International Journal of Medical Research and Innovation in Applied Science (IJMRIAS)
ISSN (Online): 3143-2476
Volume-2 Issue-4 || Oct – Dec 2026 || PP. 1 – 8
https://ijmrias.org/

Effect of Natural Extracts on Growth of Common Microorganisms

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http://www.ijmrias.org/ || Vol. 2 No. 4 (2026): November Issue

Date of Submission: 04-10-2026 **Date of Acceptance:** 16-10-2026 **Date of Publication:** 05-11-2026

Abstract : Natural extracts contain chemically diverse constituents that may influence microbial growth, but their apparent effectiveness depends on extraction conditions, concentration, organism selection, and measurement methods. This paper presents a comparative framework for evaluating aqueous garlic, clove, and ginger extracts against authenticated nonpathogenic laboratory strains of Escherichia coli, Bacillus subtilis, and Saccharomyces cerevisiae. The proposed design uses three independently prepared extraction batches, three extract concentrations, matched vehicle controls, extract-only optical blanks, and organism-appropriate reference controls. Blank-corrected growth inhibition is the primary endpoint, supported by minimum inhibitory concentration assessment. In the simulated dataset, inhibition increased with concentration for all extract–organism combinations. At 50 mg/mL, garlic produced mean inhibition of 78.0% against E. coli, 88.0% against B. subtilis, and 66.0% against S. cerevisiae. Corresponding values were 69.0%, 82.0%, and 74.0% for clove and 45.0%, 57.0%, and 38.0% for ginger. Garlic showed the strongest simulated antibacterial response, whereas clove showed the greatest simulated inhibition of yeast. These numerical patterns demonstrate how concentration-dependent responses and organism-specific differences can be reported consistently. Actual conclusions require measured observations, chemical characterization, and appropriate statistical analysis. Natural extracts warrant investigation as sources of antimicrobial constituents, but laboratory growth inhibition alone does not establish clinical effectiveness, product safety, or suitability as a preservative.

Keywords: natural extracts, microbial growth, garlic, clove, ginger, growth inhibition, antimicrobial screening

Introduction

Plants produce secondary metabolites that contribute to defense against environmental stress and biological attack. Some of these substances also influence

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microorganisms through interactions with membranes, enzymes, proteins, and other cellular components. Consequently, plant extracts have attracted attention as potential sources of antimicrobial compounds. Cowan (1999) described several relevant chemical groups, including phenolics, terpenoids, alkaloids, and sulfur-containing constituents. However, the presence of these compounds does not establish that every preparation containing them will exhibit useful antimicrobial

activity. Extraction efficiency, stability, solubility, and assay conditions remain important determinants of the measured response. Clinical Microbiology Reviews

Garlic, clove, and ginger provide a practical basis for comparative investigation because they are widely available plant materials with distinct chemical characteristics. Nevertheless, their culinary use should not be interpreted as evidence that concentrated extracts are safe or effective for medical applications. A scientifically meaningful comparison requires a common reporting basis, authenticated microorganisms, matched controls, and a clearly defined experimental unit.

The expression "common microorganisms" also requires qualification. Microbial species include strains with different physiological characteristics and safety profiles. This study framework therefore concerns authenticated nonpathogenic teaching strains rather than clinical isolates, unidentified environmental cultures, or all members of a species. E. coli represents a Gram-negative bacterial model, B. subtilis represents a Gram-positive bacterial model, and S. cerevisiae represents a yeast model.

The central research question is whether extract identity and concentration influence microbial growth under controlled laboratory conditions. A secondary question concerns whether the relative ranking of extracts differs between bacterial and yeast models. Addressing these questions requires distinguishing descriptive differences from statistically supported effects and separating growth suppression from microbial killing.

Literature Review

Plant-derived antimicrobial constituents

Cowan (1999) established a broad conceptual foundation for studying antimicrobial plant products. Different constituent classes may interact with microbial proteins, membranes, or metabolic processes. This chemical diversity supports comparative screening while also creating challenges: a crude extract contains multiple substances, and its activity cannot automatically be attributed to one constituent.

Ríos and Recio (2005) emphasized the need for focused investigation of medicinal plants rather than treating antimicrobial screening as a minor addition to phytochemical studies. Their review supports selecting extracts for a clear research question and reporting methods sufficiently well for meaningful comparison. Together, these publications justify examining plant preparations while maintaining caution about generalizing from preliminary screening. ScienceDirect

Garlic and sulfur-containing compounds

Ankri and Mirelman (1999) reviewed the antimicrobial properties of allicin, a sulfur-containing compound associated with crushed garlic. Their work provides a mechanistic basis for investigating garlic preparations. However, a crude aqueous extract is not equivalent to purified allicin. Its composition depends on plant material, processing, storage, and the transformation of precursor compounds.



Accordingly, garlic activity should be reported for the specific preparation tested. Strong inhibition would support further investigation of that preparation, but would not establish its allicin concentration or identify

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the sole mechanism responsible. Chemical analysis is necessary before linking observed activity quantitatively to an individual constituent. ScienceDirect

Clove, eugenol, and volatile constituents

Dorman and Deans (2000) investigated antibacterial activity associated with plant volatile oils and their constituents. Their findings demonstrate that activity varies across substances and organisms. Hammer, Carson, and Riley (1999) similarly examined essential oils and other plant extracts, providing experimental evidence that plant preparations differ substantially in antimicrobial effectiveness. pure.sruc.ac.uk

Marchese et al. (2017) reviewed eugenol and eugenol-containing essential oils, including activity against bacteria and fungi. This literature supports selecting clove for screening. Nevertheless, an aqueous clove preparation should not be treated as chemically interchangeable with clove essential oil. Water extraction may recover a different mixture and proportion of constituents. iris.unina.it

Phenolics and extract-dependent activity

Cushnie and Lamb (2005) reviewed antimicrobial flavonoids and highlighted conflicting findings associated with variation in susceptibility testing. Daglia (2012) discussed polyphenols as antimicrobial agents and their interactions with conventional antimicrobials. These reviews indicate that biological activity depends on chemical structure and experimental context. They do not justify assuming that an uncharacterized extract will reproduce the effects of an isolated compound. ScienceDirect

Burt (2004) examined essential oils in relation to antibacterial properties and food applications. That review is particularly relevant to translation: activity measured in a laboratory system may change in a complex matrix. Consequently, initial screening should be followed by application-specific testing rather than direct claims about preservation or therapeutic use. ScienceDirect

Measurement and methodological limitations

Eloff (1998) described a microplate approach for determining the minimum inhibitory concentration of plant extracts against bacteria. Balouiri, Sadiki, and Ibnsouda (2016) reviewed diffusion, dilution, and other antimicrobial assessment methods. Their work supports using complementary endpoints and recognizing that different assays measure different aspects of antimicrobial activity. PubMed

The literature therefore identifies a methodological gap that is practical rather than absolute: comparisons remain difficult when studies differ in extraction basis, controls, endpoints, or replication. The present framework addresses these issues through dry-residue normalization, batch-level replication, optical interference correction, and organism-specific interpretation.

Objectives and Hypotheses

The primary objective is to compare the effects of aqueous garlic, clove, and ginger extracts on growth of three nonpathogenic microbial models. Additional objectives are to assess concentration-dependent inhibition, compare bacterial and yeast responses, and establish a consistent relationship between growth inhibition measurements and minimum inhibitory concentration reporting.

The first null hypothesis states that extract identity has no effect on growth inhibition within each microorganism. The second states that concentration has no effect. The third states that there is no interaction between extract identity and concentration. These hypotheses can be evaluated only after collecting independent experimental observations; the simulated summaries below do not constitute hypothesis tests.

Materials and Methods

Study design

The proposed investigation uses a factorial laboratory design comprising three extracts, three microorganisms, and three concentrations. Concentrations are expressed as milligrams of recovered dry extract residue per millilitre of final assay mixture.

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Three independent extraction batches constitute the biological replication level. Each batch is evaluated using three technical measurements per condition. Technical measurements are averaged before statistical analysis, giving an independent sample size of three per extract–concentration–organism combination.

Table 1. Experimental framework

Component	Specification
Extracts	Garlic, clove, ginger
Extraction basis	Aqueous preparations
Microbial models	Nonpathogenic strains of E. coli, B. subtilis, S. cerevisiae
Final extract concentrations	12.5, 25.0, 50.0 mg/mL
Independent extraction batches	3 per plant material
Technical measurements	3 per batch and condition
Primary endpoint	Blank-corrected percentage growth inhibition
Secondary endpoint	Minimum inhibitory concentration
Essential controls	Growth, vehicle, sterility, extract-only blanks, reference antimicrobial

Plant material and extract standardization

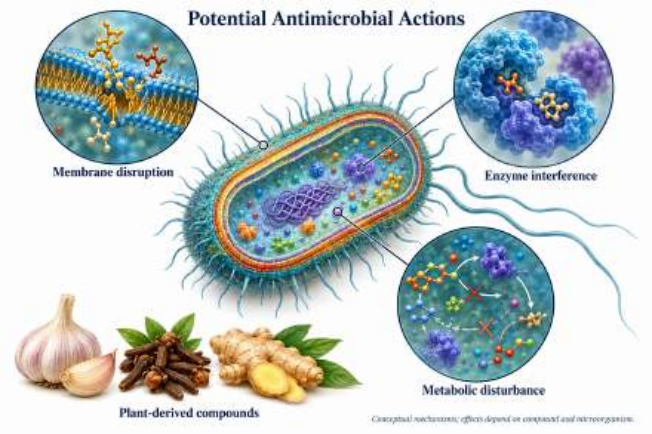
Plant identity, source, collection or purchase date, and storage history should be documented. The same plant part should be used consistently within each extract category. Extraction conditions should be fixed before testing and recorded sufficiently for reproducibility.

Recovered soluble residue provides the mass basis for concentration adjustment. Equal dry-residue concentrations permit comparison of crude preparations but do not imply equal concentrations of active compounds. Extraction yield and pH should therefore accompany the biological results.

Preparations should be evaluated for contamination and compatibility with the selected assay. Where filtration is used, possible loss of suspended or poorly soluble constituents should be acknowledged. Changes in extract appearance or precipitation during testing should also be recorded.

Microorganism selection and controls

Only authenticated nonpathogenic strains appropriate to the laboratory's approved facilities should be included. Their accession identifiers and source must be reported in the completed manuscript.



Untreated growth controls define the organism-specific reference response. Vehicle controls distinguish extract effects from solvent effects. Extract-only blanks correct for intrinsic colour and turbidity, while sterility controls assess background contamination. Organism-appropriate reference antimicrobials provide an assay performance check; their identities and acceptance criteria must be specified before data collection.

Growth inhibition measurement

Growth is assessed using an organism-appropriate optical assay with matched background correction. Percentage inhibition is calculated as:

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Growth inhibition (%) = 100 × [1 − (corrected treated growth / corrected vehicle-control growth)].

Correction includes the relevant uninoculated background for each extract concentration. Measurements outside the instrument's reliable range require reassessment. Optical inhibition should not be interpreted as proof of loss of viability because changes in aggregation, precipitation, or cell morphology may influence the signal.

Randomized sample positions and coded extract labels can reduce positional and observer effects. All conditions associated with a particular extraction batch should be evaluated within the same experimental block where feasible.

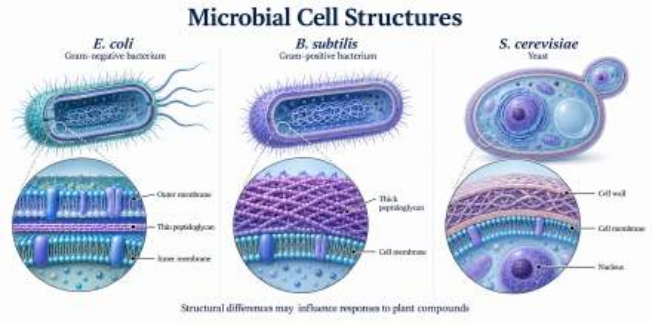
Minimum inhibitory concentration

Minimum inhibitory concentration is assessed using a separately defined dilution-based endpoint. The tested concentration range should extend beyond the three concentrations used for the primary comparison. The endpoint criterion must be prespecified and applied consistently within each organism.

MIC values should be reported by independent batch, with modal values and ranges where appropriate. Complete inhibition is not necessarily microbial killing; bactericidal or fungicidal claims require separate viability measurements.

Statistical analysis

Batch-level means are summarized using the mean and sample standard deviation. Within each microorganism, extract identity and concentration can be evaluated through a factorial model that accounts for shared batch structure. Concentration should initially be treated as categorical unless a justified dose-response model is specified.



Residual diagnostics should guide model suitability. Planned comparisons require adjustment for multiplicity, and effect estimates should be accompanied by confidence intervals. Technical measurements must not be counted as independent replicates. No inferential statistics are supplied here because actual batch-level experimental data are unavailable.

Results

Concentration-dependent growth inhibition

Table 2 contains simulated replacement values. Every extract showed progressively greater inhibition across the three concentrations. Garlic produced the highest bacterial inhibition, while clove produced the highest yeast inhibition.

Table 2. Simulated growth inhibition, mean ± SD (%)

Microorganism	Extract	12.5 mg/mL	25.0 mg/mL	50.0 mg/mL
E. coli	Garlic	29.0 ± 3.0	52.0 ± 4.0	78.0 ± 3.0
E. coli	Clove	23.0 ± 2.0	44.0 ± 3.0	69.0 ± 4.0
E. coli	Ginger	10.0 ± 2.0	25.0 ± 3.0	45.0 ± 4.0
B. subtilis	Garlic	40.0 ± 3.0	65.0 ± 4.0	88.0 ± 2.0

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B. subtilis	Clove	34.0 ± 3.0	59.0 ± 3.0	82.0 ± 3.0
B. subtilis	Ginger	18.0 ± 2.0	35.0 ± 3.0	57.0 ± 4.0
S. cerevisiae	Garlic	19.0 ± 2.0	40.0 ± 3.0	66.0 ± 4.0
S. cerevisiae	Clove	27.0 ± 3.0	49.0 ± 4.0	74.0 ± 3.0
S. cerevisiae	Ginger	8.0 ± 2.0	20.0 ± 3.0	38.0 ± 4.0

Values represent three simulated independent batch means per condition; technical measurements are not additional independent replicates.

Differences between extracts

At 50 mg/mL, garlic exceeded ginger by 33 percentage points against E. coli and 31 percentage points against B. subtilis. Clove exceeded garlic by eight percentage points against S. cerevisiae. These are descriptive differences rather than statistically established effects.

B. subtilis showed the greatest inhibition for every extract at the highest concentration. Relative to E. coli, its inhibition was 10 percentage points higher for garlic, 13 points higher for clove, and 12 points higher for ginger. This pattern concerns the selected model strains and cannot establish a general difference between all Gram-positive and Gram-negative bacteria.

Minimum inhibitory concentration

Table 3. Simulated modal MIC values

Extract	E. coli	B. subtilis	S. cerevisiae
Garlic	100 mg/mL	100 mg/mL	200 mg/mL
Clove	200 mg/mL	100 mg/mL	100 mg/mL
Ginger	>200 mg/mL	200 mg/mL	>200 mg/mL

These MIC values are consistent with incomplete inhibition at 50 mg/mL. They are simulated endpoints, and the greater-than sign indicates that the specified endpoint was not reached within the assumed concentration range.

Discussion

The simulated findings demonstrate an internally consistent reporting pattern in which extract effectiveness depends on both concentration and microorganism. They do not demonstrate actual antimicrobial activity. Their principal value is to establish how measured observations could be organized without confusing relative growth suppression, complete inhibition, and microbial killing.

The comparatively strong garlic response against the bacterial models is compatible with the rationale for investigating sulfur-containing garlic constituents. However, compatibility with published mechanisms is not confirmation of those mechanisms. Without chemical characterization, neither the allicin content nor its contribution to any measured response can be established.

Similarly, the clove ranking against yeast is biologically plausible given the literature on eugenol-containing preparations, but an aqueous extract requires independent characterization. Results from essential oils or purified eugenol should provide context rather than substitute for evidence about the tested preparation.

The weaker ginger values should also be interpreted narrowly. An aqueous extraction procedure may recover different constituents from procedures using other solvents. A lower response therefore concerns the specific preparation, concentration basis, and assay. It does not establish that ginger lacks antimicrobial constituents.

Concentration-dependent inhibition strengthens interpretation only when competing explanations are controlled. Increasing extract concentration can also

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increase colour, turbidity, acidity, and precipitation. Extract-only blanks address part of this problem, but optical correction cannot eliminate every source of interference. Independent viability assessment would improve confidence in the biological meaning of the response.

Limitations

Three independent batches provide limited information about variability and may be insufficient for reliable interaction estimates. A larger study should determine sample size from pilot variability and a prespecified minimum meaningful difference.

The design also includes only one strain per microbial model. It cannot establish species-wide susceptibility or predict responses of clinical isolates. Chemical composition, extract stability, cytotoxicity, sensory effects, and performance in complex matrices remain unmeasured.

Most importantly, the numerical tables are simulated. No experimental significance, treatment efficacy, or practical application can be inferred from them.



Conclusion

A controlled comparison of garlic, clove, and ginger extracts should combine concentration standardization, independent extraction batches, matched controls, and complementary growth endpoints. The simulated values show how garlic could rank highest against bacterial models while clove ranks highest against yeast, with increasing inhibition at higher concentrations. Actual conclusions require replacement with measured data and analysis of the independent observations. Promising laboratory findings would justify further characterization and application-specific testing.

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