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Research Article

Evaluation of Phytochemical Composition, Heavy Metal Profile, and Mosquitocidal Activity of *Amanita virosa* Lam Ethanolic Extract against Mosquito Vectors

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ABSTRACT
This study evaluated the phytochemical composition, heavy metal profile, and mosquitocidal (ovicidal, larvicidal, and adulticidal) activities of *Amanita virosa* ethanolic extract against *Anopheles*, *Culex*,and *Aedes aegypti*. *A. virosa* fruiting bodies were collected, authenticated, and subjected to cold ethanol extraction. Phytochemical analysis confirmed presence of high total alkaloid content (150.17 ± 1.53 mg/g). GC-MS identified major compounds including fatty acid esters (linoleic acid ethyl ester, 19.78%; hexadecanoic acid ethyl ester, 8.79%), 9,12-octadecadienoyl chloride (17.13%), and β-hexachlorocyclohexane (1.36%). AAS revealed significant heavy metal accumulation, with Cadmium (0.87 mg/kg), Chromium (5.58 mg/kg), and Arsenic (4.29 mg/kg) exceeding FAO/WHO permissible limits. The extract demonstrated moderate ovicidal activity when analysed using probit analyses, with HI_{50} values ranging from 89.57 ppm (*Culex*) to 96.44 ppm (*Anopheles*). Larvicidal activity was weak (LC_{50} > 90 ppm; maximum mortality 7% at 40 ppm). Adult knockdown was slow (KDT_{50}: 186.3–252.9 minutes), with 24h post-exposure mortality reaching only 12% against *Anopheles*.

Keywords: Adulticide; Alkaloids; *Amanita virosa*; GC-MS; Heavy metals; Larvicide; Mosquito control; Ovicide

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INTRODUCTION

Mosquitoes are the most significant arthropod vectors of human diseases, transmitting pathogens that cause malaria, dengue, chikungunya, Zika virus, yellow fever, and West Nile encephalitis (Caminade *et al.*, 2019; Franklinos *et al.*, 2019). Malaria alone caused an estimated 249 million cases and 608,000 deaths in 2022, with Africa bearing over 95% of the mortality (WHO, 2023). Dengue now accounts for approximately 390 million annual infections, making it the most rapidly spreading arboviral disease (Bhatt *et al.*, 2021; Kumar *et al.*, 2021).

Vector control remains the cornerstone of disease prevention, relying heavily on chemical insecticides such as pyrethroids, organophosphates, and carbamates (Hemingway *et al.*, 2016). However, the sustained and often indiscriminate use of these chemicals has led to two major crises: the widespread evolution of insecticide resistance in mosquito populations and significant environmental contamination with associated non-target toxicity (Karunaratne *et al.*, 2018; van den Berg *et al.*, 2021). Consequently, the alternative use of botanical insecticides that could strengthen an insecticide resistance management programme (WHO, 2006). Monitoring of resistance is essential to alert control programs to switch to more effective insecticides (Aymere and Laikemariam, 2006).

These challenges have intensified the search for novel, eco-friendly alternatives with

diverse modes of action (Benelli & Duggan, 2022). Natural products from plants, bacteria, and fungi offer a rich source of chemical diversity for bio-insecticide development (Atanasov *et al.*, 2021). Additionally, they hold promising potential for the development of novel pharmaceuticals, antibiotics, insecticides for pest control, and herbicides targeting unwanted plant growth (Devi et al., 2022).

The genus *Amanita* includes both edible species and some of the world's most toxic mushrooms, such as the "destroying angel," *Amanita virosa* (Bonnet & Basson, 2004; Diaz, 2018). Its toxicity in mammals is attributed to cyclic peptide toxins like α-amanitin and phalloidin (Garcia *et al.*, 2021), but its broader secondary metabolite profile and potential insecticidal properties remain largely uncharacterized (Antonyuk *et al.*, 2010; Sgambelluri *et al.*, 2014).

It is therefore, against this background that thus study was aimed at evaluating the phytochemical composition, heavy metal profile, and insecticidal activity of *Amanita virosa* ethanolic extract against *Anopheles*, *Culex*, and *Aedes.*

MATERIALS AND METHODS

Study Area

This research was conducted at the New Biology Laboratory, Department of Biological Sciences, Federal University Dutsin-Ma, Katsina State, Nigeria (12°27' N, 7°30' E) between January and March 2025.

Collection and Identification of Amanita Virosa Lam

Fresh fruiting bodies of *Amanita virosa Lam* were collected from M & D Botanical Garden, Anguwan Jaji, Potiskum Local Government Area, Yobe State, Nigeria. (11°42'19.1628'' N, 11°3'23.292'' E) The specimens were authenticated at the Department of Plant Science and Biotechnology, Federal University Dutsin-Ma, and a voucher specimen (FUDMA/PSB/00315) was deposited at the University Herbarium.

Extraction Preparation

Collected mushrooms were rinsed with distilled water to remove debris and air-dried at room temperature (28 ± 2°C) for four days. The dried samples were pulverized using a sterile mortar and pestle. One hundred and fifty grams (150 g) of the powdered material was subjected to Soxhlet extraction, using 600 ml of absolute ethanol for 72 hours with intermittent agitation. The mixture was filtered through sterile muslin cloth followed by Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator (RE-52A, Shanghai) at 40°C and then air-dried to complete solvent removal, yielding the crude ethanolic extract, which was stored at 4°C in airtight vials until use. The percentage yield was calculated as:

$$\% \text{ Yield} = \frac{Weight\ of\ crude\ extract\ obtained}{Weight\ of\ plant\ material}\ X\ 100$$

Phytochemical Analysis

Qualitative screening

The crude extract was screened for the presence of major secondary metabolite classes (alkaloids, flavonoids, tannins, phenols, saponins, terpenoids) using standard procedures as described by Harborne (2015) Sofowora (2018)

Quantitative Analysis

Total alkaloid, flavonoid, phenolic, tannin, saponin, and terpenoid contents were quantified using spectrophotometric methods. Analyses were performed in triplicate, and results were expressed as mean ± Standard Deviation (mg/g extract).

Heavy Metal Analysis by Atomic Absorption Spectroscopy (AAS)

Heavy metal content was determined following the method of Koutrotsios & Zervakis (2021). One gram of dried extract was digested with 10 mL of concentrated HNO_3 and $HClO_4$ (4:1, v/v) at 80°C until a clear solution was obtained. The digested sample was cooled, filtered, and diluted to 25 mL with deionized water. Metal concentrations were analyzed using a Flame Atomic Absorption Spectrometer (Shimadzu AA-7000, Japan) with appropriate hollow cathode lamps. Results were compared with FAO/WHO permissible limits for mushrooms and vegetables (Obeng 2009).

GC-MS Analysis

The chemical profile of the extract was determined using an Agilent 7890B Gas Chromatograph coupled with a 5977A Mass Selective Detector. Separation was achieved on an HP-5MS capillary column (30 m × 250 µm × 0.25 µm) with helium as carrier gas (1 mL/min). The oven temperature was programmed as follows: initial 70°C (hold 2 min), ramped at 20°C/min to 110°C (hold 1 min), then at 5°C/min to 230°C (hold 5 min), and finally at 20°C/min to 280°C (hold 5 min). The injector and transfer line temperatures were 250°C and 280°C, respectively. The mass spectrometer was operated in electron impact (EI) mode at 70 eV, scanning a range of 50–550 m/z. Compounds were identified by comparing their mass spectra with the NIST (National Institute of Standards and Technology) library database and by comparing retention indices with literature values.

Mosquito Rearing

Colonies of *Anopheles*, *Culex*, and *Aedes* were established from field-collected larvae and maintained under standard insectary conditions (27 ±

2°C, 75 ± 5% relative humidity, 12:12 light:dark photoperiod). Larvae were fed with cabin biscuit and yeast tablet, and adults were provided with 10% sucrose solution. For egg production, female mosquitoes were offered human hand for blood feeding by Reegan *etal* (2013).

Stock Preparation

Stock concentration was prepare given by Harris *et al.* (2016) 1gram of crude extract were dissolve in 1litre to prepare 1000ppm using the following dilution formula

C1V1 = C2V2

C1 = Concentration

V1 = Initial Volume

C2 = final Concentration

V2 = Final Volume

To find ppm:

PPM = $\frac{mass\ (mg)}{volume\ (L)}$

1gram = 1000mg

1000ml = 1L

PPM = $\frac{1000mg}{1L}$ = 1000ppm

C1V1 = C2V2

C1 = 1000ppm (Stock Concentration)

V1 = 1ml (Volume taken)

V2 = final volume (25,50,75,100 ml)

C2 = Final concentration

Dilution:

C2 = $\frac{1000\times1}{25}$ = 40ppm

C2 = $\frac{1000\times1}{50}$ = 20ppm

C2 = $\frac{1000\times1}{75}$ = 13.3ppm

C2 = $\frac{1000\times1}{100}$ = 10ppm

Ovicidal Bioassay

The ovicidal activity was assessed using the method described by WHO (2005), Elango *et al.* (2009). Twenty freshly laid eggs (<12 h old) of each mosquito species were placed in 50 mL plastic cups containing 25 mL of distilled water treated with varying concentrations of the extract (10, 13.3, 20, and 40 ppm). These concentrations were derived from serial dilutions of a 1000 ppm stock solution. A control group received only distilled water. Each concentration and control were replicated four times. Cups were maintained under insectary conditions, and the number of unhatched eggs was recorded after 72 hours. The percentage of egg hatch inhibition was calculated.Using the following formula

Ovicidal Activity (%) = $\frac{Number\ of\ unhatched\ eggs}{Total\ number\ of\ eggs\ introduced} \times 100$

Larvicidal Bioassay

Larvicidal activity was evaluated against early 4th instar larvae following WHO (2005) guidelines. Batches of 20 larvae were introduced into 200 mL plastic cups containing 100 mL of the test concentrations (10, 13.3, 20, and 40 ppm). Four replicates were performed per concentration, alongside a negative control, Larval mortality was recorded after 24 hours of exposure. Larvae were considered dead if they did not respond to gentle probing with a needle. Percentage mortality was calculated, using Abbott's formula.

Adulticidal Bioassay (Knockdown and Mortality)

Adulticidal activity was assessed using the bottle bioassay protocol given by (CDC, 2010; Ajaegbu *et al.*, 2016). Stock solution (1000 ppm) was prepared by dissolving 1 g of crude extract in 1000 mL Ethanol. Wheaton glass bottles (250 mL) were coated with 1 mL of this solution by rolling them until the Ethanol evaporated with negative Control bottles. Twenty, non-blood-fed, 2–5day old adult female mosquitoes were introduced into each bottle via a mouth aspirator. Knockdown (inability to stand or fly) was recorded at 15-minute intervals for 120 minutes. After 120 minutes, mosquitoes were transferred to holding cages with access to 10% sucrose solution, and final mortality was recorded after 24 hours. Four replicates were performed per mosquito species.

Data Analysis

Data were analyzed using SPSS version 26.0. Means and standard deviations were calculated. Probit analysis (Finley, 1971) was used to determine:

HI_{50} and HI_{90}: Concentrations inhibiting 50% and 90% of egg hatching.

LC_{50} and LC_{90}: Lethal concentrations killing 50% and 90% of larvae.

KDT_{50} and KDT_{90}: Time required to knock down 50% and 90% of adult mosquitoes.

The 95% confidence intervals (CIs) were calculated for each estimate. A chi-square test was used to assess the goodness-of-fit of the probit model.

RESULTS

Extract Yield

The cold ethanol extraction of 150 g of dried *A. virosa* powder yielded 3.1g of crude extract, corresponding to a percentage yield of 2.06%.

Phytochemical Composition of Ethanolic Extract of *Amanita virosa*

Qualitative screening confirmed the presence of all the major secondary metabolite classes such as alkaloids, flavonoids, tannins, phenols, saponin, and terpenoids.

Quantitative analysis (Figure 1) revealed a strikingly high total alkaloid content (150.17 ± 1.53 mg/g), dwarfing the concentrations of other metabolites. Phenols (5.52 ± 0.06 mg/g), tannins (8.48 ± 0.06 mg/g), and saponin (3.02 ± 0.06 mg/g) were present at moderate levels, while flavonoids (1.22 ± 0.03 mg/g) and terpenoids (2.02 ± 0.03 mg/g) were comparatively low.

Heavy Metal Content in *Amanita virosa* Ethanolic Extract

The heavy metal analysis indicated that *A. virosa* extract accumulated several toxic metals at concentrations exceeding the permissible limits set by FAO/WHO (Table 1). Most notably, Chromium (Cr) (5.58 mg/kg), Arsenic (As) (4.29 mg/kg), and Cadmium (Cd) (0.87 mg/kg) were significantly above the recommended thresholds. Lead (Pb) (2.15 mg/kg) was at the upper limit of acceptability. Mercury (Hg) and Nickel (Ni) remained within safe limits. Cobalt (Co), for which no limit is established, was detected at 1.13 mg/kg.

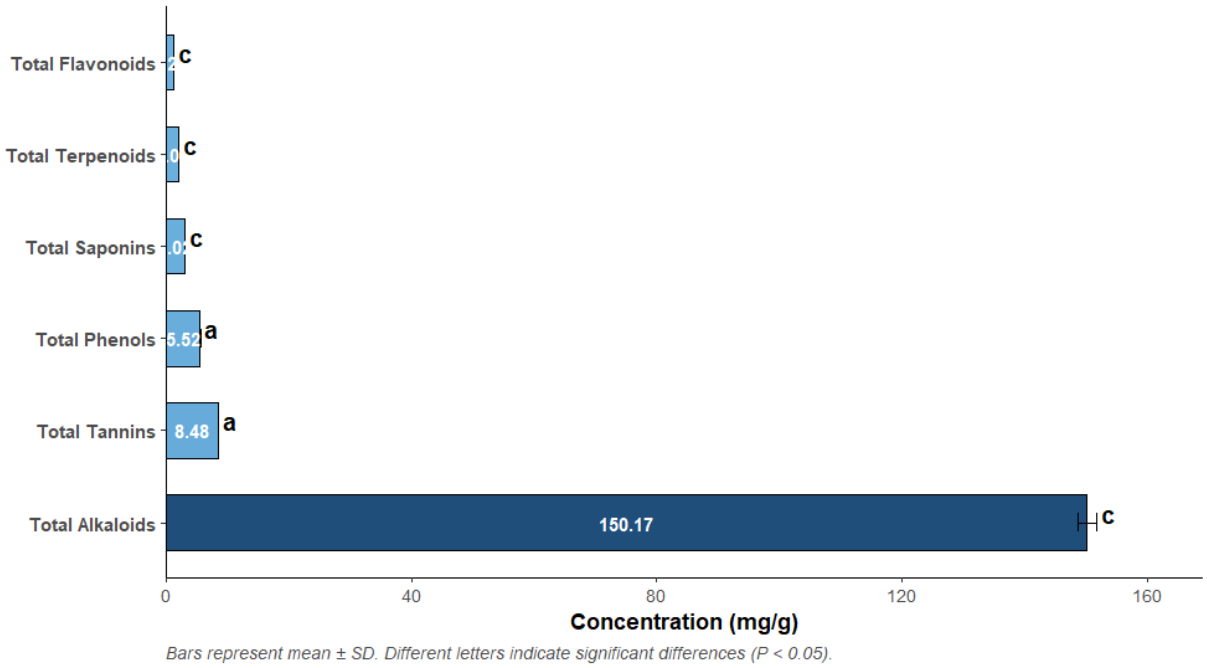

Figure 1: **Quantitative Phytochemical Composition of *Amanita virosa* Ethanolic Extract**

Table 1: Heavy Metal Concentration in Ethanolic Extract of *Amanita virosa* Compared to FAO/WHO Permissible Limits

Heavy Metal	Concentration (mg/kg)	FAO/WHO Permissible Limit (mg/kg)[1]
Lead (Pb)	2.15 ± 0.04[a]	0.3 – 2.0
Cadmium (Cd)	0.87 ± 0.02[b]	0.1 – 0.2
Chromium (Cr)	5.58 ± 0.07[a]	1.0 – 2.3
Arsenic (As)	4.29 ± 0.08[a]	0.1 – 1.4
Mercury (Hg)	0.23 ± 0.01[a]	0.1 – 0.5
Nickel (Ni)	3.47 ± 0.06[a]	10 – 67
Cobalt (Co)	1.13 ± 0.03[a]	Not established

[1]Source: Codex Alimentarius Commission (2019) – general standard for contaminants and toxins in food and feed (applicable to mushrooms and vegetables). Data was presented as Mean ± SD.

Data were analyzed using Anova followed by turkey post hoc test. Means with different superscript letter in the same column are not statistically different at 95% confidence level

GC-MS Chemical Profiling of Ethanolic Extract of *Amanita virosa*

GC-MS analysis identified a complex mixture of compounds in the ethanolic extract (Table 2). The major constituents were fatty acid esters, particularly linoleic acid ethyl ester (19.78%) and hexadecanoic acid ethyl ester (8.79%). A significant peak corresponding to 9,12-octadecadienoyl chloride (17.13%) was also detected. Other notable compounds included dibutyl phthalate (2.27%), 2-pentadecanone, 6,10,14-trimethyl- (1.22%), and the organochlorine compound β-hexachlorocyclohexane (β-HCH, 1.36%).

Ovicidal Activity of Ethanolic Extract of *Amanita virosa* against Mosquitoe

The extract exhibited a dose-dependent inhibition of egg hatching across all three mosquito species (Figure 2). At the highest concentration tested (40 ppm), the percentage of unhatched eggs was 45.2% for *Anopheles*, 53.2% for *Culex* and 50.0% for *Aedes*. Probit analysis (Table 4) yielded HI_{50} values (concentration to inhibit 50% hatching) of 96.44 ppm (*Anopheles*), 89.57 ppm (*Culex*), and 91.15 ppm (*Aedes*). The corresponding HI_{90} values ranged from 151.8 to 168.1 ppm.

Larvicidal Activity of Ethanolic Extract of *Amanita virosa* against Mosquitoes

The larvicidal activity of the extract was weak (Figure 3). Maximum mortality after 24h exposure to 40 ppm was only 7% for *Anopheles*, 5% for *Culex*, and 2% for *Aedes*. The calculated LC_{50} values were all above 90 ppm (91.73 – 104.0 ppm), and the LC_{90} 133.25 ppm (Table 4).

Adulticidal Activity (Knockdown and Mortality) of Ethanolic Extract of *Amanita virosa* against Mosquitoes

The extract caused slow knockdown of adult mosquitoes (Figure 4). The KDT_{50} values (time to knock down 50% of the population) were 210.0 minutes for *Anopheles*, 252.9 minutes for *Culex*, and 186.3 minutes for *Aedes* (Table 5). The KDT_{90} values were correspondingly longer (>247 minutes). Final mortality recorded 24hours post-exposure was low, reaching only 12% for *Anopheles*, 6% for *Culex*, and 9% for *Aedes*.

Table 2: Major Compounds Identified in Ethanolic Extract of *Amanita virosa* by GC-MS

S/N	Compound Name	RT (min)	Peak Area (%)	Molecular Formula	Molecular Weight
1	2-Pentadecanone, 6,10,14-trimethyl-	9.8	1.22	$C_{18}H_{36}O$	268.49
2	β-Hexachlorocyclohexane (β-HCH)	10.1	1.36	$C_6H_6Cl_6$	290.81
3	Hexadecanoic acid, methyl ester	10.4	7.12	$C_{17}H_{34}O_2$	270.46
4	Dibutyl phthalate	10.7	2.27	$C_{16}H_{22}O_4$	278.35
5	Hexadecanoic acid, ethyl ester	10.8	8.79	$C_{18}H_{36}O_2$	284.48
6	9,12-Octadecadienoyl chloride (Z,Z)-	11.5	17.13	$C_{18}H_{31}ClO$	298.90
7	Linoleic acid ethyl ester	11.9	19.78	$C_{20}H_{36}O_2$	308.51

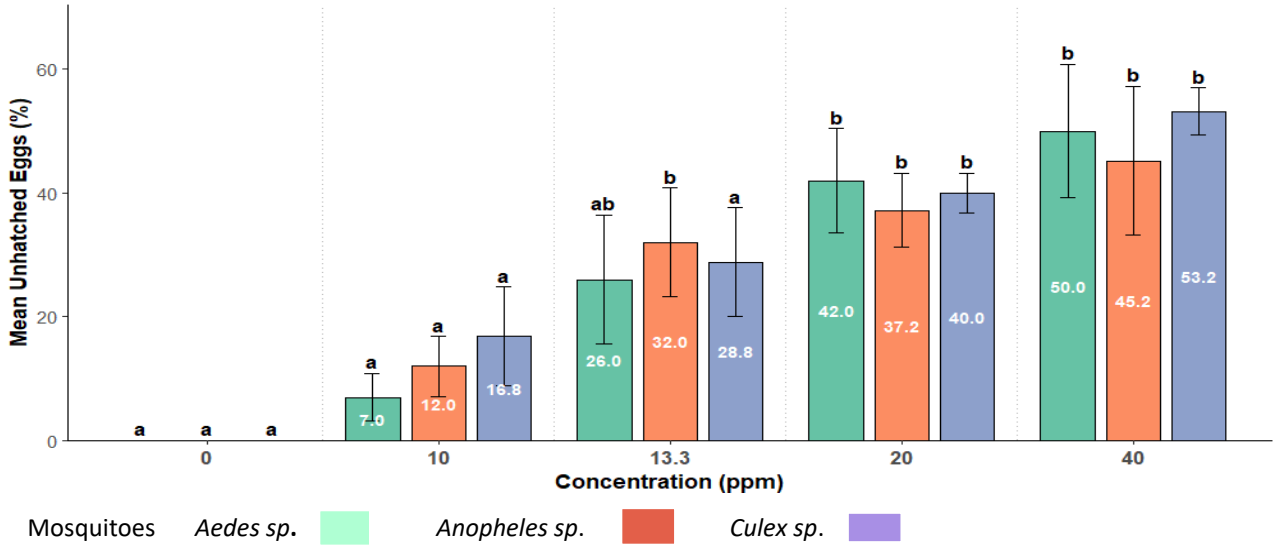

Figure 2: Ovicidal Activity of *A. virosa* Extract against Mosquitoes (Mean % Unhatched Eggs ± SD)

Table 3: Probit Analysis of Ovicidal Activity (HI_{50} and HI_{90}) Ethanolic Extract of *Amanita virosa* against Mosuitoes

Mosquito Species	HI_{50} (ppm) (95% CI)	HI_{90} (ppm) (95% CI)	χ^2 (df)	P-value
Anophels	96.44 (69.0 – 307.5)	168.1(115.7 –799.8)	15.42(3)	0.01
Culex	89.57 (67.4 – 161.8)	161.71(117.4 – 382.5)	10.68(3)	0.014
Aedes	91.15 (72.6 – 138.9)	151.80(116.3 – 284.9)	9.38(3)	0.025

Footnote: Data was presented as Estimate (Lower-upper bound) at 95% confidence interval. Observed and expected values are significantly different at $p \leq 0.05$

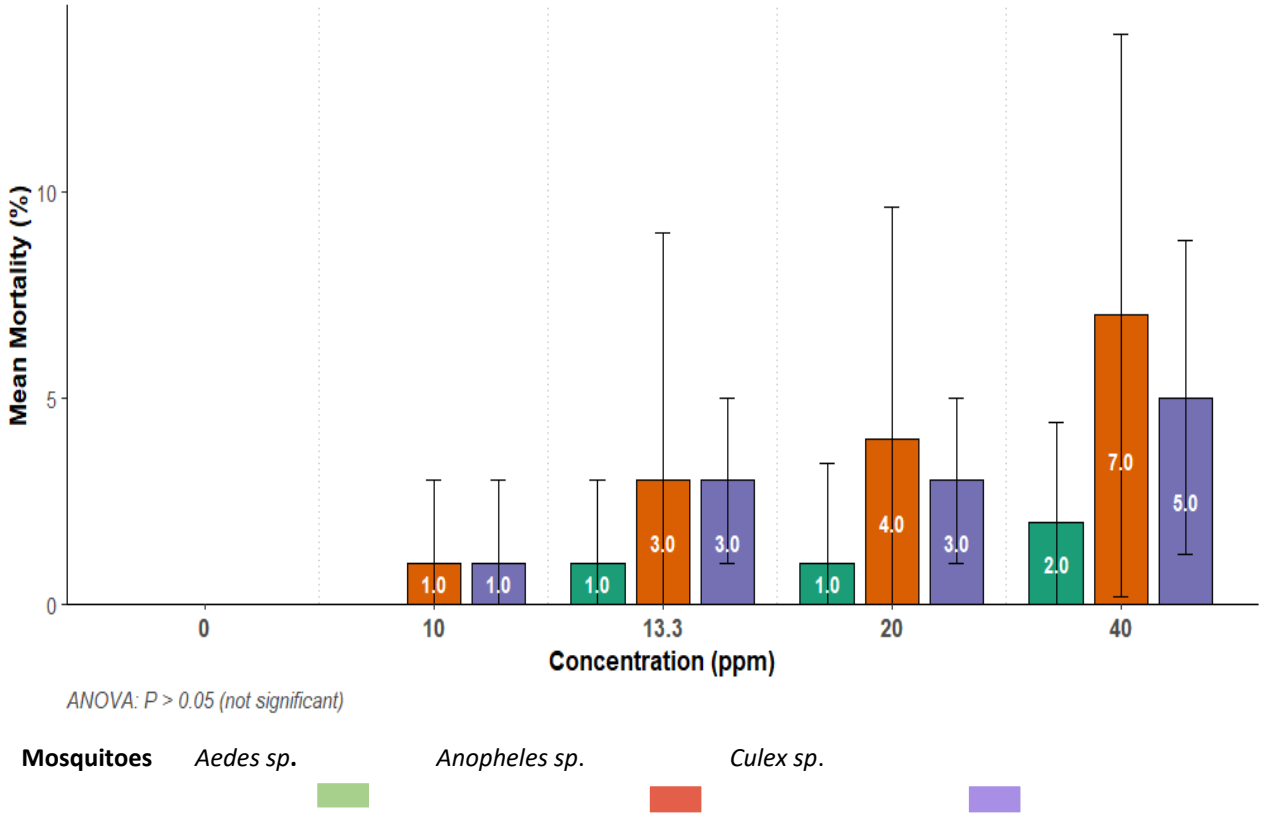

Figure 3: Larvicidal Activity of *A. virosa* Extract against Mosquitoes (Mean % Mortality ± SD)

Table 4: Probit Analysis of Larvicidal Activity (LC_{50} and LC_{90}) of *A. virosa* Extract against Mosquitoes

Mosquito Species	LC_{50} (ppm) (95% CI)	LC_{90} (ppm) (95% CI)	χ^2 (df)	P-value
Anopheles	91.73 (63.9 – 222.6)	140.25 (93.5 – 363.1)	2.2(3)	0.53
Culex	104.0 (67.5 – 414.2)	157.18 (97.5 – 671.0)	1.36(3)	0.715
Aedes	96.31 (NE)[2]	133.25 (NE)	1.36(3)	0.714

[2]NE = 95% Confidence Interval not estimated due to extreme data variability.

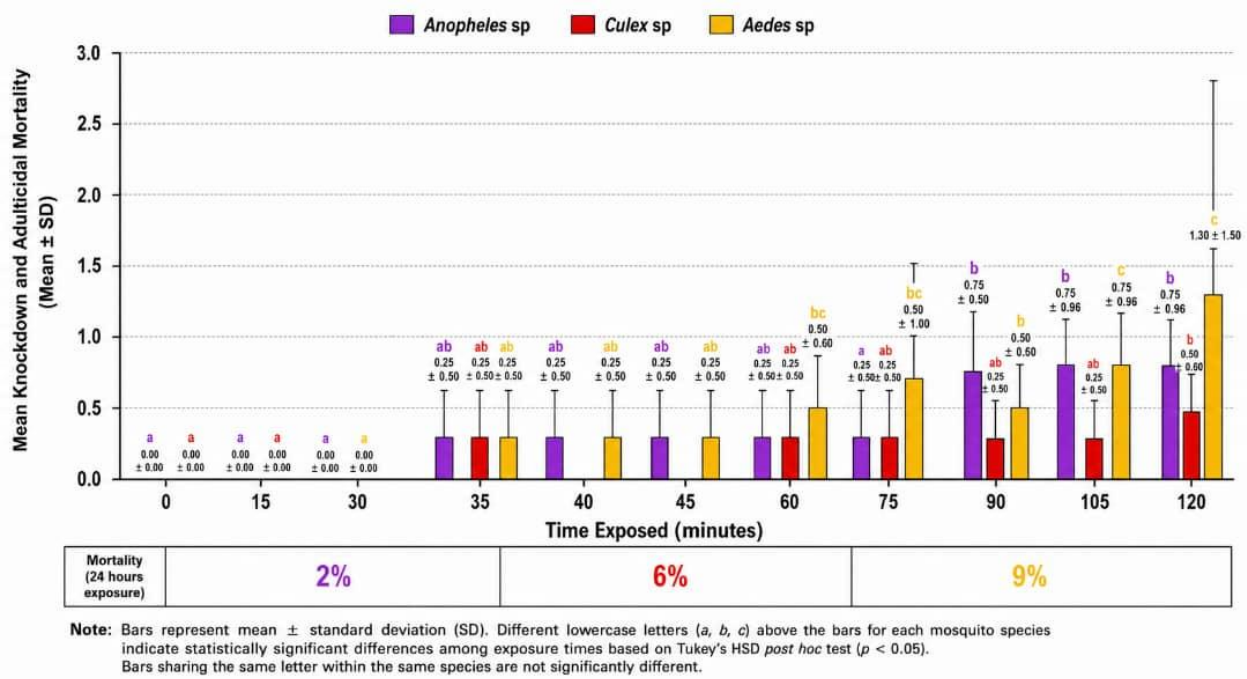

Figure 4: Adulticidal Activity of Ethanolic Extract of *Amanita virosa* against Mosquitoes (Knockdown and % Mortality ± SD)

Table 5: Probit Analysis of Adult Knockdown Time (KDT_{50} and KDT_{90}) of *A. virosa* Extract against Mosquitoes

Mosquito Species	KDT_{50} (minutes)	KDT_{90} (minutes)	χ^2 (df)	P- value
Anopheles	210.0 (163.0 – 478.1)	277.8 (203.0 – 708.8)	1.3(9)	0.99
Culex	252.9 (183.0 – 654.4)	348.9 (240.3 – 985.5)	2.0(9)	0.98
Aedes	186.3 (155.1 – 282.4)	247.0 (195.6 – 409.1)	2.18(9)	0.99

Note: Data was presented as estimate (Lowe-upper bound) at 95% confidence interval. There is no significant between expected and observed frequencies indicating model fit.

DISCUSSION

The study provides a comprehensive evaluation of mosquitocidal potential of *Amanita virosa* ethanolic extract against three major disease vectors. The findings reveal a complex chemical profile dominated by alkaloids and fatty acid derivatives, moderate ovicidal activity, but weak efficacy against larval and adult stages, compounded by significant heavy metal contamination.

The exceptionally high total alkaloid content observed in *A. virosa* was remarkable and far exceeds values reported for many medicinal plants with established insecticidal properties. For comparison, Nwabor *et al.* (2022) reported alkaloid concentrations of only 12–25 mg/g in various Nigerian medicinal plants, while El-Mahdy *et al.* (2023) found 8.5–45.2 mg/g in selected Egyptian plants with mosquitocidal activity. The concentration found in our study is consistent with the genus *Amanita sp* reputation for producing potent nitrogenous toxins (Diaz, 2018). This high alkaloid content suggests significant neurotoxic potential, as alkaloids are known to interfere with acetylcholinesterase activity and neurotransmitter receptors in insects (Matsuura & Fett-Neto, 2017).

The moderate levels of phenolics and tannins (8.48 mg/g) are comparable to those reported by Gebrehiwot *et al.* (2023) in Ethiopian wild mushrooms (4.8–9.3 mg/g for phenolics) but lower than values found in some culinary-medicinal mushrooms like *Ganoderma lucidum* (Ahmad *et al.*, 2021; 15–28 mg/g phenolics) as reported by Ahmed *etal* (2021). Furthermore, it is reported that Phenolic compounds contribute to insecticidal activity through oxidative stress induction and disruption of mitochondrial function (Zhou *et al.*, 2022).

The dominance of fatty acid esters in the GC-MS profile of *A. virosa* aligns with findings from other macrofungi studies. Ramos-López *et al.* (2020) similarly identified hexadecenoic acid esters as major constituents in entomopathogenic fungi with insecticidal activity. The high proportion of linoleic acid ethyl ester (19.78%) is particularly

noteworthy. Fouad *et al.* (2021) demonstrated that linoleic acid derivatives exhibit potent larvicidal activity against *Aedes aegypti* through cuticular disruption and desiccation, with LC_{50} values of 45–60 ppm, which is considerably lower (more potent) than the LC_{50} values obtained in our study. The presence of 9,12-octadecadienoyl chloride (17.13%) also identified in the leaves extract of Solanum. xanthocarpum has -9, 12-octadecadienoyl chloride (7.93%), Kumar et al (2022). is unusual and has not been commonly reported in mushroom extracts. However, Azmath *et al.* (2023) recently identified similar acyl chloride derivatives in fungal metabolites and suggested they may act as reactive intermediates that covalently modify insect proteins, potentially enhancing toxicity. The detection of β-hexachlorocyclohexane (β-HCH) is concerning but not unprecedented. Vijgen *et al.* (2019) documented widespread HCH contamination in environmental samples, including fungal fruiting bodies, due to historical use of lindane. This highlights the capacity of macrofungi to bioaccumulate persistent organic pollutants from their substrate.

The heavy metal accumulation profile of *A. virosa* reveals concentrations of Cd, Cr , and As that substantially exceed FAO/WHO permissible limits. These values are higher than those reported by Koutrotsios & Zervakis (2021) for cultivated *Agaricus bisporus* (Cd: 0.2–0.4 mg/kg; Cr: 1.5–2.8 mg/kg) but comparable to wild-growing *Amanita* species studied by Falandysz & Borovička (2021) in contaminated European sites. The hyperaccumulation capacity of *Amanita* species is well-documented by Mleczek *et al.* (2022) who found that *Amanita muscaria* accumulated Cd up to 5.2 mg/kg in polluted areas, exceeding safe limits by 10–20 fold.

This finding has profound implications. Direct application of this crude extract to mosquito breeding sites would risk introducing these toxic metals into aquatic ecosystems. Ntow (2022) emphasized that even low-level introduction of Cd and Cr can lead to biomagnification in food chains and chronic toxicity in non-target organisms, including fish and beneficial insects.

The ovicidal activity observed is moderate compared to previously studied natural products for instance, Prabhu *et al.* (2021) reviewed ovicidal activity of plant extracts and reported that potent ovicides like *Azadirachta indica* and *Curcuma longa* exhibit HI_{50} values below 50 ppm against *Aedes* and *Anopheles* species. However, our results are comparable to those of Cheah *et al.* (2021), who found HI_{50} values of 85–110 ppm for *Artemisia annua* extracts against the same mosquito species. The dose-dependent response observed in our study aligns with the general principle that ovicidal activity is concentration-dependent, as demonstrated by Benelli *et al.* (2022) in their work with botanical ovicides.

The differential susceptibility among mosquito species (*Culex* eggs being slightly more susceptible than *Anopheles* or *Aedes*) is consistent with reports by Dusfour *et al.* (2019), who attributed such differences to variations in egg chorion structure, thickness, and permeability. The *Aedes* egg, in particular, possesses a more robust chorion adapted to withstand desiccation, which may explain its relative resilience (Becker *et al.*, 2020).

The larvicidal activity observed was considerably weaker than that reported for many plant-based and microbial larvicides. For comparison, Govindarajan *et al.,* (2023) reported LC_{50} values of 12–28 ppm for essential oils from *Cymbopogon* species against *Anopheles* larvae. Pavela *et al.* (2021) found that numerous botanical extracts achieve LC_{50} values below 50 ppm, the threshold generally considered promising for larvicide development. Even among mushroom extracts, our results are weaker than those reported by Njogu *et al.* (2019) for *Cyptotrama asprata* metabolites (LC_{50}: 45 ppm against *Ae. aegypti*).

This weak activity suggests that the active compounds in *A. virosa* either cannot be effectively ingested or absorbed through the larval cuticle, are rapidly metabolized, or require much higher concentrations to affect larvae. Mutebi *et al.* (2022) noted that for alkaloid-rich extracts, poor cuticular penetration often limits larvicidal efficacy, as the larval integument acts as a significant barrier.

The adult knockdown times (KDT_{50}) were exceptionally slow compared to standard insecticides. Ajaegbu *et al.* (2022) reported KDT_{50} values of 35–60 minutes for *Spondias mombin* extracts against *Ae. aegypti*, while synthetic pyrethroids typically achieve knockdown within 10–30 minutes (WHO, 2022). The slow action may reflect the need for metabolic activation of the compounds or poor bioavailability at neural targets.

The 24-hour mortality is far below the WHO efficacy threshold (-100% mortality) required for candidate adulticides (WHO 2022). Farenhorst *et al.* (2021) similarly found that some entomopathogenic fungal extracts caused slow mortality, often requiring 3–5 days to achieve significant kill, which they attributed to the need for

compound bioaccumulation and delayed toxicity mechanisms.

CONCLUSION

This study demonstrated that the ethanolic extract of *Amanita virosa* possesses a rich and distinctive phytochemical profile, characterized by an exceptionally high alkaloid content and a complex mixture of fatty acid esters including linoleic acid ethyl ester and 9,12-octadecadienoyl chloride. The extract exhibited moderate, dose-dependent ovicidal activity against *Anopheles*, *Culex*, and *Aedes*, with HI_{50} values ranging from 89.6 to 96.4 ppm. However, larvicidal and adulticidal activities were weak, and far below thresholds considered promising for field application.

Based on these findings, the following steps are recommended to translate this innovation into a safe and effective tool. Significant finding of this research is the potent, dose-dependent ovicidal activity, the Amanita virosa extract. It emerged as the most potent ovicide, requiring the lowest HI50 values (89.57 - 161.71 ppm). Formulation Development Encapsulate the purified active compounds in nano-formulations (e.g., nanoparticles or emulsions) to improve their stability, solubility, and targeted delivery to breeding sites, as recommended for next-generation botanical insecticides. Comprehensive Ecotoxicological Studies Rigorously assess the safety of the purified and formulated products on non-target aquatic organisms (e.g., Daphnia, fish) to ensure environmental sustainability before any field consideration. > Investigation of Synergy Explore combinations of these purified mushroom fractions with other established botanical insecticides (e.g., from neem) to achieve synergistic effects, potentially lowering effective doses and overcoming the weakness against larvae and adults

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