

# Antimicrobial Properties of Ginger and Licorice Root and Their Synergy

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Received July 9, 2025; Revised September 29, 2025; Accepted October 14, 2025

## Abstract

This study investigated the antimicrobial potential of ginger root and licorice root extracts individually and in combination, aiming to assess whether synergistic interaction enhances their antimicrobial properties. With the escalating issue of antibiotic resistance, finding novel solutions is imperative, and natural compounds such as ginger and licorice roots pose new possibilities. This study utilized ethanol-based extraction by steeping plant powders in a 0.1% ethanol solution prepared via serial dilution of 95% ethanol, focusing on *Escherichia coli* to assess antimicrobial potential of licorice root, ginger root, and “So-Cheong-Ryong-Tang (SCRT)”, a custom mix of ginger and licorice root. Since SCRT was unavailable for ordering, a homemade version was formulated by combining ginger and licorice root to replicate the SCRT blend. Both ginger and licorice root are key components of the SCRT blend and their individual therapeutic properties are well documented. The herbal extracts were prepared and the evaluation of antimicrobial properties involved testing against a negative control using the average zone of inhibition values as a quantitative measure of *Escherichia coli* bacterial growth inhibition. The results indicated that the ginger-licorice blend demonstrated a synergistic effect, surpassing the individual extracts in potency.

**Keywords:** Antimicrobial, Susceptibility test, Synergism, Zone of inhibition, Ginger root, Licorice root, So-Cheong-Ryong-Tang

## 1. Introduction

Around 6.3 billion of the world’s population use or rely on traditional medicine to improve their health (WHO, 2022). Traditional medicine encompasses various indigenous knowledge, skills, and cultural practices that have been used over a long history to promote well-being. Traditional medicine has a long history of utilizing herbal remedies, often combining different plants to enhance their therapeutic effects. One of the key aspects of traditional medicine is its emphasis on the synergy between various herbal extracts. This synergistic effect is believed to enhance the therapeutic properties of traditional remedies. (Yuan 2017). Individuals may choose to use traditional medicine for their treatment due to dissatisfaction with conventional (Western) medicine, past good experiences, and a family history of using herbal medicines (Welz et al., 2018). Despite the widespread use of traditional medicine, there remains a need for more research into its efficacy, particularly from a Western medical perspective. While some studies have investigated the medicinal properties of isolated compounds or herbs, traditional medicines often consist of complex herbal blends that work synergistically (Leonti 2013). This study aims to address the gap by comparing the therapeutic effects of prescribed herbal blends (SCRT) with those of isolated compounds/herbs (ginger root, licorice root).

Another area of interest in traditional medicine is the potential to treat infections without raising the risk of antibiotic resistance. Undoubtedly, one of the great inventions of the 20th century was the discovery, creation, and distribution of antibiotic medicines. The advent of antibiotics is accredited to have saved over 200,000 American lives annually and extended the average American life expectancy by 5-10 years. (Gottfried 2005). However, antibiotic resistance has increased among bacteria due to the overuse and over-prescription of antibiotics. This problem has a

global impact because resistant strains have the potential to spread through people who are traveling. Not only are they over-prescribed, but it is also used as a substitute for good hygiene to maintain and grow crops and livestock. With the increasing resistance to antibiotics, it becomes harder to control the spread of infection, leading to bacteria with increased mortality rates. In 2019, antimicrobial resistance was associated with nearly 5 million deaths worldwide. (CDC 2021) (Salam 2023). To unveil the hidden role of natural compounds, including those derived from plants in treatment, one needs to look no further than the inception of antibiotics itself: the discovery of penicillin was due to a natural compound produced by the mold *Penicillium notatum*. Thus it is important to study natural medicinal compounds because they produce compounds with bioactive properties, including anti-inflammatory, antiviral, antiaging, and antimicrobial.

To assess the efficacy of Traditional Korean Medicine for the treatment of disease, this study aims to research the antimicrobial properties of ginger root, licorice root, and herbal blends used as prescribed by traditional practitioners versus how they may be tested in a laboratory setting (one pharmaceutically active ingredient- one dependent impact). Licorice root and ginger root have been selected for research due to their well documented therapeutic properties and potential antimicrobial activities. As shown in Figure 1, licorice contains numerous bioactive compounds such as alkaloids, flavonoids, tannins, terpenoids, glycosides and phenolic compounds which have been found in-vitro to have antimicrobial properties (Rodino 2015, Alfauomy 2020). Licorice root contains glycyrrhizin, a triterpenoid saponin, that disrupts bacterial cell membranes by increasing their permeability leading to cell lysis or more simply death (Pastorino 2018). Glycyrrhizin also inhibits bacterial growth by targeting enzymes that reduce pathogenicity. Additionally, it induces the production of reactive oxygen species within the bacterial cells (Li 2021), causing oxidative stress and contributing to its effects against both gram positive (eg. *Staphylococcus*, *Streptococcus*) and gram negative bacteria (eg. *E.coli*, *helicobacter pylori*)(Karaman 2021, Mamedov 2019, Fukai 2002).

Ginger is another commonly used herb in traditional medicine, known for its potent antimicrobial effects against a wide range of pathogenic bacteria, fungi, and viruses. The plant contains several bioactive compounds, primarily gingerol, shogaol, and volatile oils such as zingiberene which work synergistically to disrupt bacterial cell membranes and interfere with essential metabolic processes. These compounds work by disrupting bacterial cell membrane integrity, leading to cell death (Lantz et al., 2007). Extraction methods significantly affect the antimicrobial potency of ginger preparations, with ethanol based extractions showing more effectiveness compared to water based methods (Singh et al., 2008). One study found that "the ethanolic extract of ginger showed a broad-spectrum antibacterial effect and was more effective than the aqueous extract" (Abd-Alrahman et al., 2013), which supports the use of ethanol based herbal extraction in this research.

The antibacterial activities of flavonoids such as glabridin and glabrene from the licorice extract have been reported previously in the context of *helicobacter pylori* (Hamad 2020, Fangliang 2020). Similarly, ginger root contains bioactive compounds such as gingerol, zingerone and shogaol which also disrupt bacterial cell membranes as well as inhibit enzyme activity, and interfere with bacterial DNA replication, leading to bacterial cell death (Mao 2019).

The blend chosen for research is the most commonly prescribed medicine called So-Cheong-Ryong-Tang. So-Cheong-Ryong-Tang is a traditional Korean medicinal remedy composed of eight medicinal plant species with a long history of use across Asian countries for managing allergic conditions like allergic rhinitis and asthma (Kim 2017). SCRT may vary in composition depending on the specific traditional medicine system and formulation used. Typically, it contains various herbs, including *Pinellia ternata*, *Ephedra sinica*, *Paeonia lactiflora*, *Schizandra chinensis*, *Asarum sieboldi*, and *Zingiber officinale*. However due to a lack of sources, the SCRT mix had to be limited

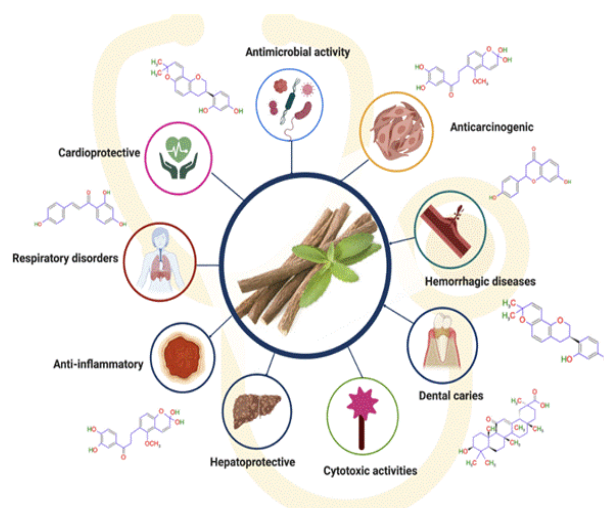


Figure 1. The different pharmacological effects licorice root exhibits (Wahab, 2021).

to just ginger root and licorice root. The herbs listed above are listed from greatest to least in amount. In terms of the efficacy of the plants, a Korean study examined the potential immunosuppressant effects of the blend on eight-week-old mice with a dose four times that of an adult human. The mice were injected with the mildly antigenic protein OVA to induce immune response. After six days of morning treatment, the treated mice demonstrated decreased levels of IL-17 (a cytokine linked to innate immune activation and inflammation) and GM-CSF (a white blood cell growth-stimulating factor) in broncho-tracheal fluid against the control. (Kim 2014 ). Thus, this traditional herbal treatment clinically reduces inflammatory responses caused by allergic rhinitis.

## 2. Methods

### 2.1 Solvent Preparation (Serial Dilution)

A serial dilution of 95 % ethanol alcohol was prepared using water as the diluent. The initial stock was prepared by combining 10 ml of 95% ethanol and 90 ml of distilled water, resulting in a 10% alcohol solution. For the first dilution, 10 mL of the 10% alcohol solution was mixed with 90 mL of distilled water using a 50 mL graduated cylinder, resulting in a 1% alcohol solution. A second dilution was performed by taking 10 mL of the 1% alcohol solution and combining it with 90 mL of distilled water to achieve a final ethanol concentration of 0.1%.

### 2.2 Solution Preparation (extracts and control)

To prepare the herbal extracts, 10 g of licorice root powder were placed in a 250 mL Erlenmeyer flask (single-herb licorice), and 10 g of ginger root powder were placed in another 250 mL Erlenmeyer flask (single-herb ginger). For the combination extract, 5 grams of licorice and 5 grams of ginger (total 10 grams) were combined in a third 250 mL Erlenmeyer flask (mass matched to single herb extracts). Each flask then received 25 mL of the 0.1% ethanol solvent (solid-to-solvent ratio of 1:2.5 w/v, with 0.40 g/mL nominal loading), was sealed with aluminum foil, gently hand-shaken for 1 minute to disperse powders, and left to steep 24 hours at room temperature (22 to 25 °C) without additional agitation. Extracts were filtered through KFP filter paper (110 mm), transferred to labeled sterile tubes, and stored at 4 °C until antimicrobial testing.

### 2.3 Cell plating & assay

Six plates were taken and sterilized with 70% ethanol to proceed with the *E. coli* bacterial culture experiment. Any flasks or vials containing unneeded materials were cleaned with distilled water. Subsequently, 20mL of prepared agar solution was poured into each plate and allowed to solidify for 24 hours. The six plates were then inoculated with *E. coli* by dipping sterile cotton swabs into the bacterial culture, removing excess liquid against the tube wall, and streaking the swabs evenly across the agar surface to produce a uniform lawn. Each plate was labeled on the bottom with a Sharpie marker, indicating sterile blank paper discs placed with treatment: (control) water, (control) 95% ethanol, (control) 0.1% ethanol, licorice root extract, ginger root extract, and ginger-licorice blend. A guide with four dots in a square formation was drawn on the back of each plate to facilitate disc placement.

Sterilized forceps were used to place each disc according to the pattern drawn back of the plate. The extracts were allowed to diffuse for 18-24 hours at room temperature. This process was repeated in three distinct trials, ensuring the reliability and consistency of the experimental results.

## 3. Results

In the study comparing the antimicrobial properties of ginger root and licorice root extracts, consistent volumes of 0.050 ml were tested for each treatment. The control (water) showed no antimicrobial activity, while licorice root extract had an average inhibition zone of 0.50 cm and ginger root extract averaged at 0.20 cm. The most potent antimicrobial effect was observed with the ginger-licorice combination solution, averaging an inhibition zone of 0.90 cm, indicating a synergistic interaction between the bioactive compounds in the two extracts. The ethanol control had an average inhibition zone of 0.90 cm.

Table 1. Zone of inhibition values for licorice root extract, ginger root extract, ginger-licorice combination solutions, water, 95% ethanol, and 0.1% ethanol solutions.

Treatment	Volume (ml) ±0.005mL	Nominal loading (g/mL)	Trial 1 (cm) ±0.05	Trial 2 (cm) ±0.05	Trial 3 (cm) ±0.05	Average (cm) ±0.05
Control (Water)	0.050	-	0.00	0.00	0.00	0.00
Licorice Root Extract	0.050	0.10	0.60	0.5	0.50	0.50
Ginger Root Extract	0.050	0.10	0.00	0.2	0.40	0.20
Ginger-Licorice Combination	0.050	0.20	1.0	0.9	0.70	0.90
Control (95% Ethanol)	0.050	-	1.0	1.0	0.60	0.90
Control (0.1% Ethanol)	0.050	-	0.00	0.00	0.00	0.00

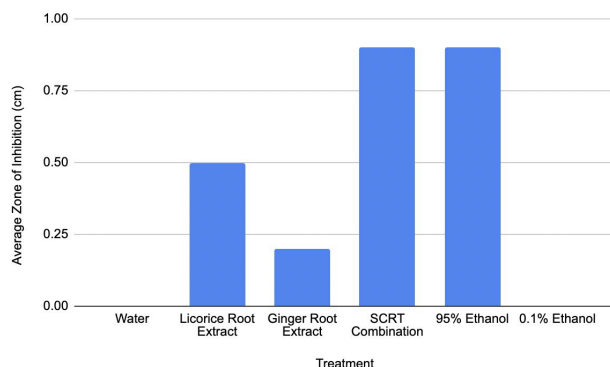


Figure 2. Average Zone of Inhibition Values from Individual Trials for Licorice Root Extract, Ginger Root Extract, Ethanol, Distilled Water and Ginger-Licorice Combination Solution.

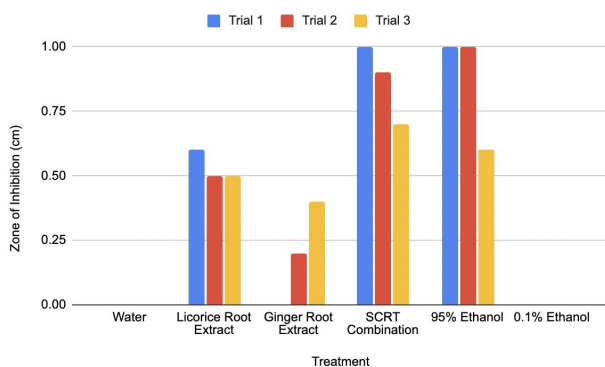


Figure 3. Zone of Inhibition Values from Individual Trials for Licorice Root Extract, Ginger Root Extract, 95% Ethanol, 0.1% Ethanol, Distilled Water, and Ginger-Licorice Combination Solution.

### 3.1 Statistical analysis

To reveal the presence of significant differences in the antimicrobial activities of the six treatment groups, a one-way ANOVA (Analysis of Variance) was performed.

The ANOVA test produced the following result of  $F(5,12) = 24.38$ , where  $p = 0.000007$ . The F-value, whose magnitude indicates the degree of substantial differences between groups, of 24.38 is a ratio that compares variance between treatment groups to variance within each group.

In this case, the variation between the treatment group means was approximately 24 times greater than the variation within groups. This large F-value indicates that the differences observed in antimicrobial activity across the treatment groups are not due to random variation, but are likely caused by actual differences in treatment effectiveness (Kim, 2017).

The numbers in parenthesis (5,12) indicate degrees of freedom, such that 5 is the degrees of freedom between each group and 12 is the degrees of freedom within the overall groups (residual values). The first number, 5, represents the between-groups degrees of freedom, which equals  $k-1$ , where  $k$  is the number of treatment groups (6 groups in total). This measures how much the group means vary from the overall mean. The second number, 12, represents the within-groups degrees of freedom, following  $N-k$ , where  $N$  is the total number of observations (18 in total, with 3 trials per group). This value represents the variation among the observed individual measurements within each group, after accounting for their group means (Kim, 2018).

Table 2. ANOVA test results for antimicrobial activity of six groups.

Variation range	Sum of squares	Degrees of freedom	F value	P value
Between groups	2.438	5	24.38	0.000007
Within groups	0.240	12	-	-
Total	2.678	17	-	-

The p-value (0.000007) indicates that the likelihood of observing these differences by chance is extremely low. Before conducting the ANOVA, the assumptions of normality and equal variances among the data were verified. Normality of data was assessed with the Shapiro-Wilk test (Mishra, 2019). The p-values greater than 0.05 obtained

from most treatment groups is indicative of a normal distribution of data without significant deviation. Equal variances of the data were also confirmed using Levene's test (with  $F = 90$  and  $p = 0.512$ ), indicating that the variability across groups was statistically similar and homogenous (Zhou, 2023).

Then, the Tukey HSD (Honestly Significant Difference) post-hoc test was conducted to identify the significant differences between treatment groups in pairs (Kim, 2018). The ginger-licorice combination exhibited significantly greater antimicrobial activity when compared individually to ginger root extract ( $p = 0.0026$ ), water ( $p = 0.0003$ ), and 0.1% ethanol ( $p = 0.0003$ ), confirming a synergistic interaction between ginger and licorice. Licorice extract alone also showed a significant difference compared to water ( $p = 0.0120$ ), indicating its independent antimicrobial effect.

However, the difference between ginger-licorice combination and licorice was not statistically significant ( $p = 0.1364$ ), suggesting that the increased effect of the ginger-licorice combination may reflect the dominant activity of licorice. No significant difference was found between ginger-licorice and 95% ethanol ( $p = 1.0000$ ), indicating similar antimicrobial activity between the combination extract and the pure ethanol control.

The 0.1% ethanol control, which matches the ethanol concentration used in the herbal extractions, revealed that this low concentration of ethanol had no antimicrobial effect (0 cm zone of inhibition from all trials). The Tukey HSD test reveals that ginger-licorice combination shows a significantly greater antimicrobial activity than 0.1% ethanol, with  $p = 0.0003$ , indicating that the observed growth inhibition was not attributable to the 0.1% ethanol solvent. Similarly, both licorice extract ( $p = 0.0010$ ) and ginger extract ( $p = 0.0375$ ) also showed significantly greater activity than 0.1% ethanol. In contrast, 95% ethanol, which has known antimicrobial properties, showed strong activity comparable to that of ginger-licorice.

Table 3. Tukey HSD post-hoc test for pairwise comparison of treatment groups.

Group 1	Group 2	Mean difference	Adjusted p-value	Lower limit (confidence interval)	Upper limit (confidence interval)
Control (0.1% ethanol)	Control (95% ethanol)	0.8667	0.0001	0.4788	1.2545
Control (0.1% ethanol)	Control (water)	0.0	1.0	-0.3879	0.3879
Control (0.1% ethanol)	Ginger root extract	0.2	0.538	-0.1879	0.5879
Control (0.1% ethanol)	Licorice root extract	0.5333	0.006	0.1455	0.9212
Control (0.1% ethanol)	Ginger-licorice combination	0.8667	0.0001	0.4788	1.2545
Control (95% ethanol)	Control (water)	-0.86667	0.0001	-1.2545	-0.4788
Control (95% ethanol)	Ginger root extract	-0.6667	0.001	-1.0545	-0.2788
Control (95% ethanol)	Licorice root extract	-0.3333	0.1088	-0.7212	0.0545
Control (95% ethanol)	Ginger-licorice combination	0.0	1.0	-0.3879	0.3879
Control (water)	Ginger root extract	0.2	0.538	-0.1879	0.5879
Control (water)	Licorice root extract	0.5333	0.006	0.1455	0.9212
Control (water)	Ginger-licorice combination	0.8667	0.0001	0.4788	1.2545
Ginger root extract	Licorice root extract	0.3333	0.1088	-0.0545	0.7212
Ginger root extract	Ginger-licorice combination	0.6667	0.001	0.2788	1.0545
Licorice root extract	Ginger-licorice combination	0.3333	0.1088	-0.0545	0.7212

#### 4. Discussion

The research focused primarily on *Escherichia coli*, a gram-negative bacterium with a distinct cell wall structure characterized by a thinner peptidoglycan layer and an outer lipid membrane (Silhavy, 2010). This narrow focus may limit the generalizability of our findings to other bacterial strains with different cell wall compositions. Gram-positive bacteria, for instance, possess a thicker peptidoglycan layer in their cell wall, which can influence their susceptibility to various antimicrobial agents. The differences in cell wall structure between gram-positive and gram-negative bacteria can be attributed to the variation in their susceptibility to certain antimicrobial agents. Gram-positive bacteria are generally more susceptible to agents that target the peptidoglycan layer due to its thicker and less complex structure compared to gram-negative bacteria (Silhavy, 2010).

The antimicrobial properties of ginger and licorice roots have been previously documented in scientific literature, supporting our findings of their potential as effective antimicrobial agents. Mao highlighted the antimicrobial activity of plant-derived compounds, emphasizing their potential as sustainable alternatives to conventional antibiotics. The bioactive compounds present in ginger and licorice roots, such as gingerol and glycyrrhizin, have been shown to



possess antimicrobial properties (Qian Qian Mao, 2019). These compounds can disrupt bacterial cell membranes, inhibit enzyme activity, and interfere with bacterial DNA replication, thereby exerting their antimicrobial effects.

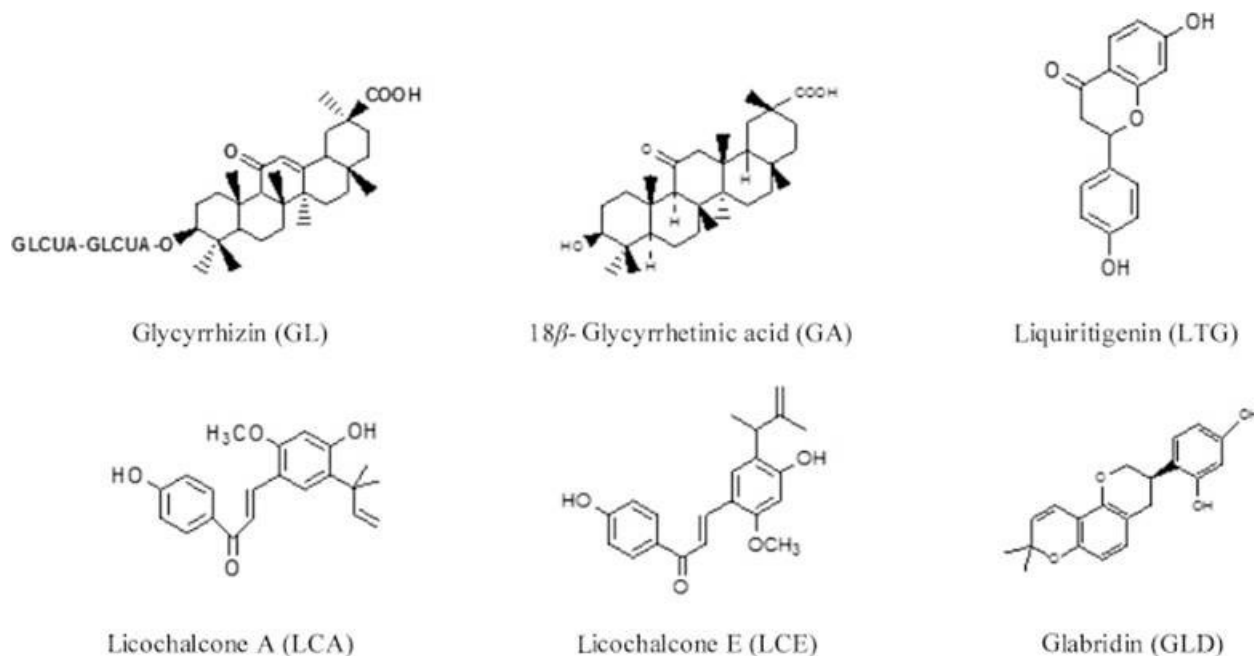


Figure 4. Various bioactive compounds in ginger and licorice, such as gingerol, shogaol, glycyrrhizin, and glabridin, that exhibit antimicrobial effects (Wang, 2015).

In understanding the antimicrobial efficacy of ginger and licorice root, it is crucial to delve into the bioactive compounds present in these plants. The antimicrobial effects of ginger and licorice root come from compounds like gingerol, shogaol, glycyrrhizin, and glabridin for instance (Wang, 2015). This observation suggests a potential synergistic effect between the bioactive compounds present in ginger and licorice root extracts. The combined action of these compounds may result in a more potent antimicrobial activity. However, since this study only tested *E. coli*, broader-spectrum effects remain a hypothesis for future research.

Furthermore, the findings from this study revealed a significant increase in the inhibition zone when the solutions derived from ginger root and licorice root were combined, compared to when they were tested separately. This observation corroborates the notion of a synergistic effect between the bioactive compounds in these plant extracts, leading to enhanced antimicrobial activity. Similar synergistic interactions between plant-derived compounds have been reported in previous studies, further supporting the potential of ginger and licorice root extracts as effective antimicrobial agents (Vaou, 2022). However, the combined solution was not as effective as the pure 95% ethanol.

The inhibition zones observed (0.2 to 0.9 cm) in this study are modest compared to values reported in the literature. For example, Abd-Alrahman et al. (2013) observed inhibition zones of 1.2 to 1.8 cm for ethanolic ginger extracts against *Staphylococcus aureus*, while licorice extracts have been reported in the 0.8- 1.5 cm range against *E. coli* and *Helicobacter pylori* (Fukai et al., 2002; Hamad et al., 2020). In contrast, standard antibiotics such as tetracycline typically yield inhibition zones greater than 2.0 cm under similar assays. Thus, while the results suggest some antimicrobial activity, the practical strength is limited, and the biological significance of zones less than 1 cm should be interpreted cautiously. One of limitations of the present study is the absence of a standard pharmaceutical antibiotic disc, such as ampicillin, which is commonly used as a benchmark in antimicrobial susceptibility testing. Including a standard antibiotic disc in future experiments would provide an external point of comparison, helping to contextualize the relatively small inhibition zones observed for herbal extracts and strengthen the external validity of the findings alongside solvent controls.

While ANOVA and Tukey HSD analyses of this study's data indicated statistically significant differences between treatment groups, it is important to interpret these findings contextually. The sample size was small, with  $n =$

3, which increases variability and limits power at the same time. Furthermore, although differences such as 0.2 cm and 0.9 cm inhibition zones reached statistical significance ( $p < 0.01$ ), the absolute differences in diameter remain biologically modest compared to zones produced by standard antibiotics. The small p-values observed (0.000007 in the ANOVA, and less than 0.01 in Tukey pairwise test) indicate that the differences between treatment groups are statistically significant and unlikely to have arisen by random variation. In practical terms, this means that the inhibition zones measured for the herbal extracts are reliably greater than those of the negative controls under the conditions tested. However, statistical significance does not necessarily imply strong biological or clinical relevance.

The actual inhibition zones observed were small (0.2 to 0.9 cm), and the experiment was conducted with a limited sample size ( $n = 3$  replicates per solution group). Therefore, while the low p-values provide confidence that the observed differences are real, the magnitude of those differences should be interpreted cautiously. The small sample sizes may also limit the experimental reproducibility, as inhibition zones can vary between trials due to subtle differences in extract preparation, disc diffusion conditions, or bacterial growth. A larger replication studies are needed to confirm the robustness and reliability of the observed antimicrobial effects. Biological significance should be judged not only by statistical detectability but also by the size of the effect relative to known benchmarks for antimicrobial activity (paper discs producing inhibition zones greater than 2.0 cm).

This distinction emphasizes that the results represent preliminary laboratory findings rather than evidence of clinically meaningful potency. Thus, the results should be seen as preliminary indications of activity rather than evidence of clinically meaningful potency. Replication with larger sample sizes and standardized antibiotic controls is needed to validate biological relevance.

## 5. Conclusion

The purpose of this experiment was to investigate the antimicrobial properties of ginger and licorice root extracts, both individually and in combination, as potential alternatives to conventional antibiotics in combating antibiotic resistance.

The findings of this study revealed measurable antimicrobial activity of ginger and licorice extracts, individually and in combination, against *Escherichia coli*. The observed synergistic effect of the combination warrants further exploration. However, since only a single gram-negative strain (*E. coli*) was tested, the results cannot be generalized to other bacterial species without additional evidence.

Although prior literature suggests ginger and licorice may act against a broad spectrum of bacteria, including gram-positive species, the present study tested only *E. coli*. Future studies should extend the scope to gram-positive bacteria and additional clinically relevant bacterial strains to evaluate whether the observed synergy holds more broadly.

Final reflections prompt us to acknowledge the lessons learned through this study. Contemplating future directions, we recognize the need for expanded research encompassing a broader range of bacteria strains, varied concentrations, and additional natural compounds. Such comprehensive studies will be crucial in advancing the field of natural antimicrobials and developing innovative strategies to combat antibiotic resistance. However, it is important to note the limitation posed by the in-vitro nature of this experiment. Extrapolating these findings to in vivo-settings is challenging due to the complex interactions of bacteria with other living organisms, which may influence the efficacy of the compounds being studied. Another thing to note is that ethanol was used as part of the combined ginger and licorice root solution. To ensure the results accurately reflect the contribution of the ginger and licorice extracts, it would be important to repeat the experiment using different solvents or varying ethanol concentrations. This would help confirm that the inhibition zone is truly caused by the active compounds in the ginger and licorice roots, not by ethanol.

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