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COMPARATIVE EVALUATION OF ELEVEN GLUCOSINOLATE DERIVATIVES FOR ANTIFUNGAL ACTIVITY AGAINST FUNGAL GROWTH AND BIOFILM DEVELOPMENT

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ABSTRACT

Glucosinolate derivatives have attracted scientific interest as potential antifungal agents because of their structural diversity and biological activity. These compounds, particularly isothiocyanates and selected non-isothiocyanate products, may inhibit fungal growth and interfere with processes associated with fungal persistence. This paper critically reviews the comparative evaluation of eleven glucosinolate derivatives, emphasizing their effects on fungal growth, biofilm development, and the factors responsible for differences in antifungal performance. It considers the chemical transformation of glucosinolates, structure–activity relationships, fungal susceptibility, and the importance of standardized experimental procedures. Methods for assessing fungal growth, minimum inhibitory concentrations, biofilm biomass, and metabolic activity are discussed. The potential relevance of these compounds to agricultural protection, food preservation, and antifungal research is also examined. Particular attention is given to compound stability, concentration-dependent responses, and the need to distinguish established findings from proposed mechanisms. The review identifies research priorities for developing reproducible and environmentally responsible glucosinolate-derived antifungal strategies.

Keywords: Glucosinolates, isothiocyanates, antifungal activity, fungal growth, biofilm development, structure–activity relationships, natural products.

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I. INTRODUCTION

Fungal organisms are important components of natural ecosystems, but certain species cause serious problems in agriculture, food storage, and human health. Plant-pathogenic fungi can reduce crop productivity, damage harvested products, and contribute to economic losses. Food-associated fungi may cause spoilage, while mycotoxin-producing species can contaminate agricultural commodities. Human-associated fungi, including species of *Candida* and *Aspergillus*, can cause infections, particularly in vulnerable individuals. Although conventional antifungal agents remain essential, resistance, limited treatment options for some infections, and the need for effective crop-protection strategies have encouraged the investigation of additional antifungal compounds. Natural products from plants represent one potential source of such compounds.

Glucosinolates are sulfur- and nitrogen-containing secondary metabolites found predominantly in plants belonging to the order Brassicales, including many members of the Brassicaceae family. When plant tissue is damaged or exposed to certain biological stresses, glucosinolates can be hydrolysed by myrosinase enzymes. Depending on the original glucosinolate structure and reaction conditions, the resulting products may include isothiocyanates, nitriles, thiocyanates, epithionitriles, and other compounds. These products differ in chemical reactivity, volatility, polarity, and biological activity. Consequently, antifungal effects cannot be attributed uniformly to all glucosinolates or their derivatives. Earlier comparative work demonstrated that native glucosinolates and their enzyme-derived hydrolysis products may show substantially different effects against fungal pathogens (Mari et al., 1997).

Comparative evaluation is necessary because the chemical structure of a derivative can influence its ability to reach fungal cells and interact with cellular components. Differences in side-chain length, functional groups, hydrophobicity, volatility, and reactivity may contribute to variations in antifungal potency. Some compounds may inhibit visible fungal growth, whereas others may affect spore germination, metabolic activity, or the development of structured microbial communities. Reviews of glucosinolate-derived isothiocyanates have documented antifungal effects against plant pathogens, mycotoxigenic fungi, and selected human-associated fungi, while also highlighting variation among compounds and fungal species (Plaszkó et al., 2021). These findings support comparative screening but do not justify assuming that every derivative has equivalent activity.

The present paper examines the comparative evaluation of eleven glucosinolate derivatives in

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relation to fungal growth and biofilm development. It considers the chemical basis of antifungal activity, the methods needed to compare compounds reliably, and the factors that influence the interpretation of experimental findings. The paper also discusses the potential applications and limitations of these derivatives. Because comparative results depend on the exact compounds, fungal strains, concentrations, and assay conditions, this review does not assign unverified rankings or numerical inhibition values to the eleven derivatives. Instead, it establishes a framework for interpreting existing evidence and designing future experiments that can determine which compounds are most effective under clearly defined conditions.

II. CHEMICAL DIVERSITY AND COMPARATIVE ANTIFUNGAL ACTIVITY OF GLUCOSINOLATE DERIVATIVES

Glucosinolates provide a chemically diverse starting point for antifungal research. Their structures contain a common glucosinolate framework linked to variable side chains, which influence the properties of the resulting hydrolysis products. The enzymatic breakdown process is affected by plant enzymes, reaction conditions, pH, and the chemical identity of the original glucosinolate. Isothiocyanates are among the best-studied products because their electrophilic carbon can react with nucleophilic groups in biological molecules. Other products, including nitriles and epithionitriles, may exhibit different chemical behaviours and biological effects. Therefore, the term glucosinolate derivative should be defined carefully in any comparative study, particularly when native compounds and their hydrolysis products are included in the same experimental panel.

The selection of eleven derivatives offers an opportunity to examine whether chemical differences correspond to differences in antifungal performance. A valid comparison should identify each compound by its precise chemical name, purity, preparation method, and concentration. Where glucosinolate hydrolysis products are generated enzymatically, the conversion efficiency and composition of the resulting mixture should be characterized. Otherwise, apparent differences between treatments could reflect differences in the amount of active product formed rather than intrinsic antifungal potency. Studies of glucosinolate-derived compounds have shown that antifungal activity varies with chemical structure, and the compounds producing the strongest effects in one system do not necessarily perform identically in another (Plaszkó et al., 2021).

Fungal growth inhibition is one of the principal endpoints used to evaluate antifungal compounds. In a standardized assay, fungal growth may be assessed through colony diameter, dry biomass,

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optical measurements, or another method appropriate to the organism. The selected endpoint should be consistent across the tested compounds and accompanied by suitable growth and solvent controls. Concentration-response experiments are especially important because a derivative may show little effect at low concentrations but stronger inhibition as exposure increases. Where data support it, investigators can estimate a minimum inhibitory concentration or another defined potency measure. Such values should be reported with the organism, culture conditions, incubation period, and method used, because results obtained using different procedures may not be directly comparable.

The fungal species selected for testing also influence the outcome. Plant pathogens such as *Fusarium*, *Rhizoctonia*, *Sclerotinia*, and *Alternaria* differ in their growth characteristics, cellular physiology, and responses to chemical stress. Yeasts and filamentous fungi may respond differently to the same derivative, while even strains within a species can vary in susceptibility. Comparative research should therefore include organisms relevant to the intended application and avoid generalizing from a single strain. Earlier research on enzyme-derived glucosinolate products demonstrated differential responses among several plant-pathogenic fungi, illustrating why compound ranking should be established experimentally for each defined test system (Mari et al., 1997).

The chemical environment can further modify antifungal activity. Volatility may affect compounds tested in vapour-phase systems, while solubility and stability influence their effective concentration in liquid media. Hydrophobic derivatives may interact strongly with biological membranes but may also have limited aqueous availability. Reactive compounds may bind to medium constituents or degrade during incubation. These factors can create differences between nominal concentrations and the exposure experienced by fungal cells. Consequently, reliable comparative studies should measure or control important physicochemical variables and report the exposure conditions clearly. Where possible, stability and recovery measurements can help determine whether reduced activity results from biological resistance or from loss of the active compound.

Overall, evaluating eleven derivatives should be treated as a structured comparison of chemical identity, fungal susceptibility, concentration-response behaviour, and assay conditions. The objective is not simply to identify the compound producing the largest apparent reduction in growth, but to determine whether the effect is reproducible, concentration-dependent, and relevant to a particular application. Comparative results become more informative when the tested

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derivatives are evaluated against common reference agents and when uncertainty is reported. This approach can reveal promising candidates while preventing overinterpretation of isolated observations or differences arising from inconsistent experimental methods.

III. BIOFILM DEVELOPMENT AND METHODS FOR EVALUATING ANTIFUNGAL EFFECTS

Biofilms are organized microbial communities in which fungal cells attach to a surface or one another and are embedded within an extracellular matrix. In clinically relevant and environmental settings, biofilm formation can influence persistence, surface colonization, and tolerance to chemical stress. Biofilm development is a multistage process that may include initial attachment, proliferation, maturation, matrix production, and dispersal. The relative importance of these stages varies by fungal species and environmental conditions. Because biofilms differ from free-floating cells, a derivative that inhibits planktonic growth may not necessarily prevent attachment or disrupt an established biofilm.

Glucosinolate derivatives could influence biofilm formation through several possible pathways. They may reduce the number of viable cells available for attachment, alter cellular surface properties, interfere with growth, or affect processes involved in extracellular matrix development. However, the observed reduction in biofilm biomass does not automatically demonstrate a biofilm-specific mechanism. If a treatment substantially inhibits growth, lower biofilm biomass may simply reflect fewer cells being produced. To distinguish these effects, researchers should compare biofilm outcomes with planktonic growth measurements and, where possible, evaluate biofilm inhibition at concentrations that have limited effects on overall cell viability.

A reliable biofilm assessment should distinguish prevention of new biofilm formation from treatment of a mature biofilm. Prevention assays expose cells to the test compound during initial attachment and development. Established-biofilm assays introduce the compound after the community has formed, thereby evaluating its ability to reduce existing biomass or viability. These experiments address different biological questions and should be reported separately. A compound that prevents biofilm establishment may have value for surface protection or prevention strategies, while a compound that reduces established biofilm viability may be more relevant to treatment research. Neither result should be inferred from the other.

Common analytical approaches include biomass staining, metabolic activity assays, microscopy, and viable-cell measurements. Biomass staining can estimate the material retained on a surface,

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but it may include both living and dead cells as well as extracellular matrix. Metabolic assays provide an indirect estimate of cellular activity and may be affected by chemical interference from the tested derivative. Microscopy can reveal biofilm architecture and cell distribution, while colony-forming measurements can provide information about viable organisms under suitable conditions. Using more than one complementary method can improve confidence in the findings. Appropriate controls are required to determine whether a compound interferes with the assay signal independently of its biological effects.

Experimental conditions must be standardized to compare the eleven derivatives fairly. Relevant variables include fungal inoculum, growth medium, incubation temperature, exposure time, surface material, solvent concentration, and washing procedures. Biofilm assays are particularly sensitive to differences in attachment surfaces and handling. Replicate experiments and suitable positive and negative controls help determine whether observed differences are reproducible. Where the study includes several species, the analysis should account for organism-specific behaviour rather than treating all biofilms as equivalent. The concentration producing a measurable reduction in biomass should not automatically be interpreted as a concentration that eradicates viable cells.

The literature on glucosinolate-derived isothiocyanates supports their investigation as antifungal compounds, while broader fungal-biofilm research demonstrates that biofilm formation involves complex interactions among cellular growth, extracellular matrix production, and regulatory processes (Plaszkó et al., 2021). Nevertheless, direct evidence for the biofilm effects of each of eleven specific derivatives must be obtained from the relevant experiments. A robust evaluation should identify whether each derivative affects initial attachment, biomass accumulation, mature-biofilm viability, or more than one endpoint. This distinction will clarify the potential value of the compounds and help prioritize candidates for further mechanistic investigation.

IV. FACTORS INFLUENCING PERFORMANCE, POTENTIAL APPLICATIONS, AND RESEARCH LIMITATIONS

Comparative antifungal performance depends on interactions between the derivative and the fungal organism. Chemical structure may affect membrane interactions, intracellular access, and reactivity with cellular components. At the same time, fungi possess different protective responses, metabolic capabilities, and capacities to tolerate chemical stress. Some fungi may transform or detoxify glucosinolate-related products, reducing the duration or magnitude of

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exposure. Research on non-isothiocyanate products has described fungal biotransformation and detoxification processes, demonstrating that fungal responses can involve both susceptibility and the ability to metabolize plant-derived compounds (Plaszkó et al., 2022). These differences support testing multiple species and examining both antifungal effects and potential routes of compound inactivation.

The concentration and duration of exposure are also important. A short exposure may delay growth without producing lasting inhibition, while longer exposure may reveal stronger effects or allow the fungus to adapt. Concentration-response curves can help distinguish weak, moderate, and strong activity within the tested range. However, the highest tested concentration should not be assumed to represent a practically useful dose. High concentrations may cause unwanted effects on plant tissues, beneficial microorganisms, food quality, or host cells. Therefore, antifungal potency should be evaluated alongside selectivity and safety rather than considered in isolation.

Agricultural protection represents one possible application of glucosinolate-derived antifungal compounds. Some derivatives may contribute to biofumigation or the suppression of soil-borne pathogens, while others could be investigated for protection of harvested produce. Their usefulness depends on persistence, volatility, application method, soil or food chemistry, and effects on non-target organisms. In food preservation, the compound must remain effective under relevant storage conditions without adversely affecting taste, odour, or product quality. Environmental applications also require consideration of degradation products and effects on beneficial soil microorganisms. Evidence of in vitro inhibition is an important starting point, but it does not establish field effectiveness or commercial suitability.

Medical applications require a separate and more demanding assessment. Antifungal activity against a human-associated fungus does not demonstrate that a derivative is safe or effective as a medicine. Potential toxicity to mammalian cells, pharmacological availability, stability, and interactions with existing drugs must be investigated. Biofilm-associated infections may present additional challenges because compounds must reach cells within a structured community. Future studies may investigate whether selected derivatives enhance the activity of established antifungal agents, but such combinations require controlled synergy testing rather than assumptions based on individual activity. Clinical relevance can only be established through progressive preclinical and clinical evaluation.

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Several limitations must be addressed when comparing the eleven derivatives. First, studies may use different fungal strains, media, incubation periods, and endpoints, limiting direct comparison. Second, some compounds may be tested as purified substances while others are generated through enzymatic hydrolysis, resulting in differences in composition and exposure. Third, biofilm biomass, metabolic activity, and viable-cell counts represent distinct outcomes and should not be treated as interchangeable. Fourth, a compound may affect growth indirectly through changes in medium chemistry or may interfere with an analytical assay. Appropriate controls and complementary measurements are therefore necessary to establish the validity of the findings.

Future research should combine standardized growth assays with separate biofilm-prevention and mature-biofilm experiments. The chemical identity, purity, stability, and effective exposure of each derivative should be documented. Mechanistic studies can then investigate whether the most promising candidates affect membrane integrity, oxidative-stress responses, metabolic functions, or other cellular processes. Safety testing and evaluation against beneficial microorganisms should accompany application-oriented studies. This integrated approach can establish a defensible comparative ranking of the eleven derivatives, identify the conditions under which each is most effective, and clarify whether their antifungal potential can be translated into useful agricultural, food-related, or biomedical applications.

V. CONCLUSION

Glucosinolate derivatives constitute a diverse group of plant-related compounds with potential antifungal properties. Their biological effects depend on chemical structure, the products formed during glucosinolate hydrolysis, and the susceptibility of the target fungus. Research has established that selected hydrolysis products, particularly certain isothiocyanates, can inhibit fungal growth, but the strength of the response varies among compounds and organisms. Consequently, the comparative evaluation of eleven derivatives requires consistent chemical identification, standardized testing, and careful interpretation rather than an assumed universal ranking.

Fungal growth and biofilm development should be assessed as related but distinct outcomes. Growth inhibition can indicate reduced fungal proliferation, whereas biofilm assays investigate attachment, community development, biomass, or viability within a structured population. A reduction in biofilm biomass does not necessarily establish biofilm eradication or a specific biofilm-targeting mechanism. Combining growth measurements with biomass, viability, and

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microscopy-based assessments can provide a more complete understanding of the effects of each derivative. Separate testing of biofilm prevention and established-biofilm treatment is especially important.

The application of glucosinolate derivatives will depend on more than antifungal potency. Compound stability, exposure, solubility, effects on non-target organisms, and possible toxicity must be considered alongside the intended use. Agricultural and food-related applications require evidence under realistic environmental or storage conditions, while medical development requires extensive safety and efficacy testing. Standardization of methods and transparent reporting are essential for distinguishing reproducible biological activity from differences caused by experimental conditions.

Future research should prioritize comparative concentration-response studies, validated biofilm assays, chemical characterization, and investigations of the mechanisms underlying differential susceptibility. The strongest candidates should be examined in relevant application models and evaluated for safety, stability, and practical feasibility. Although glucosinolate derivatives are promising subjects for antifungal research, their value will ultimately depend on robust experimental evidence showing reproducible activity under defined conditions and acceptable effects on non-target systems.

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