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β-Galactosidase activity in *Drosophila melanogaster* (Drosomycin-LacZ reporter) infected with pathogenic *Aspergillus* species and treated with solvent fractions of *Allium sativum* (garlic), *Allium cepa* (onion) and *Zingiber officinale* (ginger)

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ABSTRACT

Fungal infections caused by *Aspergillus* species pose significant health risks, especially in immunocompromised individuals. Conventional antifungals such as fluconazole remain the primary treatment, but increasing resistance necessitates alternative therapies. Plant-derived bioactive compounds have shown antifungal potential, making them promising candidates for new treatments. This study evaluated the antifungal efficacy of n-hexane, ethyl acetate, methanol, and aqueous fractions of *Allium sativum* (garlic), *Allium cepa* (onion), and *Zingiber officinale* (ginger) against *Aspergillus* infected *Drosophila melanogaster* (Drs-LacZ strain). Flies were infected with virulent strains of *A. flavus*, *A. fumigatus*, and *A. niger* and treated with plant fractions at 100–500 mg/mL. A positive control group received fluconazole (50 mg/mL), while a negative control remained untreated. Fly survival was recorded over seven days, and β-galactosidase activity was measured spectrophotometrically. The methanol fraction of *Z. officinale* produced the highest survival rate (86.67% at 500 mg/mL) in *A. flavus* infected flies, followed by the ethyl acetate fraction of *A. sativum* (68.89% at 500 mg/mL). Aqueous fractions had the weakest effects, with *A. cepa* aqueous fraction yielding 51.11% survival. β-galactosidase activity increased dose-dependently, with the methanol fraction of *Z. officinale* showing the highest activity (3.58 ± 0.02 nmol/mL/protein). The negative control confirmed pathogen virulence (8.89% survival), whereas fluconazole remained the most effective (93.33% survival, 4.84 ± 0.11 nmol/mL/protein). These findings highlight the antifungal potential of *A. sativum*, *A. cepa*, and *Z. officinale* fractions, particularly methanol and ethyl acetate extracts. Further research is needed to isolate and characterize the active compounds and elucidate their mechanisms of action.

Keywords: Antifungal activity, *Aspergillus* species, fungal infections, immunocompromised hosts, β-Galactosidase activity**.**

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INTRODUCTION

β-Galactosidase is a widely used reporter enzyme in genetic and biochemical research, particularly for studying immune responses in model organisms such as *Drosophila melanogaster* (Lemaitre and Hoffmann, 2007). In the Drosomycin-LacZ strain of *D. melanogaster*, β-galactosidase activity serves as a marker for the expression of antimicrobial peptides, including drosomycin, a key component of the insect's innate immune defense against fungal pathogens (Ferrandon et al., 2007). Monitoring β-galactosidase activity in response

to fungal infections and natural treatments provides valuable insights into host–pathogen interactions and the immunomodulatory effects of plant-based compounds.

Fungal infections pose a significant threat to human and animal health, with *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger* recognized for their pathogenic potential (Latgé and Chamilos, 2019). These fungi are associated with respiratory diseases, allergic reactions, and mycotoxin-related complications in both immunocompetent and immunocompromised hosts (Bongomin et al., 2017). *D. melanogaster* is a well-established model for studying fungal pathogenesis due to its conserved immune pathways, including the Toll pathway, which regulates drosomycin expression (Zaidman-Rémy et al., 2006). The use of β-galactosidase as a reporter gene enables quantifiable assessment of immune activation in response to fungal infections, thereby facilitating evaluation of immune modulation by natural or synthetic treatments (Neyen et al., 2014).

Natural plant-derived compounds such as ginger, garlic, and onion have long been recognized for their antimicrobial and immunomodulatory properties. Ginger contains bioactive compounds such as gingerol and shogaol, which have demonstrated antifungal and anti-inflammatory activities (Zhukovets and Özcan, 2020). Garlic is rich in organosulfur compounds, including allicin, known for its potent antimicrobial effects, while onions contain flavonoids and sulfur-containing compounds with immunostimulatory and antifungal activities (Borlinghaus et al., 2014). These bioactive constituents may enhance the immune response in *D. melanogaster* (Lopez-Ortiz et al., 2023), leading to increased antimicrobial peptide expression, which can be measured through β-galactosidase activity (Medzhitov, 2007).

This research is significant because it integrates molecular biology, immunology, and natural product research to explore novel antifungal strategies. The findings may provide insight into the potential of plant-derived compounds for immune enhancement and fungal disease management. Moreover, because β-galactosidase activity is widely used in genetic studies, this work could pave the way for future investigations into natural immune modulators and their roles in pathogen resistance.

Accordingly, this study aims to investigate β-galactosidase activity in *D. melanogaster* (Drosomycin-LacZ strain) infected with *A. flavus*, *A. fumigatus*, and *A. niger*, and subsequently treated with varying concentrations of *Zingiber officinale* (ginger), *Allium sativum* (garlic), and *Allium cepa* (onion).

MATERIALS AND METHODS

Study area

This study was conducted at the Drosophila Laboratory (Fungal Pathogens and Plant Bioactive Compounds), Department of Plant Science and Biotechnology, University of Jos, Nigeria, from January 2022 to September 2023.

Collection of plant materials

Fresh ginger (*Zingiber officinale*), garlic (*Allium sativum*), and onion (*Allium cepa*) were purchased from Farin Gada Market (Jos North LGA, Plateau State, Nigeria). Approximately 100 kg of plant material was procured in total. Plant batches were botanically verified, and voucher specimens were deposited at the University of Jos Herbarium (Voucher No. JUHN25000013PSB).

Extraction of plant materials

Plant materials were air-dried, pulverized, and sequentially macerated with n-hexane, ethyl acetate, and methanol. The remaining marc was extracted with distilled water. Fractionation followed Harborne's et al. (1998) solvent partition methodology. Extraction yields were recorded for each fraction and expressed as percentages of the dry weight.

Drosophila strains

The Drosomycin (Drs-LacZ) reporter strain of *Drosophila melanogaster* was obtained from the National Species Stock Center (Switzerland) through the Bruno Lemaitre Research Group, EPL-SV-GHI, UPLEM, Lausanne, Switzerland. Flies were maintained on standard cornmeal medium at 23 ± 1 °C and 60% relative humidity under a 12 h light/dark cycle. All experiments were performed using age-matched D. melanogaster strains (Drs-LacZ and Dpt-LacZ) as described by Abolaji et al. (2014) and Wuyep et al. (2020).

Preparation of fly food and plant extracts

Fly food was prepared to contain final concentrations of 100, 200, 300, 400, and 500 mg/mL of each plant extract. Spatulas were flame-sterilized and used to create superficial abrasions on the surface of the food to facilitate feeding. Acute toxicity was assessed by placing 15 unsexed flies per vial, in triplicate (n = 45 flies per concentration). Survival was monitored over seven days, and mortality was recorded daily (Abolaji et al., 2014; Wuyep et al., 2020).

Sexing and sorting of flies

Flies were anesthetized on ice, and males and females were distinguished based on genitalia, body size,

abdominal shape, and bristles on the forelegs. Virgin females were identified by the presence of a dark mark on the ventral abdomen, an embryonic residue excreted 8–12 h after eclosion (Lionakis and Kontoyiannis, 2010). Two- to three-day-old females were consistently used because they exhibit significantly lower mortality rates following *Aspergillus* infection compared to older flies (10–15 days old).

Establishment of fungal infections

Fungal infection was established following the ingestion method described by Wuyep et al. (2020). Flies were starved for 6 h and then exposed to yeast agar glucose media inoculated with 3-day-old cultures of *A. flavus*, *A. fumigatus* or *A. niger* for 6 h. After exposure, flies were transferred to vials containing fly food prepared with the different plant extract fractions. Survival was recorded daily for seven days (Michail and Dimitrios, 2012).

Experimental groups

Flies were divided into the following experimental groups:

A = Uninfected flies fed with normal diet (vehicle control)
B = Infected flies, untreated (negative control)
C = Infected flies treated with different concentrations of aqueous fractions of *A. sativum*, *A. cepa* and *Z. officinale*
D = Infected flies treated with different concentrations of methanol fractions of *A. sativum*, *A. cepa* and *Z. officinale*
E = Infected flies treated with different concentrations of ethyl acetate fractions of *A. sativum*, *A. cepa* and *Z. officinale*
F = Infected flies treated with different concentrations of n-hexane fractions of *A. sativum*, *A. cepa* and *Z. officinale*
G = Infected flies treated with fluconazole (50 mg/mL) (positive control).

β-Galactosidase assay

Three sets of five adult flies per treatment group were collected and stored at −20 °C. For analysis, flies were thawed on ice, homogenized in 250 µL of Buffer Z for 30 s, followed by the addition of another 250 µL of Buffer Z and vortexing. Homogenates were centrifuged at 6000 × g for 5 min, and the supernatants were collected. Protein concentrations were determined using the Bradford assay with bovine serum albumin (BSA) as the standard.

For β-galactosidase titration, 30 µL of Dpt-LacZ or 10 µL of Drs-LacZ sample was placed into a 96-well plate, followed by the addition of 250 µL of Buffer Z containing ONPG (final concentration = 0.35 mg/mL). Plates were incubated at 37 °C, and absorbance at 420 nm was measured at regular intervals (2–30 min) using a UV spectrophotometer. β-Galactosidase activity was calculated according to Griffith and Wolf (2002):

$$\beta - \textbf{Galactosidase activity} = \frac{((\textbf{OD})/\textbf{T min})\textbf{V}}{(\textbf{protein con.}(\textbf{v})/\textbf{0.0045}}$$

Where OD = optical density, T = time (min) and V = volume.

Data analysis

Survival data were analyzed using probit analysis to determine median lethal concentrations (LD_{50}) with IBM SPSS Statistics v22. Differences in survival rates between treatments were assessed using the Log-rank (Mantel–Cox) test for trend. Survival functions were compared based on pooled estimates of observed versus expected events. Statistical significance was set at $p < 0.05$. GraphPad Prism v8 software was used for graphical representation of survival curves.

RESULTS

Lethal concentration (LC_{50}) of plant extracts

Probit analysis was used to determine the lethal concentration (LC_{50}) required to induce 50% mortality in *Drosophila melanogaster* (Drs-LacZ strain) when exposed to extracts of *Allium sativum*, *Allium cepa* and *Zingiber officinale*. The results are summarized in Table 1.

Among the extracts, n-hexane fractions generally exhibited higher LC_{50} values, particularly for *A. cepa* (3240.32 mg/mL), suggesting relatively low toxicity. In contrast, the n-hexane fraction of *Z. officinale* had a much lower LC_{50} (706.36 mg/mL), indicating comparatively higher toxicity. Ethyl acetate fractions displayed intermediate toxicity levels, with *Z. officinale* again showing the lowest LC_{50} (1140.21 mg/mL). Methanol fractions had lower LC_{50} values across all plants, with *Z. officinale* showing the most toxic effect (947.08 mg/mL). Aqueous extracts produced the lowest LC_{50} values overall, particularly for *A. sativum* (855.77 mg/mL), suggesting that water-soluble bioactive compounds in garlic significantly contribute to fly mortality.

Chi-square values greater than 0.05 for most extracts confirmed a good fit for the probit model, indicating that observed mortality rates aligned well with expected values from the regression model.

Survival of Drs-LacZ flies treated with n-hexane fractions

The percentage survival of *D. melanogaster* (Drs-LacZ

Table 1. Probit analysis of the lethal concentration (LC_{50}) of the mortality rate of D*. melanogaster* Drs-LacZ (Drosomycin repeorter gene) on different plants extracts.

Extracts	Plants	Regression equation	Chi square (P > 0.05)	LC 50 (mg/ml)	Lower	Upper
	A. sativum	Y = 0.023*x + 3.70	0.158	1437.73	0.220	1.784
n-Hexane	*A. cepa*	Y = 0.018*x + 4.80	1.527	3240.32	-0.066	1.456
	Z. officinale	Y = 0.035*x + 1.50	0.653	706.36	0.739	2.361
	A. sativum	Y = 0.026*x + 3.00	0.704	1184.38	0.330	1.901
Ethyl acetate	*A. cepa*	Y = 0.017 *x+ 4.10	3.031	4826.56	-0.115	1.435
	Z. officinale	Y = 0.027*x + 1.50	0.087	1140.21	0.469	2.141
	A. sativum	Y = 0.021*x + 5.70	0.926	1321.86	0.152	1.660
Methanol	*A. cepa*	Y = 0.031*x + 0.30	0.606	983.03	0.619	2.320
	Z. officinale	Y = 0.029*x + 2.90	0.364	947.08	0.434	1.994
	A. sativum	Y = 0.035* x - 0.10	0.556	855.77	0.923	2.707
Aqueous	*A. cepa*	Y = 0.028*x – 1.00	0.523	1127.58	0.694	2.627
	Z. officinale	Y = 0.028* x +1.00	2.225	986.73	0.628	2.371

strain) infected with virulent *Aspergillus* species and treated with n-hexane fractions of *A. sativum* (ASH), *A. cepa* (ACH), and *Z. officinale* (ZOH) is presented in Figure 1.

Survival increased in a dose-dependent manner for all fractions. ZOH exhibited the highest survival rates across all fungal infections, reaching 64.44% at 500 mg/mL in *A. flavus* infected flies, 60% in *A. fumigatus*, and 51.11% in *A. niger*. ACH demonstrated moderate efficacy, while ASH had the lowest survival rates. Although all n-hexane fractions showed antifungal activity, their efficacy was lower than that of fluconazole (93.33%). The untreated negative control group (8.89%) confirmed the virulence of the fungal pathogens.

Survival of Drs-LacZ flies treated with ethyl acetate fractions

Treatment with ethyl acetate fractions of *A. sativum* (ASEA), *A. cepa* (ACEA), and *Z. officinale* (ZOEA) resulted in dose-dependent survival increase (Figure 2). ASEA showed the highest survival rates, reaching 68.89% at 500 mg/mL in *A. flavus* infected flies, 64.44% in *A. fumigatus* and 57.78% in *A. niger*. ZOEA demonstrated moderate activity, while ACEA had the lowest survival rates.

As with n-hexane fractions, the ethyl acetate fractions were less effective than fluconazole but significantly improved survival compared to the untreated control group (8.89%).

Survival of Drs-LacZ flies treated with methanol fractions

Methanol fractions of *A. sativum* (ASM), *A. cepa* (ACM), and *Z. officinale* (ZOM) produced the highest survival rates among all tested extracts (Figure 3). ZOM demonstrated the greatest efficacy, reaching 86.67% survival at 500 mg/mL in *A. flavus* infected flies, 64.44% in *A. fumigatus*, and 55.56% in *A. niger*. ACM showed intermediate effects, whereas ASM produced the lowest survival rates among the methanol extracts.

Although ZOM was highly effective, survival rates remained slightly lower than those observed with fluconazole (93.33%). The negative control group maintained significantly lower survival rates (8.89%), confirming the pathogenicity of the fungal strains.

Survival of Drs-LacZ flies treated with aqueous fractions

The aqueous fractions of *A. sativum* (ASAQ), *A. cepa* (ACAQ), and *Z. officinale* (ZOAQ) exhibited the weakest antifungal activity compared to the other solvent fractions (Figure 4). ACAQ showed the highest survival rates, reaching 51.11% at 500 mg/mL in *A. flavus* infected flies, 46.67% in *A. fumigatus*, and 40% in *A. niger*. ZOAQ produced the lowest survival rates, particularly in *A. niger* infected flies.

Overall, methanol fractions demonstrated the highest antifungal efficacy, followed by ethyl acetate fractions, n-hexane fractions, and aqueous fractions, in that order. While all plant fractions improved survival compared to the untreated control, none surpassed the standard antifungal drug fluconazole (93.33%).

β-Galactosidase activity

β-Galactosidase activity in Drs-LacZ flies treated with n-hexane fractions of *A. sativum* (ASH), *A. cepa* (ACH), and *Z. officinale* (ZOH) increased in a dose-dependent manner (Table 2). ZOH consistently exhibited the highest activity across all fungal infections, with peak values observed in *A. flavus* infected flies (1.03 ± 0.01 nmol/mL/protein at 500 mg/mL). ACH and ASH produced lower but still measurable enzyme activity.

Similar patterns were observed in *A. fumigatus* and *A. niger* infections, where ZOH displayed the strongest

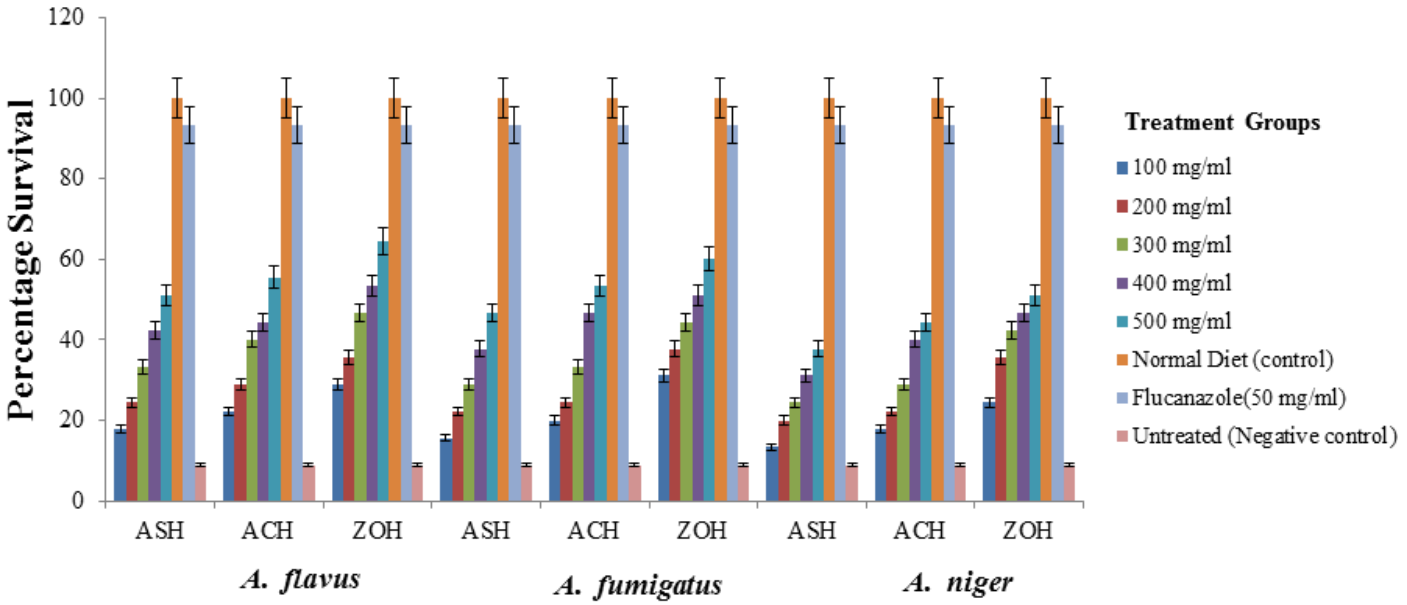

Figure 1. Percentage survival of fly strains Drs-LacZ (Drosomycin reporter gene) infected with virulent *Aspergillus* species and treated with the n-hexane fractions of *A. sativum*, *A. cepa*, and *Z. officinale.*

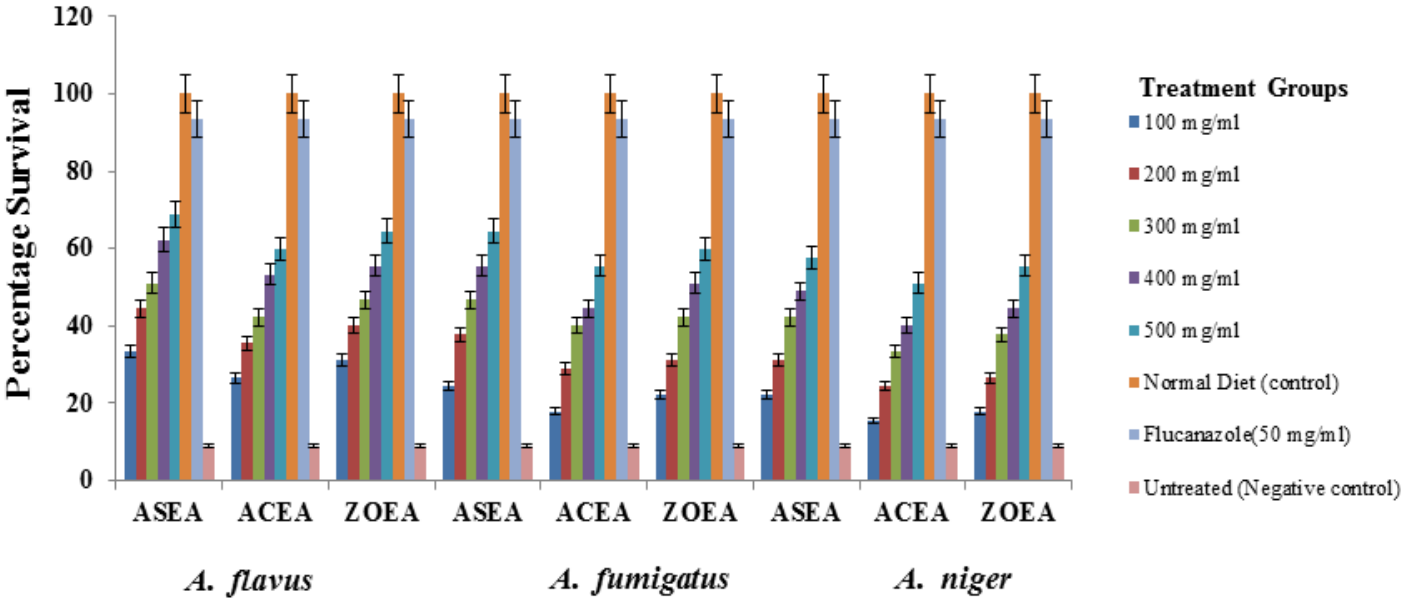

Figure 2: Percentage survival of fly strains Drs-LacZ (Drosomycin reporter gene) infected with virulent *Aspergillus* species and treated with the ethyl Acetate fractions of *A. sativum*, *A. cepa*, and *Z. officinale.*

immunomodulatory effect. Nevertheless, β-galactosidase activity in all treatment groups was significantly lower than in the fluconazole-treated group (4.84 ± 0.11 nmol/mL/protein) and normal diet control (5.25 ± 0.10 nmol/mL/ protein). The untreated negative control (0.14 ± 0.02 nmol/mL/ protein) had the lowest activity, confirming immunosuppression due to fungal infection.

β-Galactosidase activity (nmol/mL/protein) in Drs-LacZ *D. melanogaster* infected with *Aspergillus* spp. and treated with plant fractions

Ethyl acetate fractions

The β-galactosidase activity in *D. melanogaster* (Drs-

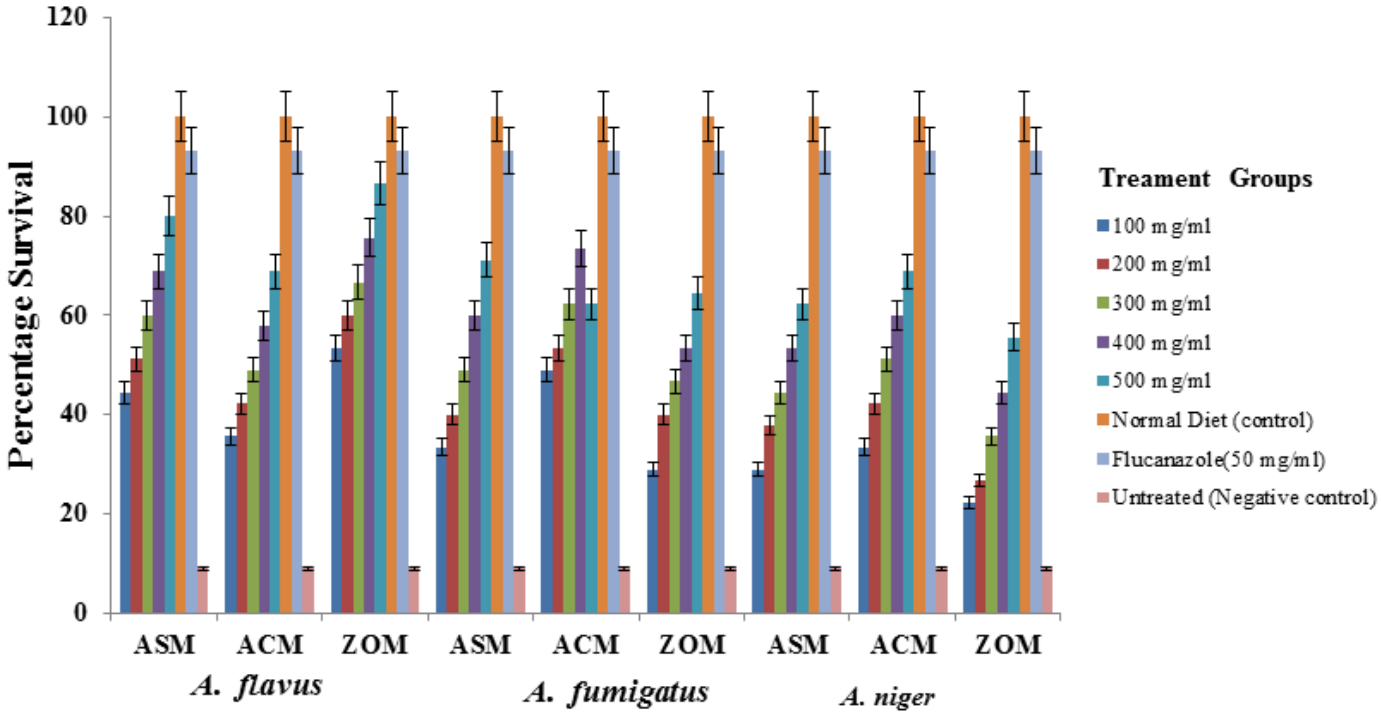

Figure 3: Percentage survival of fly strains Drs-LacZ (Drosomycin reporter gene) infected with virulent *Aspergillus* species and treated with the methanol fractions of *A. sativum*, *A. cepa*, and *Z. officinale.*

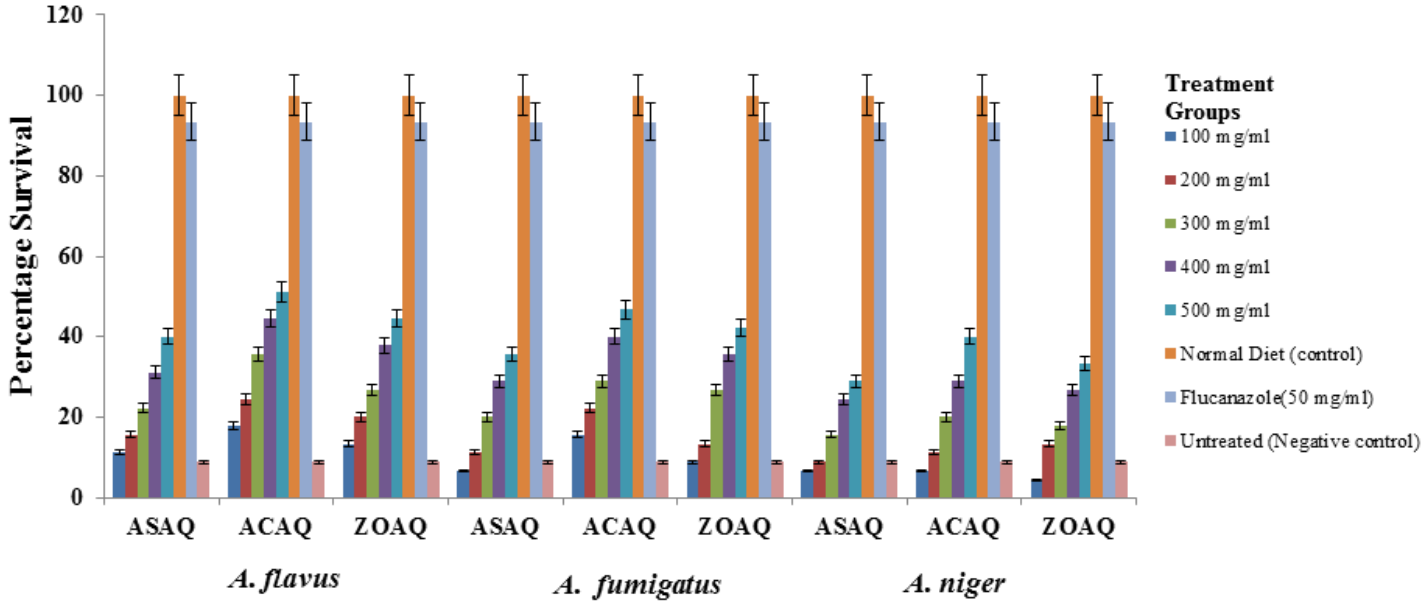

Figure 4: Percentage survival of fly strains Drs-LacZ (Drosomycin reporter gene) infected with virulent *Aspergillus* species and treated with the aqueous fractions of *A. sativum*, *A. cepa*, and *Z. officinale.*

LacZ strain) infected with *Aspergillus* spp. and treated with ethyl acetate fractions of *A. sativum* (ASEA), *A. cepa* (ACEA), and *Z. officinale* (ZOEA) exhibited a clear dose-dependent increase across all treatment groups (Table 3). Among the treatments, ASEA produced the highest β-galactosidase activity, particularly in *A. flavus* infected flies, reaching 1.96 ± 0.02 nmol/mL/protein at 500 mg/mL, followed by ZOEA (1.46 ± 0.03) and ACEA (1.10 ± 0.00). A similar trend was observed in *A. fumigatus*, where ASEA again showed the highest activity (1.57 ±

Table 2. β-Galactosidase activity (nmol/mL/protein) of fly strains Drs-LacZ (Drosomycin reporter gene) infected with virulent *Aspergillus* species and treated with the n-hexane fractions of *A. sativum*, *A. cepa*, and *Z. officinale*.

Treatment Groups	*A. flavus*			*A. fumigatus*			*A. niger*		
	ASH	ACH	ZOH	ASH	ACH	ZOH	ASH	ACH	ZOH
100 mg/ml	0.47±0.01	0.66±0.01	0.82±0.00	0.23±0.01	0.54±0.03	0.54±0.02	0.18±0.02	0.24±0.02	0.46±0.03
200 mg/ml	0.67±0.02	0.76±0.03	0.92±0.00	0.28±0.02	0.64±0.01	0.73±0.01	0.24±0.02	0.42±0.00	0.52±0.01
300 mg/ml	0.74±0.03	0.86±0.03	0.94±0.02	0.53±0.01	0.77±0.03	0.83±0.01	0.33±0.01	0.48±0.01	0.66±0.02
400 mg/ml	0.87±0.03	0.92±0.00	0.96±0.03	0.66±0.03	0.87±0.02	0.92±0.02	0.57±0.01	0.65±0.03	0.76±0.03
500 mg/ml	0.88±0.01	0.95±0.01	1.03±0.01	0.71±0.00	0.85±0.03	0.96±0.03	0.65±0.02	0.74±0.02	0.83±0.02
Normal Diet (control)	5.25±0.10	5.25±0.10	5.25±0.10	5.25±0.10	5.25±0.10	5.25±0.10	5.25±0.10	5.25±0.10	5.25±0.10
Flucanazole(50 mg/ml)	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11
Untreated (Negative control)	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02
P-value	≤0.0001								

Were ASH= *A. sativum* n-hexane fraction, ACEA= *A. cepa* n-hexane fraction, ZOEA= *Z. officinale* n-hexane fraction.

Table 3. β-Galactosidase activity (nmol/mL/protein) of fly strains Drs-LacZ (Drosomycin reporter gene) infected with virulent *Aspergillus* species and treated with the ethyl acetate fractions of *A. sativum*, *A. cepa*, and *Z. officinale.*

Treatment Groups	*A. flavus*			*A. fumigatus*			*A. niger*		
	ASEA	ACEA	ZOEA	ASEA	ACEA	ZOEA	ASEA	ACEA	ZOEA
100 mg/ml	0.96±0.02	0.43±0.01	0.55±0.03	0.45±0.01	0.33±0.03	0.37±0.02	0.63±0.02	0.33±0.02	0.42±0.01
200 mg/ml	1.26±0.02	0.67±0.01	0.76±0.03	0.65±0.04	0.53±0.01	0.66±0.03	0.84±0.02	0.35±0.02	0.38±0.23
300 mg/ml	1.36±0.03	0.88±0.02	0.95±0.02	0.83±0.01	0.77±0.03	0.77±0.03	0.97±0.02	0.45±0.01	0.58±0.01
400 mg/ml	1.56±0.03	0.94±0.01	1.1±0.00	1.05±0.02	0.84±0.02	1.02±0.01	1.02±0.00	0.56±0.03	0.77±0.02
500 mg/ml	1.96±0.02	1.1±0.00	1.46±0.03	1.57±0.06	0.87±0.02	1.14±0.01	1.14±0.01	0.74±0.01	0.84±0.02
Normal Diet (control)	5.25±0.10	5.25±0.10	5.25±0.10	5.25±0.10	5.25±0.10	5.25±0.10	5.25±0.10	5.25±0.10	5.25±0.10
Flucanazole(50 mg/ml)	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11
Untreated (Negative control)	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02
P-value	≤0.0001								

Were ASEA= *A.sativum* ethyl acetate fraction, ACEA= *A. cepa* ethyl acetate fraction, ZOEA= *Z. officinale* ethyl acetate fraction.

0.06), followed by ZOEA (1.14 ± 0.01) and ACEA (0.87 ± 0.02). In *A. niger* infected flies, ASEA maintained the highest activity (1.14 ± 0.01), with ZOEA (0.84 ± 0.02) and ACEA (0.74 ± 0.01) showing lower levels. Despite these improvements, the normal diet control (5.25 ± 0.10) and fluconazole-treated group (4.84 ± 0.11) exhibited significantly higher enzyme activities, indicating that ethyl acetate fractions provide moderate immune activation. The untreated negative control (0.14 ± 0.02) confirmed the immunosuppressive effect of fungal infection.

Methanol fractions

Treatment with methanol fractions of *A. sativum* (ASM), *A. cepa* (ACM), and *Z. officinale* (ZOM) produced a pronounced, dose-dependent increase in β-galactosidase activity across all infection models (Table 4). ZOM exhibited the highest activity in *A. flavus* infected flies (3.58 ± 0.02), followed by ASM (2.92 ± 0.07) and ACM (2.26 ± 0.02). Interestingly, in *A. fumigatus* infected flies, ACM had the highest enzyme activity (3.17 ± 0.06), followed by ASM (1.82 ±

Table 4. β-Galactosidase activity (nmol/mL/protein) of fly strains Drs-LacZ (Drosomycin reporter gene) infected with virulent *Aspergillus* species and treated with the methanol fractions of *A. sativum*, *A. cepa*, and *Z. officinale.*

Treatment Groups	*A. flavus*			*A. fumigatus*			*A. niger*		
	ASM	ACM	ZOM	ASM	ACM	ZOM	ASM	ACM	ZOM
100 mg/ml	1.96±0.01	1.45±0.02	2.16±0.03	0.82±0	1.46±0.06	0.54±0.01	0.34±0.01	0.99±0.01	0.45±0.02
200 mg/ml	2.04±0.01	1.77±0.05	2.69±0.04	0.97±0.01	1.87±0.02	0.74±0.02	0.55±0.02	1.44±0.02	0.58±0.01
300 mg/ml	2.16±0.05	1.87±0.03	2.85±0.02	1.13±0.02	2.32±0.02	0.97±0.01	0.64±0.01	1.77±0.02	0.63±0.01
400 mg/ml	2.62±0.15	2.05±0.01	3.26±0.02	1.44±0.02	2.82±0.05	1.11±0	0.79±0	2.07±0.02	0.68±0.01
500 mg/ml	2.92±0.07	2.26±0.02	3.58±0.02	1.82±0.1	3.17±0.06	1.44±0.02	0.87±0.01	2.35±0.01	0.72±0.01
Normal Diet (control)	5.25±0.10	5.25±0.1	5.25±0.1	5.25±0.1	5.25±0.1	5.25±0.1	5.25±0.1	5.25±0.1	5.25±0.1
Flucanazole (50 mg/ml)	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11
Untreated (Negative control)	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02
P-value	≤0.0001								

Were ASM= *A.sativum* methanol fraction, ACM= *A. cepa* methanol fraction, ZOM= *Z. officinale* methanol fraction.

0.10) and ZOM (1.44 ± 0.02). In *A. niger*, ACM again showed the highest activity (2.35 ± 0.01), while ASM (0.87 ± 0.01) and ZOM (0.72 ± 0.01) displayed lower responses. As observed with other fractions, the normal diet (5.25 ± 0.10) and fluconazole (4.84 ± 0.11) groups exhibited significantly higher enzyme activity than all plant treatments, indicating stronger immune activation. The untreated negative control had the lowest activity (0.14 ± 0.02).

Aqueous fractions

Aqueous fractions of *A. sativum* (ASAQ), *A. cepa* (ACAQ), and *Z. officinale* (ZOAQ) produced a modest but consistent dose-dependent increase in β-galactosidase activity across all infections (Table 5). ACAQ exhibited the highest activity across fungal species, reaching 0.83 ± 0.02 in *A. flavus*, 0.75 ± 0.01 in *A. fumigatus*, and 0.63 ± 0.01 in *A. niger*.

Table 5. β-Galactosidase activity (nmol/mL/protein) of fly strains Drs-LacZ (Drosomycin reporter gene) infected with virulent *Aspergillus* species and treated with the aqueous fractions of *A. sativum*, *A. cepa*, and *Z. officinale.*

Treatments Groups	*A. flavus*			*A. fumigatus*			*A. niger*		
	ASAQ	ACAQ	ZOAQ	ASAQ	ACAQ	ZOAQ	ASAQ	ACAQ	ZOAQ
100 mg/ml	0.28±0.01	0.36±0.01	0.34±0.02	0.23±0	0.27±0.01	0.25±0.01	0.17±0	0.27±0	0.22±0
200 mg/ml	0.35±0.03	0.45±0.02	0.36±0.01	0.28±0.01	0.31±0	0.29±0	0.23±0	0.34±0.01	0.27±0
300 mg/ml	0.55±0.02	0.68±0.01	0.66±0.03	0.44±0.01	0.55±0.01	0.53±0.01	0.27±0	0.51±0	0.32±0
400 mg/ml	0.66±0.03	0.73±0.01	0.67±0.03	0.54±0.02	0.71±0	0.57±0	0.33±0.01	0.55±0.02	0.43±0.02
500 mg/ml	0.76±0.02	0.83±0.02	0.75±0.03	0.55±0.03	0.75±0.01	0.65±0.02	0.43±0.01	0.63±0.01	0.58±0.01
Normal Diet (control)	5.25±0.1	5.25±0.1	5.25±0.1	5.25±0.1	5.25±0.1	5.25±0.1	5.25±0.1	5.25±0.1	5.25±0.1
Flucanazole(50 mg/ml)	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11	4.84±0.11
Untreated (Negative control)	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02	0.14±0.02
P-value	≤0.0001								

Were ASAQ= *A. sativum* aqueous fraction, ACAQ= *A. cepa* aqueous fraction, ZOAQ= *Z. officinale* aqueous fraction.

ASAQ and ZOAQ produced slightly lower activities, with ZOAQ performing marginally better than ASAQ in *A. fumigatus* and *A. niger* infections. Compared to controls, aqueous fractions showed the weakest immune activation, as the normal diet (5.25 ± 0.10) and fluconazole (4.84 ± 0.11) produced significantly higher β-galactosidase levels. The untreated negative control remained the lowest (0.14 ± 0.02), confirming fungal immunosuppression.

Methanol fractions produced the highest β-galactosidase activity, particularly ZOM in *A. flavus* infections. Ethyl acetate fractions showed moderate immune activation, with ASEA being the most effective across fungal species. Aqueous fractions exhibited the lowest β-galactosidase activity, suggesting weaker immunomodulatory effects compared to organic solvent fractions. All plant fractions improved immune activity relative to untreated controls but remained less potent than fluconazole or normal diet controls.

DISCUSSION

The antifungal potential of n-hexane, ethyl acetate, methanol, and aqueous fractions of *A. sativum*, *A. cepa*, and *Z. officinale* in enhancing the survival of *D. melanogaster* (Drs-LacZ strain) infected with virulent *Aspergillus* species aligns with previous research emphasizing the efficacy of plant-derived bioactive compounds against fungal pathogens. The superior efficacy of the methanol fraction particularly that of *Z. officinale*, corroborates earlier findings that methanolic extracts of medicinal plants possess enhanced antimicrobial properties due to their ability to extract a broad range of polar bioactive metabolites, including flavonoids and phenolic acids (Bekkouch et al., 2019; Aji et al., 2022).

Likewise, the notable antifungal effects of the ethyl acetate fractions especially from *A. sativum* are consistent with Kalaba et al. (2024), who reported that ethyl acetate extracts of garlic exert strong inhibitory effects against *Aspergillus* spp. due to the presence of organosulfur compounds such as allicin. Although the n-hexane fractions displayed relatively lower efficacy, *Z. officinale* still exhibited the highest survival rates among them, which supports the findings of Nweze et al. (2019), who demonstrated that non-polar extracts retain antifungal properties attributable to lipid-soluble secondary metabolites. In contrast, the relatively weak antifungal activity observed in aqueous fractions may be explained by the lower solubility and extractability of key antifungal metabolites in water (Bergman et al., 2001). Collectively, these results reaffirm the superior efficacy of fluconazole as a standard antifungal treatment (Samadi et al., 2019), but they also highlight the potential of plant-derived extracts as complementary therapeutic agents.

The β-galactosidase activity trends provide further insight into immune activation. Among all solvent fractions, n-hexane fractions elicited the lowest β-galactosidase activity, indicating moderate immune stimulation. This observation aligns with the findings of Nawaz et al. (2020), who reported that non-polar solvent extracts typically contain fewer immunostimulatory compounds. Interestingly, *Z. officinale* fractions consistently induced higher β-galactosidase activity than those from *A. sativum* and *A. cepa*, in agreement with Talib et al. (2022), who highlighted the potent immunomodulatory effects of gingerol and shogaol.

In contrast, the ethyl acetate and methanol fractions demonstrated substantially higher β-galactosidase activity, indicating a stronger immune response. The ethyl acetate fraction of *A. sativum* recorded the highest β-galactosidase activity, consistent with Kutawa et al. (2018), who reported that ethyl acetate extracts of garlic and onion are rich in organosulfur compounds, flavonoids, and polyphenols with potent immunomodulatory properties. Similarly, the methanol fractions particularly those of *Z. officinale* and *A. cepa* elicited robust immune activation, consistent with the work of Bashir et al. (2015), who reported that methanol extracts of ginger and onion contain high levels of phenolic acids and flavonoids known to enhance host immunity. The aqueous fractions, while still inducing some immune response, were the least effective, confirming earlier reports that water extracts generally yield lower concentrations of bioactive metabolites (Wong et al., 2006).

Taken together, these findings demonstrate that both antifungal activity and immune stimulation in *D. melanogaster* are strongly influenced by the solvent used for extraction and the specific plant source.

CONCLUSION

This study confirms the antifungal and immunomodulatory potential of *A. sativum*, *A. cepa*, and *Z. officinale* extracts against *Aspergillus* infections in *D. melanogaster* (Drs-LacZ strain). Methanol and ethyl acetate fractions were the most effective in enhancing fly survival and β-galactosidase activity, indicating strong antifungal and immune-boosting effects. Conversely, n-hexane and aqueous fractions showed comparatively weaker activity.

These findings suggest that bioactive compounds present in methanol and ethyl acetate fractions such as phenolic acids, flavonoids, and organosulfur compounds may play a significant role in modulating host immunity and suppressing fungal growth. Future studies should focus on isolating, characterizing, and testing these active metabolites to elucidate their mechanisms of action and assess their potential as alternative or complementary antifungal therapies.

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