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Antimicrobial Activity of *Zingiber officinale* Root Extract Against Animal-Derived Isolates of *Escherichia coli* and *Staphylococcus aureus*

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Abstract:
The purpose of this study was to determine the antimicrobial activity of *Zingiber officinale* (ginger) root extract using in vitro techniques, to measure its impact against *Escherichia coli* and *Staphylococcus aureus* isolates from fecal samples that were collected from sheep. Extracts were obtained using ethanol at the following concentrations were 60%, 70%, 80%, 90%, and 100%, and were subjected to testing using the agar well diffusion method.
The results indicated that there was a significant concentration level effect ($p < 0.05$) was observed against both tested microorganisms. *S. aureus* was more inhibited than *E. coli*, producing a range of 23 - 33 mm inhibition at the 60 and 100 percent concentrations, respectively. *E. coli* was inhibited less than *S. aureus*, inhibiting *E. coli*, with inhibition zones of 18 mm at 60% concentration and 29 mm at 100 percent concentrations, respectively.
The inhibition of *S. aureus* at concentrations greater than or equal to 70 percent (≥25 mm) and this may indicate a strong concentration and level of antimicrobial activity that approaches standard antibiotics as a result of the zone of inhibition values. a significant antimicrobial effect, there is a large level antimicrobial effect of ginger extract, and the antimicrobial effect was even greater against Gram-positive bacteria ($p < 0.01$).
These findings of this study suggest the potential use of the ginger extract as an antimicrobial for preserving foods and for topical therapeutics and the need for further specific testing for the bioactive compounds and resistant strains.

Keywords: *Zingiber officinale*; Ginger; Antimicrobial Activity; Agar Well Diffusion; Phytochemicals; Antimicrobial Susceptibility; Animals; Livestock; Gram-negative; Gram-positive; Food Preservation.

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Introduction:

The global study for new antimicrobial agents is urgent, as pathogens resistant to multiple drugs pose increasing risks to public health (Coates *et al*., 2011). The inspiring examples from defense against infection can be found in the traditional methodologies of biomedicine and the use of various plant species for treatments. Such approaches utilize the bioactive compounds from the biota of the diverse plant species (Cowan,1999). A classic example is *Zingiber officinale* Roscoe, more commonly known as ginger, a rhizome that is grown for its use in food and also has a number of medicinal functions, including being anti-inflammatory, and having antioxidant and antimicrobial properties (Mathew *et al*., 2021) Amongst numerous activities of ginger, its antimicrobial function is caused by a mixture of phenolic compounds, gingerols and shogaols, whereby the oleoresin is said to disrupt the microbial cellular membrane (Lee *et al*., 2010) When it comes to the structural features of the cell wall of bacteria, the differences are critical in the classification of the bacteria as either gram positive or gram negative. Examples of gram-negative bacteria are *E. coli*, while *S. aureus* is a gram-positive example. (Nikaido,2003, Adel& Abood, 2025) explains that *E. coli* has an additional outer membrane that contains lipopolysaccharides, and that of gram-positive *S. aureus* has but a single layer of peptidoglycan, and that makes gram negative bacteria more hydrophobic. This structural difference is important in the plant derived antimicrobials that can be used.

This study addresses the antimicrobial potential of ginger root extract by examining its efficacy in vitro against two model organisms, as well as supporting its ethnopharmacological justification and assessing its merit as a candidate for developing new antimicrobials.

Materials and Methods

Plant Material and Extraction:

Fresh rhizomes of *Zingiber officinale* were purchased from local markets, and after authentication of the plant material, they were cleaned, and subsequently thinly sliced. 100 grams of the sliced rhizomes were each separately macerated in 300 mL of absolute ethanol (100%) at room temperature (25 ± 2 °C) for 72 hours. During maceration, the mixture was agitated intermittently. After 72 hours, the mixture was filtered with Whatman No. 1 filter paper, and the ethanol was evaporated under reduced pressure using a rotary evaporator at 40 °C. The ethanol extract was obtained and the crude residue (yield: 12.5% w/w) was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution at a concentration of 200 mg/mL. 100% (v/v) DMSO was diluted with sterile distilled water to obtain working extracts at 90%, 80%, 70%, and 60% (v/v). On each plate, a negative control with DMSO (2% v/v) and a positive control with a standard 10 µg Ciprofloxacin antibiotic disc were included (Al-Khfaji *et al*.,2022).

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Bacterial Strains, Isolation, and Inoculum Preparation.

Animal-Derived Bacterial Isolation:

To determine the level of efficacy of the extract on bacteria from a natural reservoir, fresh isolates from the livestock were obtained. Fecal samples were collected from sheep in many farms. The samples were collected under sterile conditions and transported to a laboratory in sterile transport media and processed within 2 hours of the collection (Khodadadi, 2020, Singh *et al*., 2020).

Primary Processing and Enrichment:

One gram of the fecal sample was enriched in 9 mL of sterile Buffered Peptone Water (BPW) and was incubated at 37 ° C for 18 – 24 hours for non-selective enrichment.

Selective Isolation and Purification:

For *Escherichia coli*: Streaking of a loopful of enriched broth was done onto MacConkey Agar plates, and the plates were incubated at 37 °C for 24 hours. Pink colonies (which are suggestive of lactose fermentation) were then sub-cultured on Eosin Methylene Blue (EMB) agar for further purification. Subsequent colonies exhibiting a metallic green sheen were characterized as presumptive *E. coli*.

For *Staphylococcus aureus*: Enriched broth is then incubated onto Mannitol Salt Agar (MSA) plates and is then incubated for 24-48 hrs at 37°C. Yellow colonies with a surrounding yellow zone (indicating mannitol fermentation) were chosen as presumptive *S. aureus* and further subculture onto nutrient agar for purity.

Biochemical and Morphological Confirmation:

The presumptive isolates were obtained and then passed through Gram staining and other standard biochemical tests. Confirmed *E. coli* isolates showed methyl red (MR) and indole tests as positive and Voges-Proskauer (VP) and citrate tests as negative. Confirmed *S. aureus* isolates showed positive catalase and coagulase tests. For the antimicrobial assays, one confirmed isolate of each species, along with the reference strains, was selected (Fajer *et al*., 2023).

Inoculum Preparation for Assay:

To determine the level of efficacy of the extract on bacteria from a natural reservoir, fresh isolates from the livestock were obtained. These strains were sub-cultured using nutrient agar and were then incubated at 37ºC for a period of 24 hours. Using sterile 0.85% saline solution, a bacterial suspension was created, and then adjusted using a spectrophotometer to a 0.5 McFarland standard turbidity (this is approximately 1~2 x 10^8 CFU/mL).

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Antimicrobial Susceptibility Testing:

The agar well diffusion method (as outlined by standard protocols (Bauer *et al*., 1966) was used. In short, Mueller-Hinton (MHA) agar plates were evenly surface inoculated with the standardized bacterial suspensions using a sterile cotton swab. Using a sterile cork borer, 6 wells (which are 6 mm in diameter) were made in the agar, and then 100 µL of each concentration of ginger extract was placed in each of the wells. The pre-diffusion of the plates was held for 30 minutes at atmospheric temperature and then they were incubated at 37oC for a period of 24 hours. The inhibition zones (which also includes the well diameter) were used to assess and measure the levels of antimicrobial activity. This was recorded in millimeters (mm) using a digital caliper. The tests were performed in triplicate (n=3).

Statistical Analysis:

The results are shown as mean ± standard deviation. To assess the differences in the inhibition zones between the two bacterial species for each concentration, the unpaired two-tailed Student's t-test was used. Data are considered significant for p-values less than 0.05. All data were analyzed using GraphPad Prism version 9.0.

Results:

The antimicrobial properties of *Zingiber officinale* root extract towards *S. aureus* and *E. coli* are illustrated in Table 1 and Figure 1. The inhibition zones of both bacterial strains show a direct proportional relationship with the concentration of the extract.

Table 1. Comparative Antimicrobial Activity of *Zingiber officinale* Extract against the Test Bacteria.

Concentration (%)	*S. aureus* (mm) ± SD	*E. coli* (mm) ± SD	p-value
100	33.0 ± 0.5	29.0 ± 0.7	<0.01
90	30.0 ± 0.6	27.0 ± 0.5	<0.01
80	28.0 ± 0.5	24.0 ± 0.6	<0.01
70	25.0 ± 0.4	21.0 ± 0.5	<0.01
60	23.0 ± 0.6	18.0 ± 0.4	<0.01

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Table 2: Results from Assessment and Control Qualitatively.

Sample	Qualitative Assessment (Al-Khfaji,2022)	Sample	Qualitative Assessment (Fajer *et al*,2010)
S. aureus at 100%	Very Strong	*E. coli* at 100%	Strong
S. aureus at 90%	Very Strong	*E. coli* at 90%	Strong
S. aureus at 80%	Strong	*E. coli* at 80%	Moderate
S. aureus at 70%	Strong	*E. coli* at 70%	Moderate
S. aureus at 60%	Moderate/Strong	*E. coli* at 60%	Moderate
S. aureus + Ciprofloxacin (10µg)	Reference Control	*E. coli* + Ciprofloxacin (10µg)	Reference Control

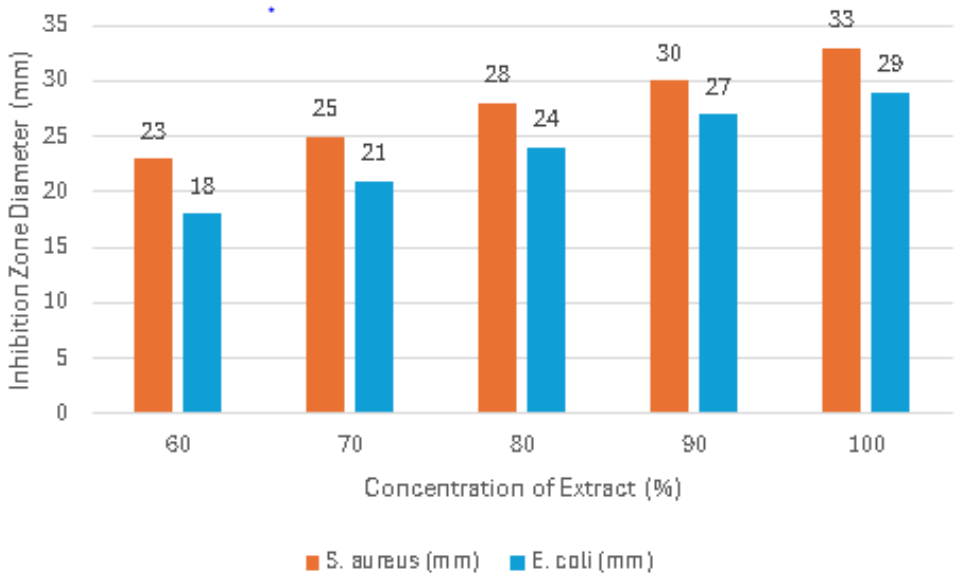

Figure 1: Response curve of Zingiber officinale ethanolic extract against *S.aureus* and *E.coli*.

Figure 1 take cares of the dose-response relationship which analyzes the mean inhibition zones in correlation to extract concentration for each of the bacterium There was a concentration-dependent increase in inhibition zones for both bacterial species, with larger zones observed at higher extract concentrations. present in the graph. As for the curves depicting *S. aureus*, it is consistently present

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above and much further than *E. coli* at all concentration levels ($p < 0.01$ at each). *S. aureus* therefore is the more susceptible bacteria.

Overall, at every single concentration tested, *S. aureus* had a much more impressive degree of sensitivity with an average of 5-6 millimeters with statistically significant results ($p < 0.01$). The positive (Ciprofloxacin) control serving to validate the testing system with high antimicrobial activity while the negative control (DMSO) showed an absence of an inhibition zone.

Discussion

The results of this study show that *Zingiber officinale* root extract demonstrates significant levels of antimicrobial activity that are dose-dependently related, and that the activity levels are potentially clinically relevant for plant extracts (zones ≥ 20 mm) (Bauer *et al*.,1966). The presence of a dose-response relationship is typical for bioactive plant extracts and indicates that the concentration of active phytochemicals is directly proportional to its antimicrobial effect (Silva,2020).
Literature regarding the essential oils of plants and phenolic extracts provides the basis for the extract's effectiveness when compared to S. aureus and E. coli (Cox *et al*,2001, Prabuseenivasan *et al*., 2006).
The difference in effectiveness for the extracts is likely tied to the differences in the cellular ultrastructure of Gram-positive and Gram-negative bacteria (Nikaido,2003). Shogaols and gingerols [e.g., (Bauer, 1966)-gingerol], the primary lipophilic active constituents of ginger, can integrate into and disrupt the phospholipid bilayer of the *S. aureus* cytoplasmic membrane Lee *et al*.,2010, Ali *et al*.,2017). Such an occurrence can lead to increase cellular membrane permeability and the leakage of K^+ ions, ATP, and nucleic acids, resulting in cell death (Sihavy,2010). On the other hand, *E. coli* has diffused hydrophobicity and asymmetric outer membrane containing lipopolisaccharides (LPS) and an efflux pump which poses a barrier to these molecules. Therefore, to achieve an antimicrobial effect, higher concentrations are required (Blair *et al*., 2015). The presence of bioactivity of the extracts towards *E. coli* indicates that the extracts may contain substances that can overcome this barrier through self-promoted uptake or by synergistic action to disrupt the membrane (Helander, 1998).
The ethanolic extract employed in this study contains, among others, (Bauer *et al*., 1966) -gingerol, (Cox *et al*., 2001) -gingerol, (Oliveira *et al*., 2020)-gingerol, and zingiberene (Wang *et al*., 2019). The antimicrobial activity is probably attributable to a combination of these compounds rather than a single one (Hemaiswarya *et al*., 2008). The extract's antimicrobial activity, as noted by (Hemaiswarya *et al*.,) "This is one of the major benefits of employing whole extracts because it may minimize the risk of developing resistance in contrast to single-target antibiotics."

Conclusions

The results of the present study provide definitive proof of the ability of ethanolic Zingiber officinale root extract to perform antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. The antimicrobial activity manifested the concentration-dependent manner of the ethanolic extract with higher statistically significant potency against the Gram-positive bacterium. Particularly, the extract's

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activity against *S. aureus*, with levels of strong inhibition, is consistent with its traditional medicinal use. Thus, with the result, ginger can be considered as one of the natural antimicrobial sources, which can be used as a natural antimicrobial food preservative to prevent food spoilage, antimicrobial components of dressings for wounds, and antiseptic skin formulations. The strong differential activity also demonstrates the dominant role of the bacterial cell wall structure and composition in determining susceptibility of the bacteria to the antimicrobial activity of *Z. officinale*. Extensive studies are recommended for the isolation of active principles in the extract, the determination of their specific molecular mechanisms of action, and the assessment of the in vivo activity and safety, to complete the necessary studies prior to the commercialization of a consistent phytomedicine and/or preservative product.

Recommendations:

Future studies should focus on isolating the specific bioactive compounds responsible for the antimicrobial effect. It is also recommended to test the ginger extract against multidrug-resistant clinical isolates and conduct in vivo trials to evaluate its safety and therapeutic potential, particularly for topical applications and food preservation.

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Authors Contributions: M.H.M.: conceptualization, methodology, writing-original draft. S.B.J., A.I.: resources, data curation, writing-review & editing.

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