923 strains were obtained from culture collection from the Department of Microbiology, University of Kelaniya. The bacteria were maintained on nutrient agar slant at 4 °C and subculture periodically within 2–3 weeks on fresh medium.

2.4. Determination of Antimicrobial Activity via Agar Well Diffusion Method

The antimicrobial activity of extracts obtained by *Zingiber officinale* was tested according to the method described by Akintobi [7] with modifications. From maintained cultures of *Escherichia Coli*, *Staphylococcus aureus*, and *Salmonella typhi*, slants were made and incubated at 32 °C for 24–48 hours. Loops from each slant culture were transferred to a sterile saline solution tube (5.0 mL) to prepare the suspension of each culture. These suspensions were swabbed evenly on pre-solidified sterile Muller Hilton Agar plates to obtain uniform microbial lawns. The wells were cut. Three wells were bored on each plate, using a sterile borer: one well for the extract of *Zingiber officinale* (250 μ L), the second well for the chloramphenicol, the

positive control (20 µg/mL, 250 µL), the third well for the negative control, distilled water (250 µL) and these wells was filled after syringe filtering each. They were allowed to stand for one hour for proper diffusion and then were incubated (37 °C, 24 hours). Similarly, the process was duplicated. The sensitivity of the test organisms to ethanol and water extract of *Zingiber officinale* was indicated by a clear zone of inhibition around the wells. The diameter of the clear zone (Zone of inhibition) was measured to the nearest mm using a transparent ruler. This (Zone of inhibition) was taken as an index of the degree of sensitivity of the test organisms to ethanol and aqueous extracts.

2.5. Determination of the Minimum Inhibitory Concentration (MIC) by Agar Dilution Method

The Minimum inhibitory concentration of the extracts of *Zingiber officinale* was determined as described by Akintobi [7]. Each extract was added at concentrations of 0 (control), 1.25, 2.5, 5, 10, 20, 40, 80, 160, and 200 mg/ml into molten Nutrient agar and poured into Petri dishes. The overnight broth cultures of *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* were spot-inoculated on the plates, so each inoculum contained 10⁶ CFU. Plates were incubated and observed (28 °C, 24 hours).

2.6. Statistical Analysis

All experiment results were performed in triplicates and expressed as mean ± Standard Deviation (± SD). Calculations were done by using MINITAB 21.4 software. Analysis of variance was performed, and the significant difference recorded between mean values was determined by Tukey's pairwise comparison test (level of significance

of $p < 0.05$). Statistical analyses were conducted using MINITAB 21.4 software.

3. RESULTS

3.1. Sample Collection and Preparation

Chinese cultivar consisted of larger rhizomes when compared with the local cultivar rhizomes and less number of nodes (Fig. 1). After slicing the rhizomes, Chinese ginger contained light yellow coloured flesh with high water consistency. Meanwhile, the local cultivar had off-white coloured flesh with less watery consistency when compared to the Chinese cultivar. After drying, dried Chinese ginger pieces were smaller than the local cultivar (Fig. 1).

3.2. Determination of Antimicrobial Activity via Agar Well Diffusion Method

Ethanol extracts of ginger powder revealed that each cultivar had a certain inhibition towards *Staphylococcus aureus* and *Salmonella typhi*; meanwhile, neither TG nor CG ethanol extracts do not show any inhibition towards *Escherichia coli*. Aqueous ginger powder extracts revealed no inhibition zones were observed from TG's aqueous extracts. Aqueous extracts of CG showed a zone of inhibition only towards *Salmonella typhi*, which was nearly similar to the zone of inhibition of the positive control, chloramphenicol (Table I, Fig. 2).

3.3. Determination of the Minimum Inhibitory Concentration (MIC) by Agar Dilution Method

Salmonella typhi in ethanol extracts of TG showed total inhibition at a ginger extract concentration of 10 mg/ml.

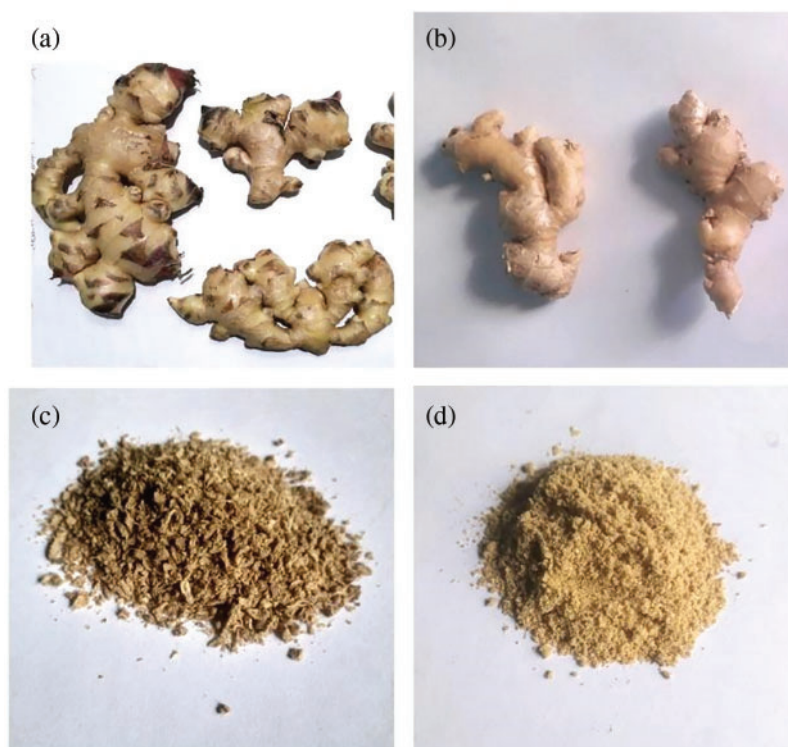
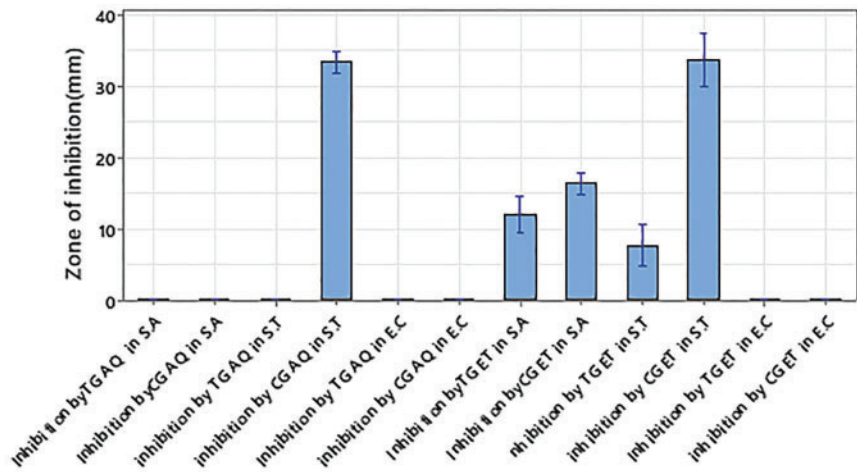


Fig. 1. Collected *Zingiber officinale* samples and powder form after processing: a) Fresh rhizomes of *Zingiber officinale* Chinese cultivar (CG), b) Fresh rhizomes of *Zingiber officinale* Sri Lankan cultivar (TG), c) Powder formed after grinding dried *Zingiber officinale* Chinese cultivar (CG), and d) Powder formed after grinding dried *Zingiber officinale* Sri Lankan cultivar (TG).



Individual standard deviations are used to calculate the intervals.

Fig. 2. The Zone of Inhibition (mm) formed by all types of extracts of ginger variants is shown in an interval plot. TGET-Traditional Ginger ethanol extract, CGET-Chinese ginger ethanol extract, TGAQ-Traditional Ginger Aqueous extract, CGAQ-Chinese ginger Aqueous Extract S.A-*Staphylococcus aureus*, S.T-*Salmonella typhi*, E.C-*Escherichia coli*.

TABLE I: SENSITIVITY PATTERN OF TEST ORGANISMS TOWARDS ETHANOL AND AQUEOUS EXTRACTS OF TRADITIONAL AND CHINESE GINGER												
Test organism	Ethanol extract Zone of inhibition (mm)						Aqueous extract Zone of inhibition (mm)					
	TG	(+)	(-)	CG	(+)	(-)	TG	(+)	(-)	CG	(+)	(-)
<i>Staphylococcus aureus</i>	12.0 (±1.00)	26.3 (±1.15)	1.07 (±0.12)	16.3 (±0.58)	40.3 (±1.53)	1.13 (±0.15)	0	44.7 (±0.58)	0	0	46.3 (±1.53)	0
<i>Salmonella typhi</i>	7.7 (±1.15)	23.3 (±1.53)	2.20 (±0.10)	33.7 (±1.53)	39.3 (±1.15)	1.07 (±0.25)	0	31.7 (±1.53)	0	33.3 (±0.58)	34.3 (±0.58)	0
<i>Escherichia coli</i>	0	25.3 (±0.58)	0	0	24.7 (±0.58)	0	0	32.0 (±1.00)	0	0	27.0 (±1.00)	0

Note: TG-Traditional Ginger, CG-Chinese ginger (+)-Chloramphenicol, (-)-for Ethanol extracts- Ethanol, for Aqueous extracts-Distilled water.

In comparison, *Staphylococcus aureus* and *Escherichia coli* growth were inhibited at a ginger extract concentration of 20 mg/mL. Meanwhile, *Staphylococcus aureus* growth was inhibited at a 10 mg/mL concentration of ginger extract. Growth of *Salmonella typhi* and *Escherichia coli* were inhibiting at a ginger extract concentration of 20 mg/mL. Only *Salmonella typhi* showed total growth inhibition in aqueous extracts CG (at 200 mg/mL of aqueous extract concentration).

4. DISCUSSION

Due to the misemployment of antibiotics, microorganisms have developed resistance mechanisms towards antibiotics [18]. Plant-derived medications have gained much interest as chemotherapeutic medication alternatives for treating diseases [19]. Depending on the area and the environmental conditions in which the cultivation occurs, the chemical composition and morphological characteristics of the ginger rhizomes could vary. However, multiple studies have been carried out in many other countries to evaluate the antibacterial activity of ginger cultivated within their countries; in Sri Lanka, very few such studies have been carried out.

The fresh forms of *Zingiber officinale* rhizomes, Chinese cultivars, and some stand-out characteristics like more extensive rhizomes and several nodes of Chinese cultivar rhizomes, may be due to breeding and selection practices

used in countries which often cultivate Chinese cultivars, like China and Vietnam [20], [21].

The antimicrobial properties of ginger and its extracts are primarily due to the phenolic compounds such as eugenols, gingerols, shogaols and zengenols. The level of antimicrobial activity of ginger extracts majorly depends on the chemical composition of the ginger, the type of solvent used for the extraction process, the technique used for the process, and the process it undergoes [9]. Also, solvent polarities have a crucial role in the extraction of plant secondary metabolites and, as a result, in their antimicrobial activity. This study evaluated the antimicrobial activity of ethanol and aqueous extracts of two ginger cultivars, traditional ginger (TG) and Chinese ginger (CG), against several bacterial strains.

The level of antimicrobial activity of each ginger extract was determined using the standard agar well diffusion method by measuring the diameter of the zone of inhibition formed by each extract against *Staphylococcus aureus*, gram-positive, an opportunistic pathogen that can cause a wide range of illnesses, from sepsis and deadly pneumonia to moderately severe skin infections [22]. *Salmonella typhi* is a gram-negative bacterium that causes typhoid fever, which is common in children and young adults and has plagued developing countries for decades [23]. *Escherichia coli* is a gram-negative bacterium. Typically, *E. coli* can be found in your intestines. Most strains are typically harmless. A few strains develop vomiting, nausea, stomach

cramps, diarrhoea and bloody diarrhoea. The well diffusion was carried out using the Muller Hinton Agar (MHA) as compared to most other media; MHA is a loose agar that is loose agar that, compared to most other media, provides for improved antibiotic diffusion. A truer zone of inhibition is produced by enhanced diffusion [24].

Ethanol extracts of TG exhibited inhibitory effects on *Staphylococcus aureus* and *Salmonella typhi*, with the highest against *Staphylococcus aureus*. In the case of CG ethanol extracts, they displayed inhibitory effects against both *Staphylococcus aureus* and *Salmonella typhi*, with the highest against *Salmonella typhi*. None of the ethanol extract types were effective against *Escherichia coli*. When considering aqueous extracts, CG displayed a significant level of inhibition, primarily against *Salmonella typhi*, with inhibition levels comparable to the positive control, chloramphenicol (Table I, Fig. 2). The minimum inhibitory concentration (MIC) of ginger extracts was also determined. The MIC for TG ethanol extract against *Salmonella typhi* was 10 mg/mL, while it was 20 mg/mL for *Staphylococcus aureus* and *Escherichia coli*. In the case of CG ethanol extract, the MIC for *Escherichia coli* and *Salmonella typhi* was 20 mg/mL, and for *Staphylococcus aureus*, it was 10 mg/mL. Additionally, only *Salmonella typhi* showed total growth inhibition in CG aqueous extracts at 200 mg/mL, while the other organisms continued to thrive at various ginger extract concentrations. Although the growth of *E. coli* was inhibited at low concentrations of ethanolic extracts in MIC, it didn't show any inhibition zones during the agar well diffusion method, where each well consisted of ethanol extracts of each cultivar at a concentration of 1000 mg/mL. This may be caused due to evaporation of the rapid evaporation of the solvent ethanol during the agar well diffusion method. These results highlight the potential of ginger extracts in combating specific bacterial strains, with variations in efficacy based on the type of ginger and the bacterial species. The experiment could not be carried out with higher concentration levels of ginger extracts since when increasing the concentration level, a larger volume of ginger extract should be added to the prepared agar, which dilutes it, inhibiting its solidification. Therefore, it is ideal to carry out this using the gradient method, which uses strips infused with an antibiotic concentration gradient or a broth dilution method [25].

5. CONCLUSION

The antimicrobial efficacy of dried *Zingiber officinale* varies, while the ethanol extracts are more effective than aqueous against the studied pathogens (*Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*). Chinese cultivar extracts outperformed Sri Lankan cultivar extracts in terms of antimicrobial activity against the studied pathogens (*Staphylococcus aureus*, and *Salmonella typhi*).

CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

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