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Investigating the effects of *Allium sativum* (garlic), *Allium cepa* (onions) and *Zingiber officinale* (ginger) extracts on *Drosophila melanogaster* drosomycin and diptericin reporter gene strain

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ABSTRACT

The growing interest in natural products as potential therapeutic agents has drawn attention to commonly used plants such as *Allium sativum* (garlic), *Allium cepa* (onion), and *Zingiber officinale* (ginger), which are rich in bioactive compounds. However, despite their widespread use in traditional medicine, the safety and toxicity profiles of these plants remain poorly understood. *Drosophila melanogaster*, a widely used model organism in biomedical research, provides a cost-effective and efficient system to evaluate the toxicity and bioactivity of plant extracts. This study aims to assess the acute toxicity and bioactivity of *A. sativum*, *A. cepa*, and *Z. officinale* using *D. melanogaster* as a model organism. The plant extracts were prepared using the maceration method with solvents of varying polarity, n-Hexane, ethyl acetate, methanol, and water. Phytochemical screening was performed using standard methods. Acute toxicity was assessed by monitoring mortality rates at various extract concentrations (100–500 mg/mL), and statistical analysis was conducted to evaluate model fit. Toxicity was determined through probit analysis to calculate the lethal concentration (LC_{50}) required to cause 50% mortality in *Drosophila melanogaster* strains (drosomycin and diptericin). Phytochemical screening revealed that methanol extracts had the highest diversity of compounds, including alkaloids, flavonoids, and carbohydrates. Ethyl acetate extracts showed moderate levels of flavonoids and steroids, while aqueous extracts were limited to alkaloids and flavonoids. n-Hexane extracts demonstrated the lowest phytochemical diversity, containing only steroids. Probit analysis of LC_{50} values indicated that methanol and aqueous extracts exhibited lower LC_{50} values, signifying higher toxicity. Specifically, the aqueous extract of *A. sativum* had the lowest LC_{50} (762.24 mg/mL), while hexane extracts had the highest LC_{50} values, reflecting lower toxicity. Mortality rates increased with higher extract concentrations, with methanol and aqueous extracts consistently causing greater mortality in both *Drosophila* strains. The higher toxicity of methanol and aqueous extracts was attributed to their efficient extraction of potent bioactive compounds. These findings underscore the significance of solvent selection in phytochemical studies and highlight the potential of these plants as sources of bioactive agents for pharmacological applications.

Keywords: Acute toxicity, lethal concentration, mortality rate, phytochemicals, reporter genes.

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INTRODUCTION

The use of medicinal plants for health benefits has gained significant interest due to their rich phytochemical content and diverse biological activities. Among these plants, *Allium sativum* (garlic), *Allium cepa* (onion), and *Zingiber officinale* (ginger) are widely recognized for their traditional uses and therapeutic potential. These plants are rich sources of bioactive compounds, such as organosulfur compounds in garlic, flavonoids in onions,

and gingerols in ginger, all of which possess antioxidant, anti-inflammatory, and antimicrobial properties (Rahman and Lowe, 2018; Oyawoye et al., 2022). Research has demonstrated that these phytochemicals contribute to various health-promoting effects, supporting their use as natural alternatives for disease prevention and treatment (Singla and Kaur, 2018).

The use of plants with therapeutic properties to treat pathological conditions is a common practice. However, this practice may occasionally lead to intoxication due to the complex array of components in plants, including compounds with toxic properties (Maag et al., 2015). Plants are constantly exposed to environmental stresses, such as predators and pathogens. To survive under these unfavorable conditions, they have developed adaptations, including the synthesis of bioactive compounds as defense mechanisms. Some of these compounds, such as alkaloids, terpenoids, tannins, and glycosides, are potentially toxic (Dang et al., 2015; Maag et al., 2015).

Understanding the safety profile of these plants requires rigorous evaluation of their potential toxic effects, particularly at higher doses or during chronic exposure. While garlic, onion, and ginger are generally considered safe for human consumption, there is evidence that certain compounds may exert cytotoxic or genotoxic effects at specific concentrations (Guldiken et al., 2018). Assessing their toxicity is crucial to establishing safe consumption levels, especially since these plants are commonly used in medicinal preparations. Acute toxicity studies, which evaluate the immediate adverse effects of a substance, are a foundational step in toxicity assessment for human health applications (Pognan et al., 2023).

Drosophila melanogaster, the common fruit fly, is an increasingly popular model organism for toxicity studies due to its genetic similarities to humans, short life cycle, and ease of maintenance in laboratory settings (Pandey and Nichols, 2011). Research using *Drosophila* as a model to study the toxic effects of natural products such as those derived from garlic, onion, and ginger, has demonstrated the model's ability to reveal crucial toxicological and pharmacological data. The fruit fly shares approximately 75% of disease-related genes with humans, making it an ideal model for understanding how certain plant-based compounds might impact human health (Himalian et al., 2022; Yang et al., 2019).

Additionally, *Drosophila* serves as an excellent model for studying both acute toxicity and the bioactivity of plant-derived compounds. It enables the assessment of immediate adverse effects as well as long-term health impacts (Kushalan et al., 2022). Recent studies have utilized *Drosophila melanogaster* to investigate the effects of various natural products on survival, reproductive capacity, and biochemical pathways. These studies have helped establish safety profiles and therapeutic potential (Bellen et al., 2019). This model has proven effective in assessing toxicity mechanisms that may be relevant to human physiology, offering valuable data for health management (Calap-Quintana et al., 2017).

This study aims to provide a comprehensive assessment of the safety profiles of these commonly consumed plants, contributing to a deeper understanding of their bioactivity and toxicity, with applications in human health management.

MATERIALS AND METHODS

Study area

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

Collection of plant parts

A total of 100 kg of fresh onions, garlic, and ginger were purchased from the Farin Gada Market in Jos North Local Government Area, Plateau State, Nigeria.

Extraction of plant parts

Plant extracts were prepared using the maceration method, based on solvent polarity. The solvents used included n-hexane, ethyl acetate, and distilled water, following the method described by Puri (1998).

Phytochemical screening

The plant extracts were screened for their phytochemical constituents to determine the presence of alkaloids, saponins, tannins, flavonoids, carbohydrates, steroids, anthraquinones, cardiac glycosides and terpenoids. This was done using standard phytochemical screening procedures as described by Sofowora (2008) and Trease and Evans (2002).

Test for alkaloids

The test for alkaloids was conducted according to the method reported by Trease and Evans (2002). A 0.5 g sample of each extract was stirred with 5 ml of 1% aqueous HCl on a steam bath. The mixture was then filtered using Whatman filter paper No. 42 (125 mm). Next, 1 ml of the filtrate was treated with 2–3 drops of Mayer’s reagent. Another 1 ml of the filtrate was treated

with Dragendorff's reagent. The formation of turbidity or precipitation with either of these reagents was taken as evidence of the presence of alkaloids.

Test for saponins

The test was performed according to the method described by Trease and Evans (2002). A total of 0.5 g of different fractions was dissolved in 25 mL of distilled water and then filtered using Whatman filter paper No. 42 (125 mm). An additional 10 mL of distilled water was added, and the mixture was shaken vigorously to produce a stable, persistent froth. The froth was collected, mixed with three drops of olive oil, and shaken. The formation of an emulsion indicated the presence of saponins.

Test for tannins

The test was conducted following the method described by Edeoga et al. (2005). A quantity of 0.5 g of the fractions was stirred with 10 mL of distilled water and filtered using Whatman filter paper No. 42 (125 mm). To the filtrate, a solution of ferric chloride was added. The appearance of a blue-black, green, or blue-green precipitate indicated the presence of tannins.

Test for anthraquinones

The Borntrager's test, as described by Sofowora (2008), was used to detect anthraquinones. A total of 0.5 g of each fraction was placed into a dry test tube, and 5 mL of chloroform was added. The mixture was shaken for 5 minutes and then filtered. The filtrate was shaken with an equal volume of 100% ammonia solution. The presence of a pink, violet, or red color in the ammoniacal layer (lower layer) indicated the presence of free anthraquinones.

Test for cardiac glycosides

The test was performed according to the method reported by Sofowora (2008). A total of 100 mg of the extracts was dissolved in 70% alcohol and filtered. Approximately three drops of lead subacetate were added to the filtrate, which was then filtered again. The resulting filtrate was extracted with 10 mL of chloroform in a separating funnel and concentrated to dryness. The residue obtained was dissolved in 1 mL of glacial acetic acid containing one drop of ferric chloride solution. This mixture was underlaid with 1 mL of concentrated sulfuric acid. The formation of a brown ring at the interface indicated the presence of a deoxysugar, characteristic of cardenolides.

Test for steroids and terpenes

The test was performed following the method described by Trease and Evans (2002). One hundred milligrams of each extract was dissolved in chloroform, followed by the addition of 1 mL of acetic anhydride. Two drops of concentrated sulfuric acid were then added. The appearance of a pink color, which changes to bluish-green upon standing, indicates the presence of steroids and terpenes.

Test for flavonoids

The test was performed using the method described by Sofowora (2008). A total of 0.5 g of the extract was dissolved in 30 mL of distilled water, stirred, and filtered using Whatman filter paper number 42 (125 mm). To 10 mL of the filtrate, 5 mL of 1 M dilute ammonia solution was added, followed by 10 mL of concentrated sulfuric acid. The formation of a yellow precipitate, which disappears upon standing, is indicative of the presence of flavonoids. Additionally, 5 mL of dilute ammonia was mixed with 5 mL of the extract, and 5 mL of concentrated sulfuric acid was added. The development of a yellow color confirms the presence of flavonoids.

Test for carbohydrates

The test was conducted according to the method reported by Sofowora (2008). A total of 0.5 g of the extract was dissolved in 30 mL of distilled water and filtered using Whatman filter paper number 42 (125 mm). A few drops of Molisch's reagent were added to the filtrate, followed by the careful addition of 1 mL of concentrated sulfuric acid down the side of the inclined test tube. This procedure allowed the acid to form a distinct layer beneath the aqueous solution without mixing. The appearance of a reddish or violet ring at the interface of the two layers indicates the presence of carbohydrates.

Drosophila melanogaster fly stock selection

The diptericin (Dpt-LacZ) and drosomycin (Drs-LacZ) reporter gene strains of *Drosophila melanogaster* were obtained from the National Species Stock Center in Switzerland. The flies were maintained and reared on a cornmeal medium at a temperature of 23 ± 1°C and 60% relative humidity under a 12-hour dark/light cycle. All experiments were conducted using the same *D. melanogaster* strains (Dpt-LacZ and Drs-LacZ) provided by the Bruno Lemaitre Research Group, EPL-SV-GHI, UPLEM, Lausanne, Switzerland, as described by Abolaji et al. (2014) and Wuyep et al. (2020).

Preparation of fly food and plant extracts for acute toxicity testing

The acute toxicity testing followed the methods described by Abolaji et al. (2014) and Wuyep et al. (2020). Fly food was prepared at varying concentrations of 500 mg/mL, 400 mg/mL, 300 mg/mL, 200 mg/mL, and 100 mg/mL. A flame-sterilized spatula was used to make superficial abrasions on the surface of the food. For each concentration, 15 unsexed flies were placed in triplicate vials, resulting in a total of 45 flies per concentration. Survival was recorded daily over a seven-day period, and mortality data were collected.

Data analysis

The survival rates of the flies at different concentrations were analyzed using probit analysis to determine the lethal concentration (LD50) values. This analysis was performed using SPSS software (version 22). Percentage mortality rates were calculated and presented in graphical format.

RESULTS

Phytochemical screening of plant extracts

The phytochemical screening of extracts from *Allium sativum* (garlic), *Allium cepa* (onion), and *Zingiber officinale* (ginger) revealed distinct patterns of compound presence across various solvents, suggesting differences in solubility and extraction efficiency (Table 1). Methanol extracts (ASM, ACM, ZOM) exhibited the greatest diversity of phytochemicals, with a strong presence of alkaloids, flavonoids, and carbohydrates, underscoring methanol's ability to dissolve a broad spectrum of compounds. Ethyl acetate fractions (ASEA, ACEA, ZOEA) also contained notable phytochemical constituents, showing moderate to high levels of flavonoids and steroids but relatively fewer alkaloids and tannins, indicating a preference for less polar compounds.

Aqueous extracts (ASAQ, ACAQ, ZOAQ) displayed limited phytochemical diversity, primarily consisting of alkaloids, flavonoids, and cardiac glycosides, reflecting water's selectivity for polar compounds. Conversely, hexane extracts (ASH, ACH, ZOH) showed minimal phytochemical presence, with steroids being the only consistently detected compounds. This suggests hexane's limited efficacy in extracting polar bioactive compounds.

Probit analysis of lethal concentration (LC_{50}) of the extracts

Probit analysis was used to determine the lethal concentration (LC_{50}) values of various extracts from *A. sativum*, *A. cepa*, and *Z. officinale* for the mortality rate of the *Drosophila melanogaster* Dpt-LacZ strain. The LC_{50} values represent the concentration required to achieve 50% mortality in the fly strain. Methanol and aqueous extracts demonstrated greater toxicity, with lower LC_{50} values compared to hexane and ethyl acetate extracts (Table 2).

Among the aqueous extracts, *A. sativum* had the lowest LC_{50} value (762.24 mg/mL), indicating the highest toxicity, whereas hexane extracts generally showed the highest LC_{50} values, reflecting their lower toxicity. The regression equations for each extract demonstrated a positive linear relationship between concentration and mortality rate, with the slopes indicating the strength of the concentration-dependent toxic effect.

Additionally, chi-square values for all extracts were above 0.05, confirming that the observed mortality rates were statistically consistent with the model's predictions. Methanol extracts of *Z. officinale* and *A. cepa* showed relatively low LC_{50} values (1065.64 mg/mL and 1177.17 mg/mL, respectively), highlighting methanol's effectiveness in extracting bioactive compounds responsible for higher toxicity.

The probit analysis of the lethal concentration (LC_{50}) required inducing 50% mortality in *Drosophila melanogaster* Drs-LacZ strain (reporting the drosomycin gene) when exposed to various extracts of *Allium sativum*, *Allium cepa*, and *Zingiber officinale* is presented in Table 3. Among the extracts, n-hexane extracts generally exhibited higher LC_{50} values, particularly for *A. cepa* (3240.32 mg/mL), suggesting relatively low toxicity. Conversely, the n-hexane extract of *Z. officinale* displayed a much lower LC_{50} value (706.36 mg/mL), indicating higher toxicity compared to other n-hexane extracts.

Ethyl acetate extracts demonstrated intermediate toxicity levels, with *Z. officinale* again showing lower LC_{50} values (1140.21 mg/mL) compared to *A. sativum* and *A. cepa*. Methanol extracts exhibited the highest toxicity, with LC_{50} values as low as 947.08 mg/mL for *Z. officinale*, highlighting methanol's efficiency in extracting toxic compounds from this plant. Aqueous extracts showed the lowest LC_{50} values, particularly for *A. sativum* (855.77 mg/mL), indicating higher toxicity and suggesting that water-soluble compounds in *A. sativum* contribute significantly to mortality in Drs-LacZ flies.

Chi-square values greater than 0.05 for most extracts indicate a good model fit, suggesting that the observed mortality rates align well with the expected values predicted by the regression model.

Percentage mortality of fly strains in acute toxicity testing

The acute toxicity testing of *A. sativum*, *A. cepa*, and *Z. officinale* extracts in *Drosophila melanogaster* strains

Table 1. Phytochemical screening of plants extracts.

Phytochemicals	Plants extracts											
	ASH	ACH	ZOH	ASEA	ACEA	ZOEA	ASM	ACM	ZOM	ASAQ	ACAQ	ZOAQ
Alkaloids	-	-	-	+	-	+	+++	+++	+++	+	+	+
Saponins	-	-	-	-	-	-	-	+	-	-	-	+
Tannins	-	++	-	-	++	-	-	+	-	-	+	-
Flavonoids	-	+	-	+	+	+	++	++	+++	-	+	++
Carbohydrates	+	+++	+	+++	+	+	++	+++	+++	-	-	-
Steroids	++	++	+++	++	++	+++	+	+	+	-	-	-
Terpenes	-	-	-	-	-	-	-	-	+	-	-	-
Anthraquinones	-	-	-	-	-	++	-	-	-	-	-	+
Cardiac glyscosides	++	+	++	-	-	++	-	-	++	-	-	+

Key: ASH – *A. sativum* (Hexane), **ACH** – *A. cepa* (Hexane), **ZOH** – *Z. officinale* (Hexane), **ASEA** – *A. sativum* (Ethylacetate), **ACEA** – *A. cepa* (Ethylacetate), **ZOEA** – *Z. officinale* (Ethylacetate), **ASM** – *A. sativum* (Methanol), **ACM** – *A. cepa* (Methanol), **ZOM** – *Z. officinale* (Methanol), **ASAQ** – *A. sativum* (Aqueous), **ACAQ** – *A. cepa* (Aqueous), **ZOAQ** – *Z. officinale* (Aqueous).

Table 2. Probit analysis of the lethal concentration (LC_{50}) of the mortality rate of *D. melanogaster* Dpt-LacZ (diptericin repeorter gene) on different Plants extracts.

Extracts	Plants	Regression equation	Chi square (P > 0.05)	LC_{50} (mg/mL)	Lower	Upper
n-Hexane	*A. sativum*	Y = 0.027*x + 2.10	1.628	1287.30	0.328	1.917
	A. cepa	Y = 0.025*x + 2.70	0.112	1264.29	0.334	1.940
	Z. officinale	Y = 0.018*x + 4.00	0.602	2778.96	0.014	1.594
Ethyl acetate	*A. sativum*	Y = 0.021*x + 4.90	0.083	1464.78	0.159	1.698
	A. cepa	Y = 0.020*x + 5.00	0.141	1837.19	0.072	1.595
	Z. officinale	Y = 0.024*x + 3.60	0.394	1355.90	0.239	1.797
Methanol	*A. sativum*	Y = 0.020*x + 5.40	0.562	1936.16	0.027	1.526
	A. cepa	Y = 0.027*x + 1.10	0.394	1177.17	0.482	2.177
	Z. officinale	Y = 0.028*x + 1.40	0.065	1065.64	0.511	2.182
Aqueous	*A. sativum*	Y = 0.035*x - 0.30	1.357	762.24	0.885	2.647
	A. cepa	Y = 0.017*x + 0.50	0.038	2573.69	0.223	2.196
	Z. officinale	Y = 0.018*x + 2.40	0.425	2189.69	0.183	1.921

(Dpt-LacZ and Drs-LacZ) revealed a clear trend of increasing mortality with increasing extract concentrations across all plant extracts. Figure 1 summarizes the overall mortality percentages for both strains over seven days, indicating that methanol and aqueous extracts consistently exhibited higher toxicity compared to ethyl acetate and n-hexane extracts.

At lower concentrations (100 mg/mL), as shown in Figure 2, methanol extracts were the most toxic to both strains, followed by ethyl acetate and n-hexane extracts, with aqueous extracts demonstrating the lowest toxicity. This trend remained generally consistent across concentrations, with methanol extracts causing the highest mortality rates at every concentration level (Figures 3 to 5). By 500 mg/mL (Figure 6), both methanol and aqueous extracts of *A. sativum* and *A. cepa* exhibited near-maximal toxicity, resulting in the highest mortality rates in both fly strains.

Ethyl acetate and n-hexane extracts consistently showed the lowest toxicity, suggesting a possible correlation between solvent

Table 3. 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
n-Hexane	*A. sativum*	Y = 0.023*x + 3.70	0.158	1437.73	0.220	1.784
	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
Ethyl acetate	*A. sativum*	Y = 0.026*x + 3.00	0.704	1184.38	0.330	1.901
	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
Methanol	*A. sativum*	Y = 0.021*x + 5.70	0.926	1321.86	0.152	1.660
	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
Aqueous	*A. sativum*	Y = 0.035*x - 0.10	0.556	855.77	0.923	2.707
	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

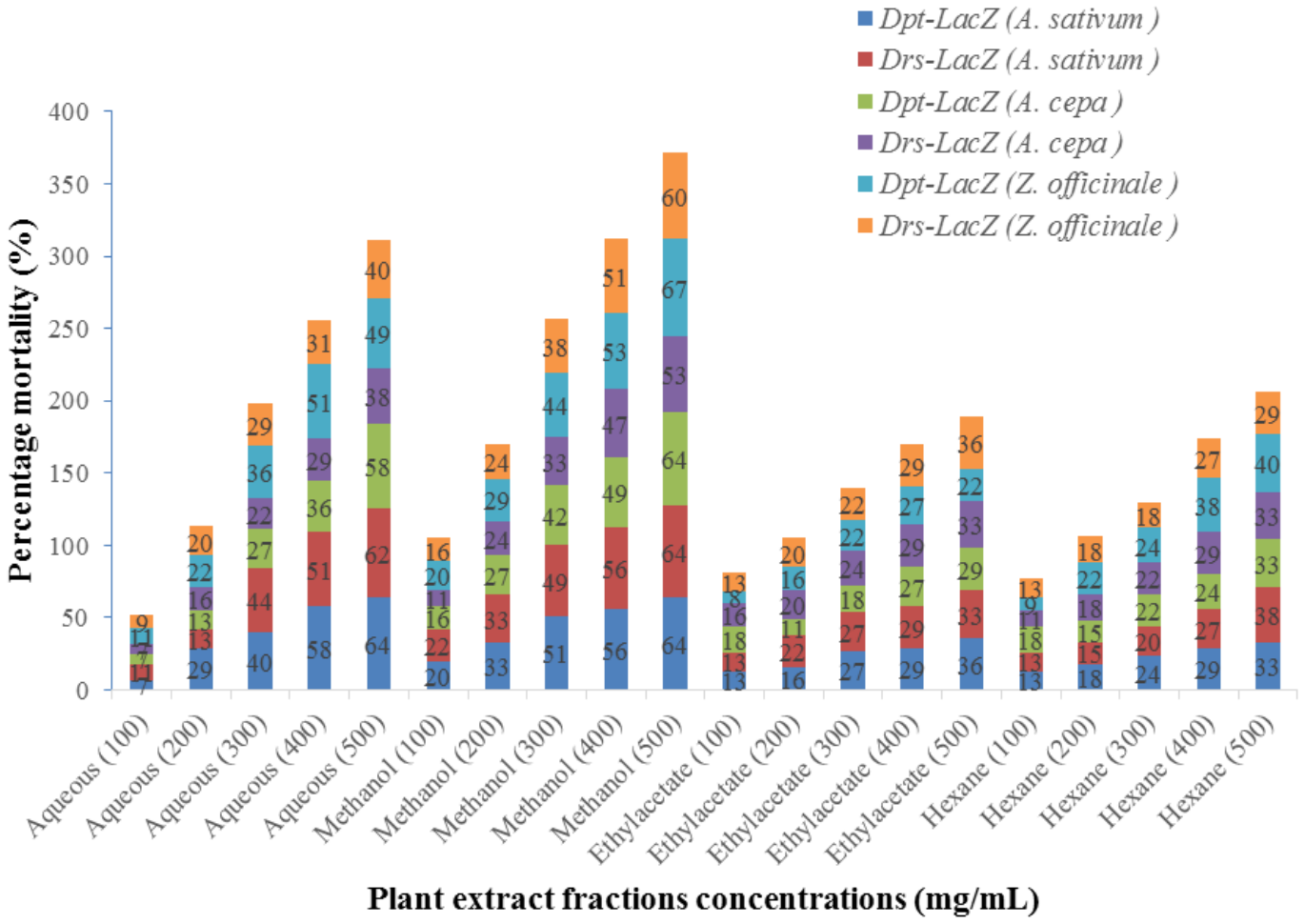

Figure 1. Stacked column bar chart presenting the entire percentage mortality (%) of Dpt-LacZ and Drs-LacZ Fly Strains when subjected to treatment concentrations of plants extracts for 7 days.

polarity and the efficacy of extracting bioactive, toxic compounds. The findings indicate that methanol and aqueous solvents are more effective at extracting highly toxic bioactive compounds, particularly in *A. sativum* and *A. cepa*. These results provide valuable insights into the comparative potency of plant extracts in toxicity studies.

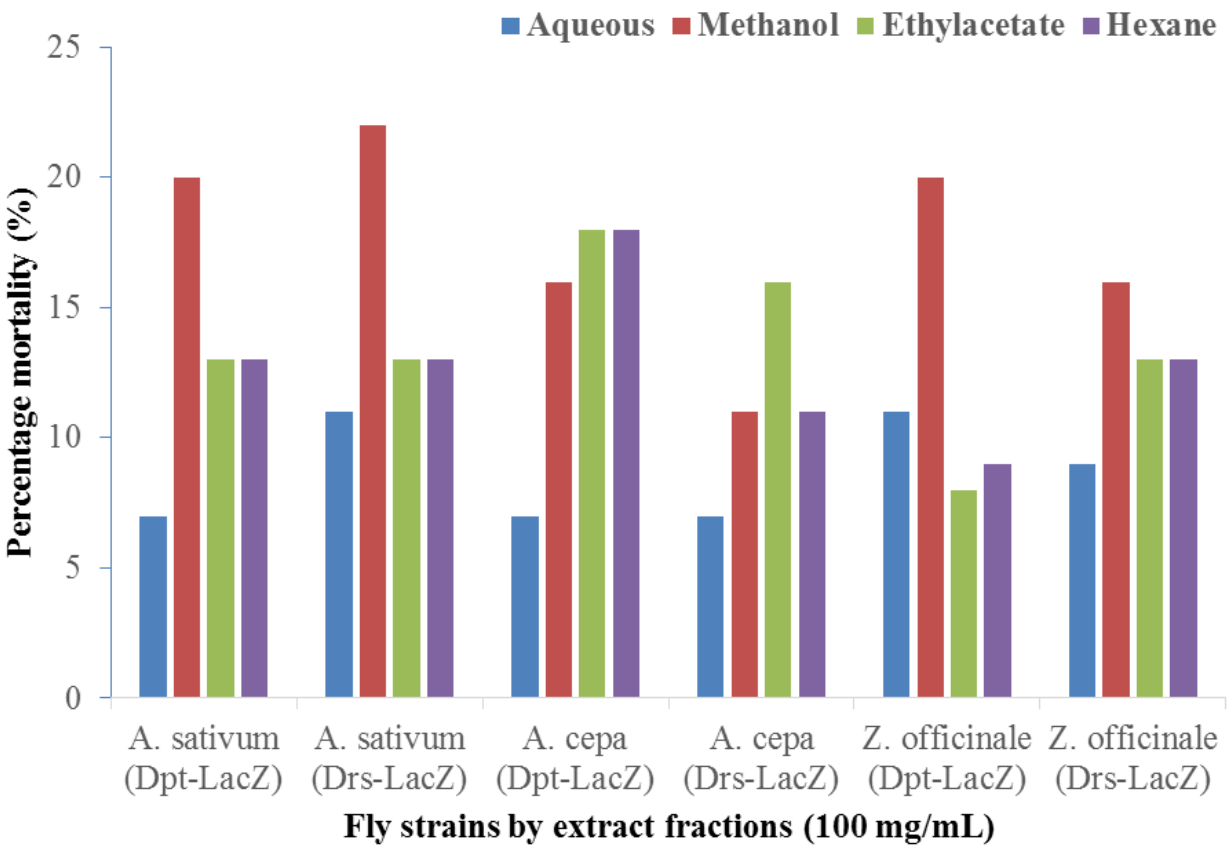

Figure 2. Percentage mortality of fly strains Dpt-LacZ (diptericin reporter gene) and Drs-LacZ (drosomycin reporter gene) from acute toxicity testing with 100 mg/mL of plants extract fractions.

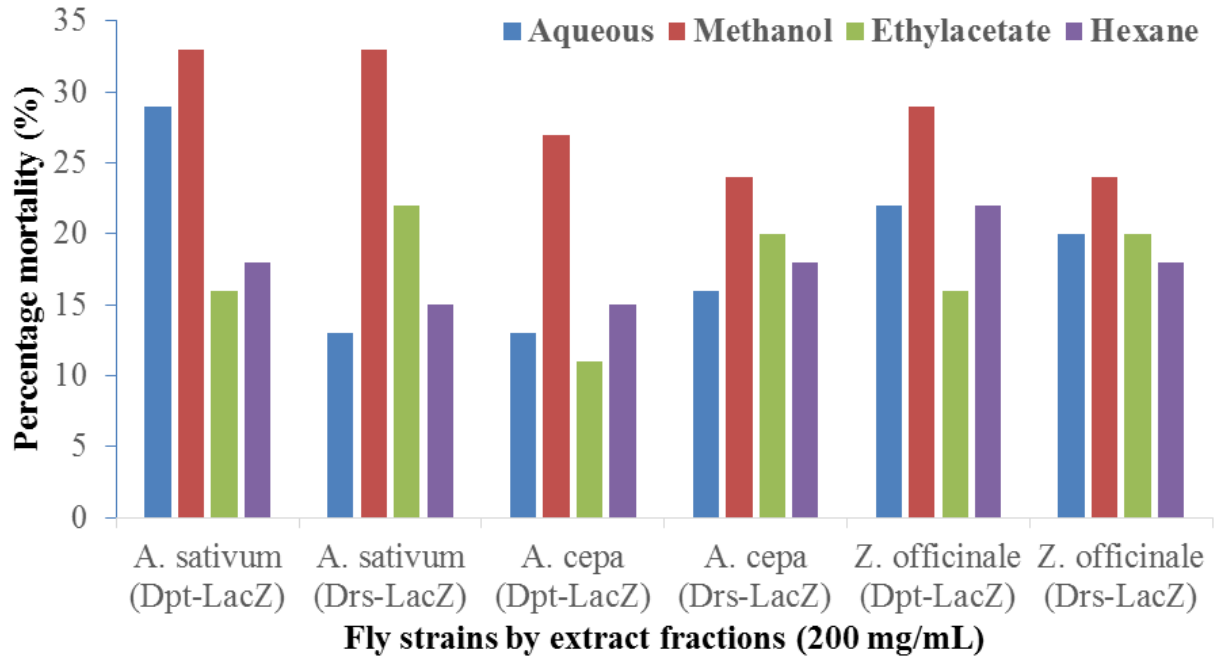

Figure 3. Percentage mortality of fly strains Dpt-LacZ (diptericin reporter gene) and Drs-LacZ (drosomycin reporter gene) from acute toxicity testing with 200 mg/mL of plants extracts.

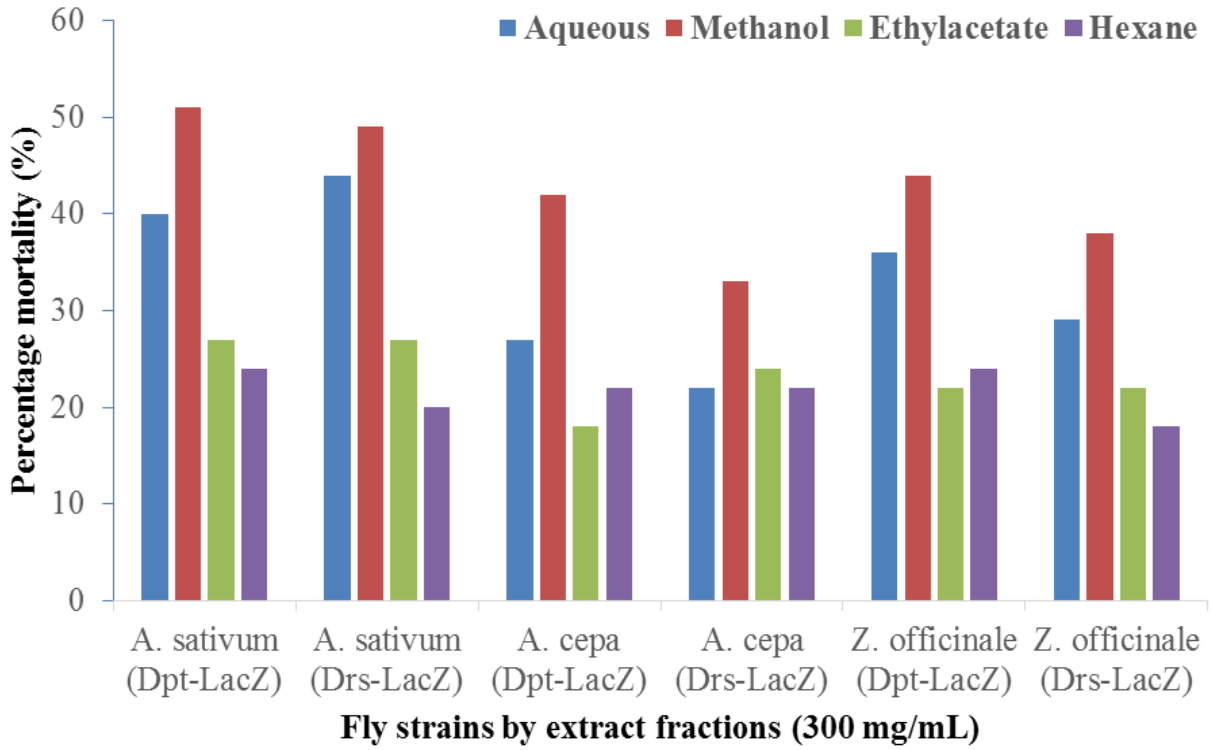

Figure 4. Percentage mortality of fly strains Dpt-LacZ (diptericin reporter gene) and Drs-LacZ (drosomycin reporter gene) from acute toxicity testing with 300 mg/mL of plants extracts.

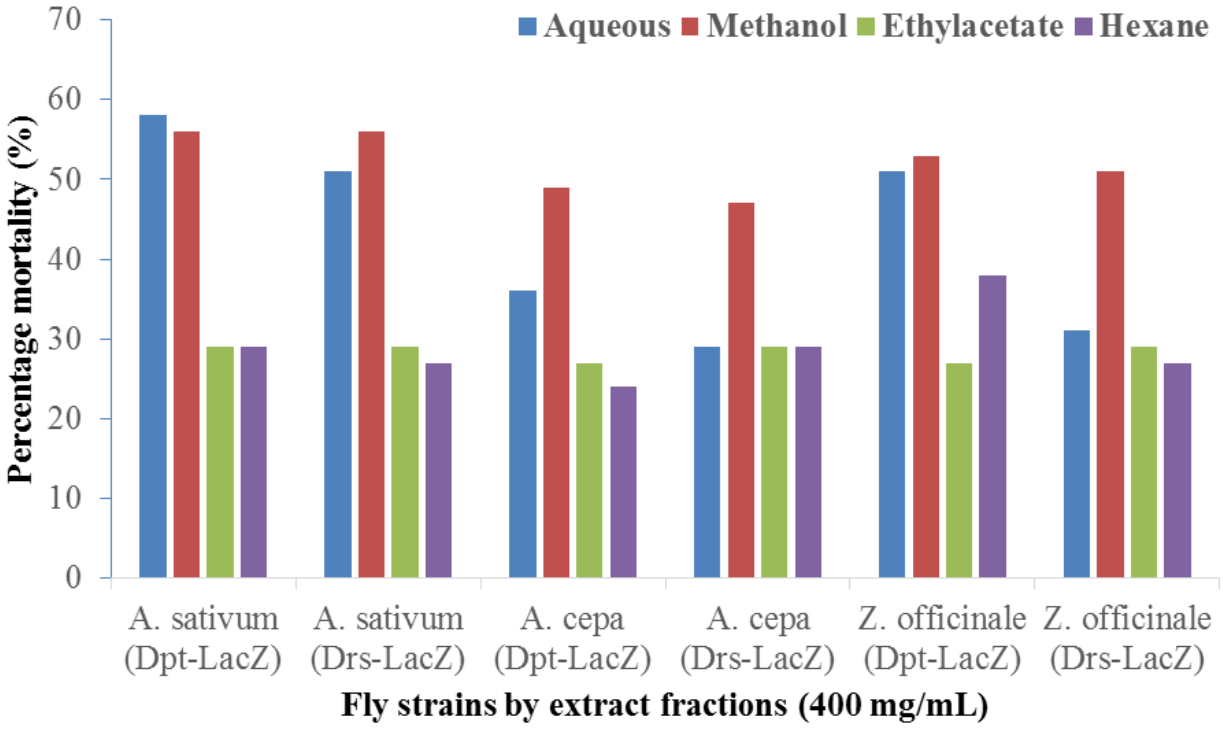

Figure 5. Percentage mortality of fly strains Dpt-LacZ (diptericin reporter gene) and Drs-LacZ (drosomycin reporter gene) from acute toxicity testing with 400 mg/mL of plants extracts.

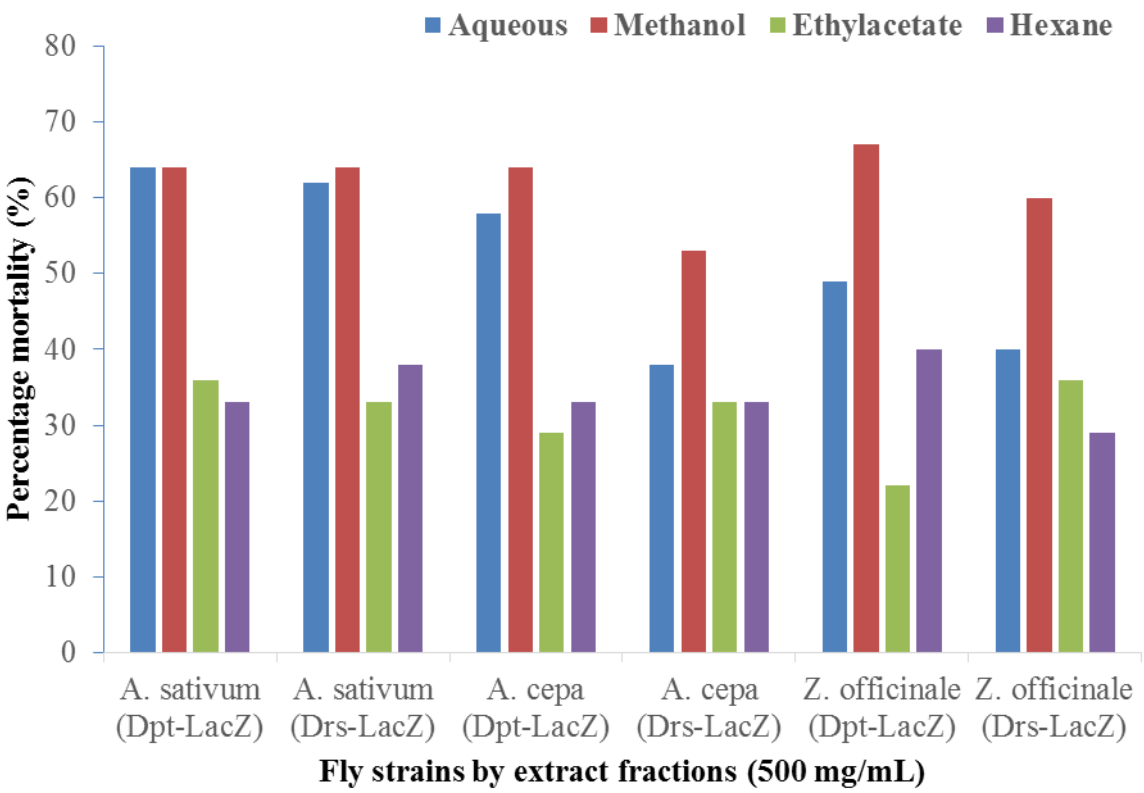

Figure 6. Percentage mortality of fly strains Dpt-LacZ (diptericin reporter gene) and Drs-LacZ (drosomycin reporter gene) from acute toxicity testing with 500 mg/mL of plants extracts.

DISCUSSION

The findings from the phytochemical screening of *A. sativum*, *A. cepa*, and *Z. officinale* extracts align with previous research, emphasizing solvent-dependent variations in extractable phytochemicals. Consistent with these results, Shehu et al. (2023) reported that methanol, a polar organic solvent, efficiently extracts a wide range of bioactive compounds, such as alkaloids and flavonoids, due to its ability to penetrate plant cell walls and dissolve various phytochemicals. This efficiency is reflected in the observed high phytochemical diversity in methanol extracts, highlighting methanol's effectiveness in extracting these compounds.

In contrast, the lower phytochemical content observed in *n*-hexane extracts aligns with findings by Chukwudebe (2020) and Khalili et al. (2022), who noted that non-polar solvents primarily extract lipophilic compounds, such as steroids, but are generally ineffective for polar compounds like alkaloids and flavonoids.

The probit analysis of lethal concentration (LC50) for *D. melanogaster* mortality indicates that methanol and aqueous extracts exhibit higher toxicity. This observation is supported by earlier studies, such as Ahmed et al. (2020), which suggest that high-polarity solvents tend to yield extracts containing more potent bioactive compounds. Specifically, methanol and water extracts of *A. sativum* and *Z. officinale* demonstrated particularly low LC50 values, reflecting significant toxicity. This aligns with findings by Shukla et al. (2015), who reported that sulfur-containing compounds in methanol extracts of garlic contribute to enhanced antimicrobial and toxic activities. Similarly, the high toxicity of aqueous garlic extracts may be attributed to water-soluble sulfur compounds, which likely contribute to their biological activity.

Comparative analysis of toxicity levels revealed that *Z. officinale* extracts consistently exhibited lower LC50 values, suggesting higher bioactivity. Supporting this, Prasad and Tyagi (2015) documented that active compounds in ginger, such as gingerol, shogaol, and zingerone, are efficiently extracted in methanol and exhibit strong biological activities, including toxicity against microbial and insect models. The high mortality observed in *Drosophila melanogaster* strains (*Drs-LacZ* and *Dpt-LacZ*) treated with methanol ginger extracts further underscores the potency of these compounds.

Furthermore, the lower toxicity of ethyl acetate and hexane extracts observed in this study aligns with findings by Thakur et al. (2020), who noted that intermediate-polarity solvents like ethyl acetate extract a moderate range of phytochemical compounds,

particularly flavonoids and some lipophilic components. These compounds tend to exhibit lower toxicity in insect models. The consistently lower mortality of flies exposed to these fractions suggests that they primarily contain less toxic, intermediate-polarity compounds, potentially limiting their bioactive applications compared to methanol and aqueous extracts.

Overall, the increased mortality rates observed in *Drosophila melanogaster* treated with methanol and aqueous extracts across plant types reflect these solvents' ability to extract diverse, highly bioactive phytochemicals. This finding is consistent with recent literature. The comparative toxicities observed in this study underscore the importance of solvent selection in bioactivity studies and highlight the potential of *A. sativum*, *A. cepa*, and *Z. officinale* extracts as sources of potent bioactive agents, particularly when polar solvents are used.

CONCLUSION

This study demonstrates that the toxicity of *A. sativum*, *A. cepa*, and *Z. officinale* extracts is significantly influenced by the solvent used for extraction. Methanol and aqueous extracts exhibited higher toxicity due to their efficiency in extracting potent bioactive compounds. The findings underscore the critical role of solvent choice in phytochemical studies and highlight the potential of these plants as sources of bioactive agents for pharmacological applications.

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