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Research Article

Phytochemical Screening of Garlic (*Allium sativum* L.) Extracts and Their Antifungal and Anti-aflatoxigenic Activity against *Aspergillus flavus*

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ABSTRACT

Garlic (*Allium sativum* L.) is recognized for its rich phytochemical composition and antimicrobial activity. This study assessed the effects of different extraction solvents and methods on phytochemical recovery from garlic cloves and evaluated the antifungal and anti-aflatoxigenic activity of the extracts against *Aspergillus flavus*. The study aimed to identify an efficient, eco-friendly extraction strategy to maximize bioactive compounds for fungal control and aflatoxin B1 (AFB1) reduction, supporting food safety and crop protection. Garlic cloves were extracted using five solvents of varying polarity (ethyl acetate, acetone, ethanol, ethanol/water 50%, and water) through three extraction methods: KSM, USM, and MSM. Phytochemical screening revealed marked variation in constituent profiles depending on both solvent and method, with ultrasound-assisted extraction (USM) yielding the highest diversity of phytochemicals. The 50% ethanol extract was particularly rich in flavonoids, tannins, phenols, alkaloids, saponins, steroids, and glycosides. Antifungal assays confirmed that the 50% ethanol extract showed the strongest inhibitory effect on *A. flavus*, suppressing mycelial growth by up to 98%, completely inhibiting spore production at the lowest concentration tested, and achieving nearly complete biomass reduction. This extract also resulted in the greatest reduction of AFB1 production, lowering toxin levels to below 9 µg/ml. In contrast, aqueous extracts exhibited weaker activity at low concentrations but improved significantly at higher doses. In conclusion, extraction using 50% ethanol combined with USM is highly effective for recovering antifungal and anti-aflatoxigenic compounds from garlic. It is recommended that this extraction approach be further explored for developing natural fungicidal formulations and for application in food and feed safety management.

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Introduction

Aspergillus flavus contamination of grain crops is very common and poses a significant threat to food security. It is a common opportunistic fungus that infects maize, cereals, nuts, and dried fruits and results in significant quantitative and qualitative losses (Mateo et al., 2017; Taniwaki et al., 2018). Beside direct crop damage, *A. flavus* is able to produce a number of toxic secondary metabolites, especially aflatoxins (Xing et al., 2016). These include aflatoxin B1 (AFB1), which is considered

to be one of the most hazardous naturally occurring carcinogens threatening human and animal health (Caceres et al., 2020; Moral et al., 2020).

Even though synthetic fungicides have the capability of inhibiting the growth of *A. flavus*, the widespread usage of the fungicides has led to issues of contamination of the environment, human safety, as well as emergence of drug-resistant strains of the fungi (Brenneman et al., 1993). As a result, more focus has been paid to natural compounds of plant origin as alternative antifungal agents. These are mostly safer, biodegradable, and effective compounds because they contain a variety of bioactive constituents (Atanasov et al., 2015; Tang et al., 2018; Mohammed and Mustafa, 2020; Mustafa and Abdulaziz, 2020, 2021; Abid and Tawffiq, 2022).

The interest of scientists in natural products and their biological activity is long-standing, and they can find applications in crop protection and the improvement of human health (Khalil and Mustafa, 2020; Oglah et al., 2020; Fakri Mustafa et al., 2021) The increase in the literature evidences an international tendency toward using natural products as an alternative to synthetic chemicals to increase the quality of crops and minimize the use of chemicals (Mahmood et al., 2014; Aldewachi et al., 2020; Mustafa et al., 2020). Besides, natural compounds can be used as useful chemical platforms to produce new bioactive agents with antifungal and antitoxigenic properties (Bashir et al., 2020).

Garlic (*Allium sativum* L.), belonging to the family Liliaceae, has important nutritional and medical significance. It contains many important biochemical compounds that are used for treating many common diseases. It also possesses antimicrobial properties that increase the safety and shelf-life of food products by combating spoilage and foodborne diseases (Habib et al., 2021). When tested against the fungal pathogens, *Aspergillus* and *Cryptococcus*, garlic significantly inhibited the growth of these fungi comparable to the growth inhibition achieved by the treatment of ketoconazole (Shams-Ghahfarokhi et al., 2006). In another study, aqueous, ethanolic, methanolic, and petroleum ether extracts of *A. sativum* showed significant antifungal effectiveness against pathogenic fungi, such as *Botrytis cinerea*, *Candida* species, *A. niger*, *A. flavus*, *Rhizopus stolonifera*, *Alternaria alternate*, and *Penicillium expansum* (Fufa, 2019).

The objective of the present study was to prepare garlic extracts with five solvents of various polarity indices using three different methods and compare their antifungal efficacy against *A. flavus*. Furthermore, the study aimed at determining the effectiveness of these garlic extracts in inhibiting the production of AFB1 by *A. flavus*.

Materials and Methods

Preparation of natural sample

Garlic cloves were peeled, cut and dried at room temperature for four weeks. The garlic cloves were crushed with a coffee blender and sieved to make a fine powder. Each sample was placed in a separate beaker. The powder was extracted using a variety of solvents with varying polarity index (PI) values. Ethanol (PI = 5.2), ethanol/water (50%), ethyl acetate (PI = 4.4), acetone (PI = 5.1), and water (PI = 10.2), in a 10:1 ratio, were mixed with powder and used in the following methods: kinetic-supported maceration, ultrasound-supported maceration, and microwave-supported maceration.

Kinetic-supported maceration (KSM)

Using a shaker water bath at 30°C for 72 h, a kinetic maceration of the sample powder (two grams) in 20 ml of extraction solvent was performed. Before being utilized in the phytochemical analysis, the mixture was filtered, and then the resultant solution was kept in a refrigerator (Majoumouo et al., 2019).

Ultrasound-supported maceration (USM)

A sonicator water-bath (Power sonic 410, 40 kHz, 350 W) was used to macerate the mixture of garlic powder (2 g) in the extracting solvent (20 ml) for 30 min at 30°C. The macerated mixture was filtered, and the filtrate was kept in the refrigerator for later use (Montero-Calderon et al., 2019).

Microwave-supported maceration (MSM)

A microwave-oven (Moulinex, MW531070) was used to macerate the mixture of garlic powder (2 g) in the extracting solvent (20 ml) for 5 min at 100 W. After that, the irradiated mixture was filtered, and the filtrate was kept in the refrigerator (Yusoff and Leo, 2017).

Phytochemical investigation

The fifteen prepared extracts were investigated for the presence of different phytochemical groups, including flavonoids, alkaloids, tannins, terpenoids, carbohydrates, phenolic compounds, proteins, steroids, saponins, and glycosides, using standard phytochemical screening methods (Harborne, 1998).

Isolation of fungus

The fungal isolate, used in the study, was identified on the basis of morphological features, described by

Thatana et al. (2017). An isolate of toxin-producing *A. flavus* strain was obtained from the Plant Pathology Laboratory of the Department of Plant Protection. The fungus was cultured on potato dextrose agar (PDA) at 28°C for 7 days, and fresh cultures were used in antifungal efficacy tests and aflatoxin production studies.

Effect of garlic extracts on mycelial growth

Mycelial growth of *A. flavus* was evaluated using 85 mm Petri plates containing PDA medium supplemented with different concentrations of garlic extracts (12.5, 25, and 50 mg/ml). Each Petri plate was inoculated in the center with a 5 mm disc of 7 days old culture of *A. flavus*. The plates were incubated at 27°C for six days. After incubation, colony diameter was measured using ImageJ software. Inhibition percentage of mycelial growth (IPM) was calculated using the given below formula:

$IPM = [(Ac – At) \times 100] / Ac$

Where Ac and At represent the colony diameter in the control and in the treatment respectively.

Effect of garlic extracts on *A. flavus* sporulation

The inhibitory effect of garlic extracts on *A. flavus* spore production was evaluated in the similar way as the effect of garlic extracts were assessed on mycelial growth. Spore production was quantified using a counting slide, and the inhibition percentage was calculated according to the following equation.

$IPM = [(Sc - St) \times 100] / Sc$

Where Sc and St represent spore count in the control and in the treatment respectively.

Effect of garlic extracts on biomass growth

Erlenmeyer flasks (200 ml) containing potato dextrose broth (PDB) were used to evaluate the effect of garlic extracts on fungal biomass growth. For each tested concentration of garlic extract (12.5, 25, and 50 mg/ml), 100 ml of PDB medium was prepared and transferred to a separate Erlenmeyer flask. Each flask was then inoculated with 1 ml of fungal spore suspension at a concentration of 10^6 spores/ml. The flasks were incubated for 10 days at 27°C. On the 10^{th} day, fungal biomass was separated from the medium by filtration. The collected biomass was then dried at 60°C until a constant weight was achieved. The dry weight of biomass from each treatment was compared to that of the control to calculate the percentage of growth inhibition induced by each garlic extract at different concentrations according to the following equation.

$IPM = [(Bc - Bt) \times 100] / Bc$

Where Bc and Bt represent the biomass production (g) in the control and in the treatment respectively.

Evaluation of the effect of garlic extracts on AFB1 production by *A. flavus*

To evaluate the effect of garlic extracts in reducing aflatoxin B1 production, 100 ml of liquid aflatoxin production medium containing 5% sucrose, 0.1% $MgSO_4 \cdot 7H_2O$, 1% KH_2PO_4, and 0.0176 g/L $ZnSO_4 \cdot 7H_2O$ was inoculated with four fungal discs taken from the edge of a newly growing colony. Garlic extracts were added to the medium at the tested concentrations, and the cultures were incubated for 7 days at 27°C under controlled conditions. After the incubation period, the fungal mass was removed by filtration using sterile filter paper, and the resulting liquid filtrate was collected and stored at -20°C until analysis. The amount of aflatoxin B1 was quantified using high-performance liquid chromatography (HPLC) Shimadzu LC-2010HT equipped with a Phenomenex C18 reverse-phase column (250 × 4.6 mm, 5 µm). The acetonitrile, distilled water, and glacial acetic acid solution [(180:820:10) v/v/v], delivered at a flow rate of 0.5 ml/min, was used in the mobile phase, and detected at a wavelength of 365 nm.

The concentration of toxin in the samples was calculated using the following equation:

$C_{toxin} = (A_{sample} / A_{standard}) \times C_{standard} \times D$

Where:

C_{toxin} = concentration of toxin in the sample,

A_{sample} = area of the sample curve,

$A_{standard}$ = area of standard toxin curve,

$C_{standard}$ = concentration of the toxin standard, and

D = dilution factor (dilutions number)

Data analysis

Each experiment had five replicas of treatments. The statistical analysis of data was done with the Experimental Analysis System of the SAS software (2012 version) in completely randomized design (CRD). Means were compared and differences between treatments were tested to ascertain the significance of the differences at a probability level of 0.05 using Duncan Multiple Range Test. Results were expressed as means ± standard error (Mean ± SE).

Results

Phytochemical investigation

The garlic cloves were extracted using five different solvents: ethyl acetate, acetone, ethanol, ethanol /water (50%), and H_2O. The extraction was carried out using three different methods viz., KSM, USM, and MSM. The

presence of selected major and secondary phytoconstituents in the fifteen extracts obtained from the extraction procedures was determined using a phytochemical screening test. The findings of the phytochemical screening assays are shown in Tables 1, 2, and 3. Based on these findings, the USM technique of extraction performed the best regarding release of phytochemical compounds.

USM screening tests (Table 1) revealed that the contents of garlic clove extracts varied according to the extractant used. With the exception of terpenoids, most reactions in the ethanol/water (50%) of *A. sativum* extract were positive. Both acetone and ethanol extracts contained flavonoids, tannins, phenols, alkaloids, and saponins, but terpenoids, carbohydrates, and proteins were not found. Steroids were present in ethanol extract but not in acetone extract. Ethyl acetate contained flavonoids, tannins, phenols, and alkaloids, but no terpenoids, carbohydrates, proteins, steroids, or glycosides. Flavonoids, carbohydrates, alkaloids, proteins, saponins, steroids, and glycosides were found in the water extracts, but tannins, phenols, and terpenoids were not.

Antifungal activity

Table 4 indicates that there is a great difference between the response of *A. flavus* to the various concentrations and types of garlic extracts. The extract of garlic made using 50 percent ethanol had the greatest inhibitory activity in comparison to other extracts which showed very high inhibitory rates of the mycelial growth of 96.35 percent to 98.05 percent at all concentrations. It was also found to inhibit 100% spore production with the lowest concentration (12.5 mg/ml). Moreover, the extract showed the greatest rates of biomass inhibition (99.12%), especially at the concentration of 50 mg/ml, which indicates the great effectiveness of the active compounds obtained with 50% ethanol.

Table 1. Phytochemicals extracted using USM.

Phytochemical	Name of Test	Solvent				
		Ethyl	Acetone	Ethanol	50%	Water
Flavonoids	Lead acetate test	Pi	Pi	Pi	Pi	Pi
Tannins	Braymer's test	Pi	Pi	Pi	Pi	Ni
Terpenoids	Liebermann-Burchard test	Ni	Ni	Ni	Ni	Ni
Carbohydrates	Molish's test	Ni	Ni	Ni	Pi	Pi
Alkaloids	Mayer's test	Pi	Pi	Pi	Pi	Pi
Phenols	Ferric chloride test	Pi	Pi	Pi	Pi	Ni
Proteins	Xanthoproteic test	Ni	Ni	Ni	Pi	Pi
Steroids	Salkowski test	Ni	Ni	Pi	Pi	Pi
Saponins	Olive oil test	Pi	Pi	Pi	Pi	Pi
Glycosides	Liebermann's test	Ni	Pi	Pi	Pi	Pi

The symbols Pi and Ni represent the positive and negative results respectively.

Table 2. Phytochemicals extracted using MSM.

Phytochemical	Name of Test	Solvent				
		Ethyl	Acetone	Ethanol	50%	Water
Flavonoids	Lead acetate test	Ni	Ni	Pi	Pi	Ni
Tannins	Braymer's test	Pi	Ni	Ni	Ni	Ni
Terpenoids	Liebermann-Burchard test	Ni	Ni	Ni	Ni	Ni
Carbohydrates	Molish's test	Pi	Pi	Pi	Pi	Pi
Alkaloids	Mayer's test	Pi	Ni	Pi	Ni	Ni
Phenols	Ferric chloride test	Ni	Ni	Ni	Ni	Ni
Proteins	Xanthoproteic test	Pi	Pi	Pi	Pi	Pi
Steroids	Salkowski test	Ni	Ni	Ni	Pi	Pi
Saponins	Olive oil test	Pi	Ni	Ni	Pi	Pi
Glycosides	Liebermann's test	Ni	Ni	Pi	Ni	Ni

The symbols Pi and Ni represent the positive and negative results respectively.

Table 3. Phytochemicals extracted using KSM.

Phytochemical	Name of Test	Solvent				
		Ethyl	Acetone	Ethanol	50%	Water
Flavonoids	Lead acetate test	Ni	Pi	Ni	Pi	Pi
Tannins	Braymer's test	Ni	Ni	Pi	Ni	Ni
Terpenoids	Liebermann-Burchard test	Ni	Ni	Ni	Ni	Ni
Carbohydrates	Molish's test	Ni	Ni	Ni	Pi	Pi
Alkaloids	Mayer's test	Pi	Ni	Pi	Pi	Pi
Phenols	Ferric chloride test	Ni	Pi	Ni	Ni	Ni
Proteins	Xanthoproteic test	Pi	Pi	Pi	Pi	Pi
Steroids	Salkowski test	Ni	Ni	Pi	Pi	Pi
Saponins	Olive oil test	Pi	Ni	Ni	Pi	Pi
Glycosides	Liebermann's test	Ni	Ni	Ni	Ni	Ni

The symbols Pi and Ni represent the positive and negative results respectively.

The water extract showed the minimum inhibitory effect at lower concentrations, particularly regarding spore production, with an inhibition level not more than 35.65% at a concentration of 12.5 mg/ml. However, its efficacy improved significantly at the higher concentration (50 mg/ml), showing 88.76% inhibition of mycelial growth and 82.71% inhibition of spore production, indicating that water-soluble compounds require higher concentrations to attain a noticeable effect.

Acetone and ethyl acetate extracts were also found to have moderate efficacy, indicating moderate inhibition levels at low and medium concentrations. They were found significantly more effective at 50 mg/ml and showed more than 85% inhibition of mycelial growth and spore production in some treatments. Absolute ethanol extract also exhibited significant inhibitory properties although it was not as effective as 50% ethanol extract indicating that a combination of polar and nonpolar solvents might be more effective in extraction of antifungal compounds.

Table 4. Sensitivity of *A. flavus* to different concentrations of *A. sativum* extracts.

Extracts	Concentration of extract (mg/ml)	% Inhibition of mycelium growth	% Inhibition of spore production	% Inhibition of biomass
50% Ethanol	12.5	96.35 ± 3.2 a	100 ± 1.3 a	89.64 ± 5.4 c
	25	97.08 ± 3.6 a	100 ± 1.9 a	94.61 ± 3.9 ab
	50	98.05 ±2.9 a	100 ± 2.2 a	99.12 ± 4.7 a
Water	12.5	53.82 ± 1.3 e	35.65 ± 4.1 d	84.38 ± 1.4 d
	25	61.78 ± 2.2 d	37.45 ± 4.9 d	88.54 ± 1.9 c
	50	88.76 ± 2.5b	82.71 ± 3.8 b	94.91 ± 2.2 ab
Acetone	12.5	63.73 ±4.1 d	45.25 ± 2.8 d	75.14 ± 4.5 e
	25	65.92 ± 3.8 d	47.4 ± 3.5 d	79.64 ± 4.2 e
	50	89.24 ± 3.9 b	85.457 ± 3.8 b	94.35 ± 4.9 ab
Ethyl acetate	12.5	63.52 ± 1.9 d	41.25 ± 2.8 d	81.24 ±5.7d
	25	61.82 ± 2.8 d	41.25 ± 3.1 d	83.94 ± 4.9 b
	50	88.92 ± 3.1 b	83.4 ± 3.7 b	88.94 ± 5.1 c
Ethanol	12.5	79.78 ± 4.2 c	78.45 ± 5.2 c	80.70 ± 6.1 d
	25	81 .92 ± 4.7 c	79.4 ± 4.9 c	82.56 ± 5.8 d
	50	89.24 ± 4.6 b	84.42 ± 2.8 b	87.14 ± 5.9 c

Duncan's Multiple Range Test reveals a non-significant difference ($p > 0.05$) among the average values of inhibition followed by the same letter in the same column.

Effect of different garlic extracts on AFB1

Table 5 shows that all garlic extracts reduced AFB1 concentrations compared to the control treatment, which recorded 30.25 µg/ml. The 50% ethanol extract recorded the lowest values at different concentrations, reaching 8.24, 7.18, and 7.57 µg/ml at concentrations of 12.5, 25, and 50 mg/ml respectively. There were no significant differences between the 25 and 50 mg/ml concentrations according to DMRT. In contrast, the aqueous extract recorded higher values reaching 10.25, 11.29, and 11.47 µg/ml at the same concentrations, while the acetone, ethyl acetate, and absolute ethanol extracts showed intermediate values ranging between 8.87 and 10.42 µg/ml.

Table 5. Effect of different garlic extracts on AFB1.

Extract	Concentration of extract mg.ml^{-1}	Ug.ml^{-1}
50% Ethanol	12.5	8.24 ± 0.21 d
	25	7.18 ± 0.27 e
	50	7.57 ± 0.16 e
Water	12.5	10.25 ± 0.11 bc
	25	11.29 ± 0.16 b
	50	11.47 ± 0.18 b
Acetone	12.5	9.85 ± 0.14 c
	25	9.14 ± 0.16 cd
	50	10.42 ± 0.18 b
Ethyl acetate	12.5	8.87 ± 0.17 d
	25	9.41 ± 0.06 c
	50	9.12 ± 0.11 cd
Ethanol	12.5	9.74 ± 0.09 c
	25	9.27 ± 1.02 c
	50	9.62 ± 1.1 c
Control	0	30.25 ± 0.18a

Duncan's Multiple Range Test reveals a non-significant difference ($p > 0.05$) among the average values of inhibition followed by the same letter in the same column.

Discussion

A. flavus is regarded as one of the most significant pathogenic fungi of crops which can cause huge losses both in the fields and during storage. The incidence, prevalence and severity of this deleterious fungus vary from filed to field in the same area. The qualitative phytochemical analysis of the five garlic extracts made using the USM approach showed that there are a variety of bioactive compounds in the extracts, such as saponins, flavonoids, alkaloids, and so on, which were consistently found in all the extracts. These results are consistent with previous literature that found comparable phytochemical profiles in *Allium* species (Ahmad and Beg, 2001; Enejiyon et al., 2020).

The antifungal effect observed in this research is largely attributed to the phenolic compounds and hydrogen-donating bioactive molecules present in the extracts of garlic. These compounds react with fungal cell walls and membrane proteins resulting in the disruption of membrane, enzyme inactivation, and inhibitory effect on fungal cell division. The findings showed that fungal growth and sporulation were significantly inhibited, which is also in line with the previous evidence of the antifungal effect of garlic-derived extracts (Fontenelle et al., 2007).

Out of the solvents tested, the 50% ethanol extract collected by the USM technique had the best antifungal activity. It has increased effectiveness due to the increased polarity of the ethanol-water mixture which enables the easy extraction of polar bioactive compounds (Tiwari et al., 2011).

The polar solvents are known to enhance the extraction of phenolics, flavonoids, proteins and carbohydrates and consequently increase extract yield and biological activity (Do et al., 2014). The 50 percent ethanol-water extract exhibited the strongest effect of inhibiting *A. flavus* in the current research because it had the highest amounts of phenolic and flavonoid compounds. The activity of phenolics is due to the interaction not only with the cell wall but also with proteins, which eventually leads to cell damage and prevents growth (Banik et al., 2014; Coppo and Marchese, 2014; Lee et al., 2024; Sun et al., 2025).

An evident concentration-dependent antifungal activity was found, which was probably explained by the enhanced access to bioactive constituents when the extract concentration was higher (Mahmoud et al., 2011; Kiswii et al., 2014).

It is noteworthy that the 50 percent ethanol extract substantially inhibited the development of *A. flavus* as well as a significant decrease in aflatoxin B1 (AFB1) synthesis. Allicin and aguanidine are other sulfur-based bioactive compounds found in garlic that make it an antimicrobial and antitoxic agent (Bayan et al., 2014; Redondo-Blanco et al., 2020). These antimicrobial compounds affect the fungal metabolism by disrupting the enzyme systems and regulatory pathways that participate in the aflatoxin biosynthesis (Sarfraz et al.,

2020; Anjorin et al., 2022).

There are reports which confirmed that garlic extracts notably decreased the concentrations of AFB1 in maize and peanuts (Negera and Washe, 2019; Vargas-Ortiz et al., 2020). The extract has also been reported to restrict early fungal developmental processes, thereby limiting progression to aflatoxin-producing stages and supporting its antitoxic activity (Daniel et al., 2015).

Garlic antifungal effect is mainly due to allicin (diallyl thiosulfinate), which is the most abundant sulfur-based product in allicin, which is formed when tissues are disrupted. Allicin has a wide antifungal spectrum against many plant pathogens, such as *B. cinerea*, *Phytophthora infestans*, and *Alternaria* spp. (Curtis et al., 2004; Daniel et al., 2015; Hosseini et al., 2019). Allicin disrupts the fungal cell wall integrity at the cellular level by interfering with the ergosterol biosynthesis pathway, especially at later stages, as shown in *Saccharomyces cerevisiae* (Jordá and Puig, 2020). Also, allicin oxidizes thiol (-SH) groups in protein molecules of the cells, disrupting vital metabolic processes. It is also linked to the rise of intracellular reactive oxygen species (ROS) production resulting in oxidative stress and cell death by apoptosis or necrosis based on concentration and exposure time (Chin et al., 2014; Gruhlke et al., 2016).

Conclusion

The findings of this research proved that garlic extracts made through the USM method possessed high antifungal activity against *A. flavus*, especially at high concentrations. Phytochemical screening established the presence of bioactive components like saponins, tannins, alkaloids, and flavonoids which are probably the cause of the observed antifungal activities. Out of the tested solvents, the 50 percent ethanol-water extract exhibited the highest level of inhibition of mycelial growth, biomass production, and sporulation in relation to the rest of the solvent extracts. More flavonoids and phenolic compounds of *A. sativum* powder were also extracted under this solvent system.

The high total phenolic concentration of the 50 percent ethanol extract may have been responsible to the improved biological activity. Also, the 50 percent ethanolic and aqueous extracts markedly decreased the AFB 1 production. The present results indicate the potential of garlic extracts as natural, environmentally friendly antifungal and antitoxic agents and propose their potential deployment as alternative antifungal agents to address the problem of *A. flavus* contamination of maize and other cereal grains.

Authors' Contributions

BAA conceived and designed the study, conducted the data analysis, and prepared the initial draft of the manuscript. BYI and YFM contributed critical insights, constructive feedback, and valuable suggestions that enhanced the quality and clarity of the manuscript. All authors reviewed and approved the final version and accept responsibility for the accuracy and integrity of the work.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 3: Good Health and Well-Being
SDG 12: Responsible Consumption and Production

Reference

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