

Derivatized Extracts from *Aframomium melegueta* K. Schum. and *Vernonia amygdalina* Delile Contain Organic Compounds that Showed Antiviral Effects against Atypical Fowl Pox Virus (FPV Kabete)

Oladunmoye MK¹, Afolami OI^{1*}, Oladejo BO¹, Amoo IA², Osho BI³

¹Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria

²Department of Chemistry, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria

³Department of Animal Production and Health, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria

ABSTRACT

In this study, we tested for the antiviral potentials of derivatized extracts from two plants *Aframomium melegueta* K. Schum. and *Vernonia amygdalina* Delile against atypical Fowl pox virus (FPV Kabete). Preliminary study on derivatized extracts from the plants showed that *A. melegueta* extract contain two abundant phenols (benzaldehyde-3-hydroxy-4-methoxy and butan-2-one-4-(3-hydroxy-2-methoxyphenyl) while the *V. amygdalina* extract contained Phytol and a nucleoside analogue Methyl-2-O-benzyl-d-arabinofuranoside as most abundant compounds. We determined the prophylactic and therapeutic efficacy of the derivatized extracts against the FPV in Embryonated Chick Eggs (ECEs) and also linked their antiviral properties to the activity of the most abundant compounds they contain. Results showed that the extracts had strong therapeutic and prophylactic efficacy against FPV. Summarily, the extract from *A. melegueta* had stronger prophylactic efficacy against the FPV with an inhibition concentration (IC₅₀) of 159.49 ± 2.16 µm, it reduced the mortality of embryos (from 21.46 ± 2.31% to 6.89 ± 0.29%), diminished the FPV Log₁₀EID₅₀ titer from 3.86 to 2.78 and its percentage viral reduction index for prophylactic assay was at 91.7%. However, the *V. amygdalina* extract showed better therapeutic potential with inhibition concentration (IC₅₀) of 179.90 ± 2.74 µm, it strongly reduced the embryo mortality (from 21.46 ± 2.31% to 7.72 ± 0.34%) and the FPV Log₁₀EID₅₀ titer diminished from 3.86 to 3.08 while it had a viral percentage reduction index of 83.4% for the therapeutic assay. This study demonstrated the antiviral efficacy of derivatized extracts from the two test plants against FPV and that compounds contained in the extracts can serve as molecular leads in subsequent study for biosynthesis of novel anti-poxvirus inhibitors.

Keywords: Phenols; Nucleoside analogues; Antiviral potential; Fowl pox virus; Phytol; ECEs

INTRODUCTION

Fowl pox is an important disease of poultry and other domestic birds caused by fowl pox virus, a non-segmented DNA virus. The outbreak of the disease in poultry leads to important economic losses to the owners. Nyaga et al. reported the occurrence of an “atypical” Fowlpox virus (FPV) strain in Kabete, Kenya, and the virus strain caused heightened epithelial proliferation, mild paralysis, endotheliosis and lympho-proliferative damages of

matured birds [1]. Other studies in recent years affirmed that the atypical FPV might cause mild infections in man [2]. More so, Zhao et al. [3] characterized the atypical FPV whole genome and confirmed that the atypical FPV (Kabete) strain reported by Nyaga et al. [1] had reticulo-endotheliosis virus (REV) genes integrated into its whole genome. Hence, these reports underscore the need for alternative trials of natural products derived from antiviral compounds to combat the FPV strain

*Correspondence to: Afolami Olufemi Ifeoluwa, Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria, Tel: +2347069438529; E-mail: oiafolami@futa.edu.ng

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since live attenuated vaccines might not confer absolute immunity.

The recent strides made in pharmacopeia delineated the potency of phyto-compounds such as alkanols, nucleoside base analogues and phenols as drug leads against re-emerging infectious strains of viruses. Scientists regard nucleoside analogues as suitable antiviral drug leads since they block the replication of many enveloped DNA viruses in living tissues [4] or actively hinder the entry of viruses into living tissues [1]. In his holistic study, Nittya and Suaresh [5] suggested that the plant *V. amygdalina* Del; might possess antiviral compounds since local respondents in parts of Western Africa use it against measles invasion while another recent report from Faeji et al. [6] showed that extract from the plant have antiviral activity against Newcastle Disease Virus strain (NDV N1E). Moreover, *Aframomium melegueta* K. Schum. is another plant with a rich source of phenols and past findings showed the plant possess stellar antimicrobial efficacy, although, there exist scant data to demonstrate its efficacy against viruses [7]. Recent studies involving the testing of antiviral activity of plants have sought to use derivatized forms of plant extracts containing known organic compounds in verified quantities [4]. This has helped scientists to be more precise as regards dose dependent analysis of antiviral drug compounds and more importantly, to encourage compound biosynthesis.

In this study, we tested the antiviral efficacy of derivatized extracts from *A. melegueta* and *V. amygdalina* against an atypical Fowlpox virus strain (FPV Kabete). Preliminary studies on the derivatized extracts already showed that the *V. amygdalina* extract contain two abundant compounds (Phytol and a nucleoside analogue Methyl-2-O-benzyl-d-arabinofuranoside) while the *A. melegueta* extract contain two abundant phenols (benzaldehyde-3-hydroxy-4-methoxy and butan-2-one-4-(3-hydroxy-2-methoxyphenyl)). We determined the multiple therapeutic and prophylactic effects of these compounds in derivatized extracts from the two plants against FPV on Embryo Chick Egg cultures (ECEs) and linked the possible mechanisms of action responsible for the antiviral efficacy exhibited by these compounds in the extracts.

MATERIALS AND METHODS

A brief on the major organic compounds present by mass in derivatized extracts

Extracts from the test plants *A. melegueta* and *V. amygdalina* were obtained by adopting procedures described by Huang et al. [8]. After extraction process, protocols native to the Agilent Turbo Mass Spectrophotometer 7890A GC with chemstation NIST was adopted for derivatization of the extracts obtained [9]. Identification and quantification of the major organic compounds in the derivatized extracts were carried out using Agilent Turbo Mass Spectrophotometer 7890A GC with chemstation NIST for Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The two concentrates from the plants have been analyzed and clarified prior to antiviral assay. Full details of these procedures are already in a publication (short

communication) currently undergoing peer review but summarized in Figures 1 and 2.

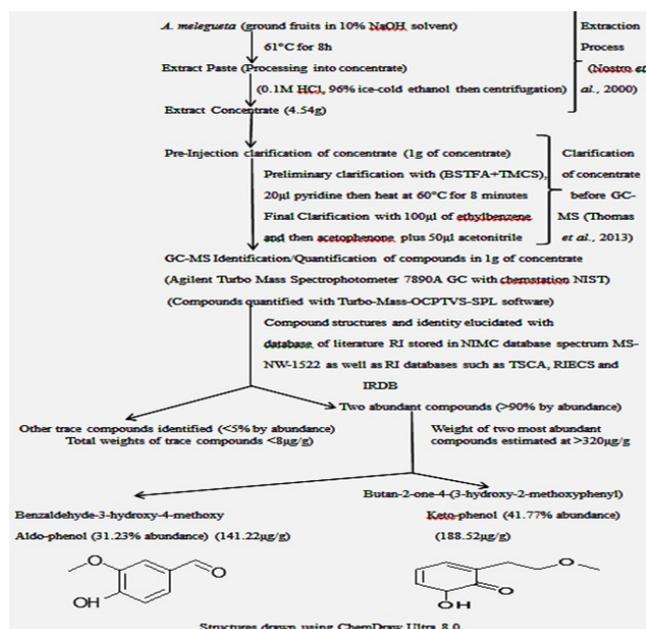


Figure 1: Preliminary chart on the GC-MS analysis of derivatized extract from *A. melegueta*.

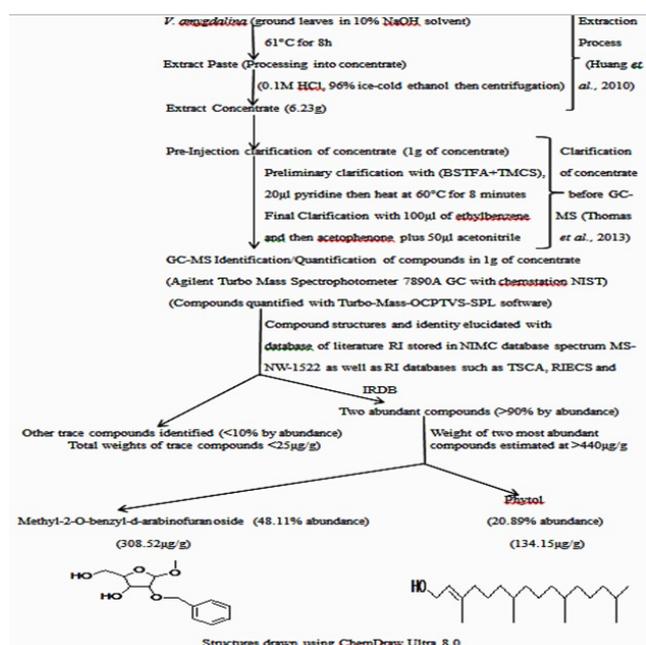


Figure 2: Preliminary chart on the GC-MS analysis of derivatized extract from *V. amygdalina*.

Virus source and Egg Cultures (ECEs)

The atypical strain of Fowlpox virus (FPV Kabete) used in this study was obtained from the Viral Vaccines Production Division of National Veterinary Research Institute (NVRI), Vom and Embryonated Chick Eggs (ECEs) aged 12 days were from the poultry division of NVRI, Vom, Nigeria. Before use, the embryonated eggs were candled to check for viability. The viable eggs were punctured at the chorio-allantoic region and at a region about 3 mm below the air sac, using pressure from a

vacuum pump (Z844-V Weitzler, Germany) to create artificial space and collapse the chorioallantoic membrane for inoculation of the extracts into Chorioallantoic membrane (CAM). The in-ovo extract toxicity assay, determination of embryo infectious dose (EID₅₀), inhibitory and therapeutic effects of derivatized extracts using ECEs were carried out at viral research division of the NVRI, Vom, Nigeria.

Reconstitution of derivatized extracts before antiviral assay

A 50 mm stock solution of the derivatized extracts were prepared by reconstituting 0.5 g of the each extract obtained after the GC-MS into 10 ml of the diluent consisting of Penicillin, Streptomycin, Gentamycin, and Amphotericin B (PSGA X5) adjusted to pH 7.2. The resulting solutions were filtered using 0.23 µm membrane filters *via* suction pressure filtration into sterile vials and stored at -4°C until needed.

Acute toxicity assay of derivatized extracts on ECEs

The toxic effects of derivatized extracts on chick embryos were tested to determine its minimum toxic concentration in 13 days old ECEs. The 13 days old ECEs were divided into four groups of five ECEs per group. The ECEs carried labels as appropriate in biosafety cabinets and acclimatized ECEs in humidified incubator (Schumboldt CR-8V CO₂ pressure Incubator). From the 50 mm stock earlier prepared, three different dilutions of the derivatized extracts (450 µm, 300 µm, and 150 µm) were obtained. The eggs were swabbed with 70% ethanol and a 0.2 ml of each dilution was separately inoculated into the allantoic cavity of labeled ECEs in their respective groups while the control groups received 0.2 ml PBS+PSGA X5 [6]. The inoculation sites were sealed with paraffin wax and the eggs were incubated for 120 hours at 37 ± 1°C. The eggs were candled daily and dead embryos were removed. At the end of the incubation period, embryos were harvested and the toxic effect of the various dilutions of the concentrate on the embryos were determined by examining them for hemorrhages and evaluating percentage mortality of embryos in each group [6].

Determination of Initial Egg Infectious Dose (EID₅₀) of FPV

The determination of the mean embryo infectious dose (EID₅₀) of FPV Kabete was according to the methods of Nyaga et al. [1] and Young et al. [10]. A 0.9 ml of PBS was dispensed into labeled sterile tubes and a 0.1 ml of the virus stock was used to prepare serial dilutions of 10⁻¹ to 10⁻⁷. The 13 days old ECEs were divided into seven groups of five Eggs per group and labeled appropriately under biosafety cabinets. The eggs were swabbed with 70% ethanol and inoculated ECEs with 0.1 ml of each virus dilution *via* the CAM. Inoculation sites were sealed with paraffin wax and eggs were incubated for 120 hours at 37 ± 1°C. The inoculated eggs were candled daily to check for mortality. At the end of the incubation period, the chorioallantoic membranes (CAMs) of surviving ECEs were harvested aseptically and examined for pock lesions. The Spearman-Kärber formula (1931) was used to calculate the Egg infectious dose 50 (EID₅₀) of the virus.

Antiviral assays of derivatized extracts against FPV

The antiviral efficacy of extracts against FPV using ECEs was according to the methods of Nyaga et al. [1] and Faeji et al. [6]. From the toxicity assays, the minimum toxic concentration threshold of 300 µm was used and a 100 EID₅₀ virus stock solution was prepared. The 13 days old ECEs were divided into six test groups of fifteen ECEs each while the control groups had six ECEs. The eggs were labeled as appropriate and sites of inoculation were swabbed with 70% ethanol under biosafety cabinets. To perform the inhibition assay, a 0.1 ml/300 µm of each extract was first inoculated into the ECEs *via* the CAM infection route and incubated for at 37°C for 90 minutes for the concentrate to adsorb to the CAM. At the end of 90 minutes, 100 EID₅₀/0.1 ml FPV was inoculated into the eggs. The eggs were sealed with paraffin wax and incubated at 37°C for 120 hours. The eggs were candled daily and dead embryos were removed. To perform the therapeutic assay, a 100 EID₅₀/0.1 ml FPV was first inoculated into the eggs *via* the CAM and incubated for 90 minutes for virus adsorption. At the end of 90 minutes 0.1 ml/300 µm of each extract was inoculated into the eggs. The eggs were sealed with paraffin wax and incubated at 37°C for 120 hours. The eggs were candled daily and dead embryos were removed. At the end of the incubation periods, CAM and embryos were aseptically harvested from the surviving eggs. The embryos were examined for deformities and the mortality rates of the chick embryos were recorded for each group. The dose dependent concentration (IC₅₀) of each derivatized extract against FPV was also obtained for therapeutic and prophylactic assays.

Determination of final FPV EID₅₀ titer after treatment assays

The CAMs from the treatment groups were harvested separately into sterile cruise vessels and CAMs were soaked in PBS for 4 hours. The soaked CAMs were crushed and homogenized with a pestle. The marshed CAMs were centrifuged at 5000 rpm for 30 minutes to obtain homogenates of the CAMs. From this, a 20% of the homogenate was prepared as the virus stock for final FPV EID₅₀ determination.

The same procedures earlier described for the initial EID₅₀ determination as described by Nyaga et al. [1] and Young et al. [10] was repeated and the final EID₅₀ FPV titer was determined using the Spearman-Kärber formula (1931).

Data analysis

Different statistical tools in GraphPad Prism 5.0 were used to achieve all data analyses in this study. The reduction index of FPV EID₅₀ titer (initial and final) after treatments assays, the activity curves of extracts against FPV EID₅₀ and the average mortality of eggs across different treatment groups were analyzed.

Furthermore, the antiviral activity of derivatized extracts by dose dependent 50% inhibition concentration (IC₅₀) was determined using non-linear regression analyses.

RESULTS

Toxicity of extracts in ECEs

The derivatized extracts contained different compounds that have been characterized in our preliminary studies. However, the major compounds had >70% abundance by weight after compound recovery by GC-MS in the derivatized extracts from the two plants. The two compounds most abundant in *V. amygdalina* extract were Phytol with 20.89% abundance (a recovery index of 134.15 µg per 1 g) and the nucleoside analogue Methyl-2-O-benzyl-d-arabinofuranoside with 48.11% abundance (recovery index of 308.52 µg per 1g) while two

compounds most abundant in *A. melegueta* extract were the aldo-phenol benzaldehyde-3-hydroxy-4-methoxy with 31.23% abundance (a recovery index of 141.22 µg per 1 g) and the keto-phenol butan-2-one-4-(3-hydroxy-2-methoxyphenyl) with 41.77% abundance (recovery index of 188.52 µg per 1 g). The information on the toxicity assay of the extracts from the plants is in Table 1. Evaluations on the probable toxicity of the extracts in ECEs were done by juxtaposing the mortality rates and occurrence of deformities in the embryos with the control groups. More so, the mortalities across the different dilutions of the derivatized extracts from both plants were also compared with the standard index set for atypical Fowl pox virus.

Table 1: The mean mortality of ECEs with respect to toxicity of derivatized extracts.

Test Plants	Test Groups (Extract dilutions)	Mean Mortality in test groups (%)	Mean Mortality in Control (Ct. G) (%)	Mean Mortality threshold set for ECE toxicity (%)	Mortality Deformities for ECEs (Test groups)	Deformities in ECEs (Control Ct. G)
<i>V. amygdalina</i>	ED ₁	22.22 ± 2.05 ^d	00 ± 00 ^a	30.00 ± 1.00 ^e	Absent	Absent
	ED ₂	12.59 ± 2.06 ^{bc}	00 ± 00 ^a	30.00 ± 1.00 ^e	Absent	Absent
	ED ₃	9.40 ± 1.19 ^b	00 ± 00 ^a	30.00 ± 1.00 ^e	Absent	Absent
<i>A. melegueta</i>	ED ₁	20.37 ± 1.40 ^d	00 ± 00 ^a	30.00 ± 1.00 ^e	Absent	Absent
	ED ₂	7.72 ± 0.34 ^b	00 ± 00 ^a	30.00 ± 1.00 ^e	Absent	Absent
	ED ₃	5.34 ± 1.67 ^b	00 ± 00 ^a	30.00 ± 1.00 ^e	Absent	Absent

The second dilution (ED₂) was chosen as the concentration to use for the antiviral assays in both plants

Keys: ED₁-450 µm dilution, ED₂-300 µm dilution, ED₃-150 µm dilution, Ct. G-Control group having no treatment with extracts, values with same superscript have no significant difference at p ≤ 0.05.

The mean Embryo Infectious Dose (EID₅₀) of FPV in ECEs

The Table 2 shows how the EID₅₀ of FPV was determined in ECEs using the Spearman-Karber formula. The result suggests

that the atypical FPV has high virulence in ECEs since comparably lower dilutions of the FPV stock still had mortalities across the treatment groups observed in this study.

Table 2: Egg Infectious Dose determination (EID₅₀ Determination).

Dilutions factors of 10	(in Pock (P)	Positive Pock (N)	Negative	Cumulative Positive (C _p)	Cumulative Negative (C _n)	Overall positive Cumulative (%)	Extrapolation
A	5	0	13	0	100	~	
B	4	1	8	0	80	~	
C	3	2	4	2	66.7	P.D.	
D	1	4	1	6	14.29		
E	0	5	0	11	0	~	
F	0	5	0	16	0	~	

G	0	5	0	21	0	~
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Keys: A: Dilution 1, B: Dilution 2, C: Dilution 3, D: Dilution 4, E: Dilution 5, F: Dilution 6, G: Dilution 7, P.D.: Partial difference. Authors harvested all the ECEs and observed for pock formation after incubation period of 120 hours.

The EID₅₀ was determined using the Spearman-Kärber formula of $\text{LogEID}_{50} = \{X + \frac{1}{2}(\log d) - \{(\sum B/n) + 1 + P.D.\}$

Where

X is the range of all dilutions used,

d is the square of dilution factor constant (10),

B is the negative cumulative below 50%,

n is dilution factor below 50% and

P.D. is the partial difference.

Partial Difference (P.D)

$$= \frac{\text{Next above 50\%} - 50\%}{\text{Next above 50\%} - \text{Next below 50\%}} \times \text{Log}_{10}10$$

$$\{66.7 - 50 / 66.7 - 14.29\} = \{16.7 / 52.4\} = 0.32$$

Hence, Partial Difference (P.D)=0.32.

$$\text{Log}_{10}\text{EID}_{50\%} = \{X + \frac{1}{2}(\log d) - \{(\sum B/n) + 1 + P.D.\}$$

From the table, X=6, d=100, B=11, P.D.=0.32.

$$\text{LogEID}_{50} = 6 + \frac{1}{2}(\text{Log}_{10}100) - \{(11/6) + 1 + 0.32\}$$

$$\text{LogEID}_{50} = (6 + 1) - \{1.82 + 1 + 0.32\}, \text{LogEID}_{50} = 7.0 - 3.14. \text{LogEID}_{50} = 3.86. \text{EID}_{50} = \text{Antilog } 3.86$$

Hence, EID₅₀=7244.4 ml⁻¹

Therefore Viral Titer EID₅₀=Log103.86/0.1 ml of FPV or 0.01: 72.44(1/7244.4 ml)

Evaluation of antiviral efficacy of derivatized extracts against atypical FPV

The results in Tables 3 and 4 showed that the extracts drastically reduced the lethality of the atypical FPV in ECEs as evidenced in the mortality rates. The tables also showed that the extract's activity against the lethality of the FPV depends on the type of treatments used whether prophylactic or therapeutic. In Table 3, the *A. melegueta* extract reduced the lethality of the FPV more in the prophylactic treatment (reduction of mortality from 21.46 ± 2.31% to 6.89 ± 0.29%) while Table 4, shows that the *V. amygdalina* extract reduced the lethality of the virus more in the therapeutic treatment (mortality was reduced from 25.09 ± 2.14% to 7.72 ± 0.34%) (Table 5). In the Figures 3 and 4, the virus titer curves of the extracts against the FPV virus showed the reduction in the FPV viral activity caused by the administration of the extracts both in prophylactic and therapeutic assays.

Table 3: Reduction of mortality rates in ECEs by derivatized *A. melegueta* extract against FPV.

Test Plant	Treatment Types	Test Groups	Mean Mortality in test groups (%)	Mean mortality virus group (G ₂) (%)	Mean Mortality threshold set for antiviral assays (%)	Deformities in ECEs (Test groups)	Deformities in (virus control G ₂)
<i>Aframomium Melegueta</i>	Therapeutic Assay	G ₁	10.47 ± 1.46 ^c	21.46 ± 2.31 ^d	50.00 ± 1.00 ^e	Present	Present
		G ₃	3.39 ± 0.57 ^{ab}	21.46 ± 2.31 ^d	50.00 ± 1.00 ^e	Absent	Present
		G ₄	00 ± 00 ^a	21.46 ± 2.31 ^d	50.00 ± 1.00 ^e	Absent	Present
	Prophylactic Assay	G ₁	6.89 ± 0.29 ^b	21.46 ± 2.31 ^d	50.00 ± 1.00 ^e	Absent	Present
		G ₃	3.39 ± 0.57 ^{ab}	21.46 ± 2.31 ^d	50.00 ± 1.00 ^e	Absent	Present
		G ₄	00 ± 00 ^a	21.46 ± 2.31 ^d	50.00 ± 1.00 ^e	Absent	Present

The *A. melegueta* extract reduced the embryo mortality in both assays, but the extract was more potent for the prophylactic inhibition rather than therapy

Dose dependent activity of derivatized extracts against atypical FPV

The summary of the dose dependent antiviral efficacy of the derivatized extracts from the two test plants against the atypical

FPV for both treatment assays (prophylactic and therapeutic) are in Table 4. The Table 4 shows have the percentage reduction index for *A. melegueta* extract (69.5% viral reduction for therapeutic assay, 91.7% viral reduction for prophylactic assay) and the percentage reduction for *V. amygdalina* (83.4% viral

reduction for therapeutic assay, 61.1% reduction for prophylactic assay). Finally, the Table 4 also showed the effective dose for the derivatized extracts from the two plants. The *A. melegueta* extract had a better dose dependent average (IC₅₀ of 159.49 ± 2.14 μm

for prophylactic and IC₅₀ of 216.85 ± 4.18 μm for therapeutic) compared to that of *V. amygdalina* (IC₅₀ of 179.90 ± 2.74 μm for therapeutic assay and IC₅₀ of 240.50 ± 4.68 μm for the prophylactic assay).

Table 4: Reduction of mortality rates in ECEs by derivatized *V. amygdalina* extract against FPV.

Test Plant	Treatment Types	Test Groups	Mean Mortality in test groups (%)	Mean mortality in virus control group (G ₂) (%)	Mean Mortality threshold for antiviral assays (%)	Deformities in ECEs (Test groups)	Deformities in ECEs (virus control G ₂)
<i>V. amygdalina</i>	Therapeutic Assay	G ₁	7.72 ± 0.34 ^b	25.09 ± 2.14 ^d	50.00 ± 1.00 ^e	Absent	Present
		G ₃	3.95 ± 0.62 ^{ab}	25.09 ± 2.14 ^d	50.00 ± 1.00 ^e	Absent	Present
		G ₄	00 ± 00 ^a	25.09 ± 2.14 ^d	50.00 ± 1.00 ^e	Absent	Present
	Prophylactic Assay	G ₁	12.72 ± 1.22 ^c	25.09 ± 2.14 ^d	50.00 ± 1.00 ^e	Present	Present
		G ₃	3.95 ± 0.62 ^{ab}	25.09 ± 2.14 ^d	50.00 ± 1.00 ^e	Absent	Present
		G ₄	00 ± 00 ^a	25.09 ± 2.14 ^d	50.00 ± 1.00 ^e	Absent	Present

The *V. amygdalina* extract reduced the embryo mortality rate more for the therapeutic assay rather than for prophylactic inhibition

Keys: G₁-test group with 300 μm concentrate plus 100EID₅₀ FPV, G₃-Virus control with 100EID₅₀ FPV alone, G₃-concentrate control with 300 μm concentrate alone, G₄-ECE control with no treatment.

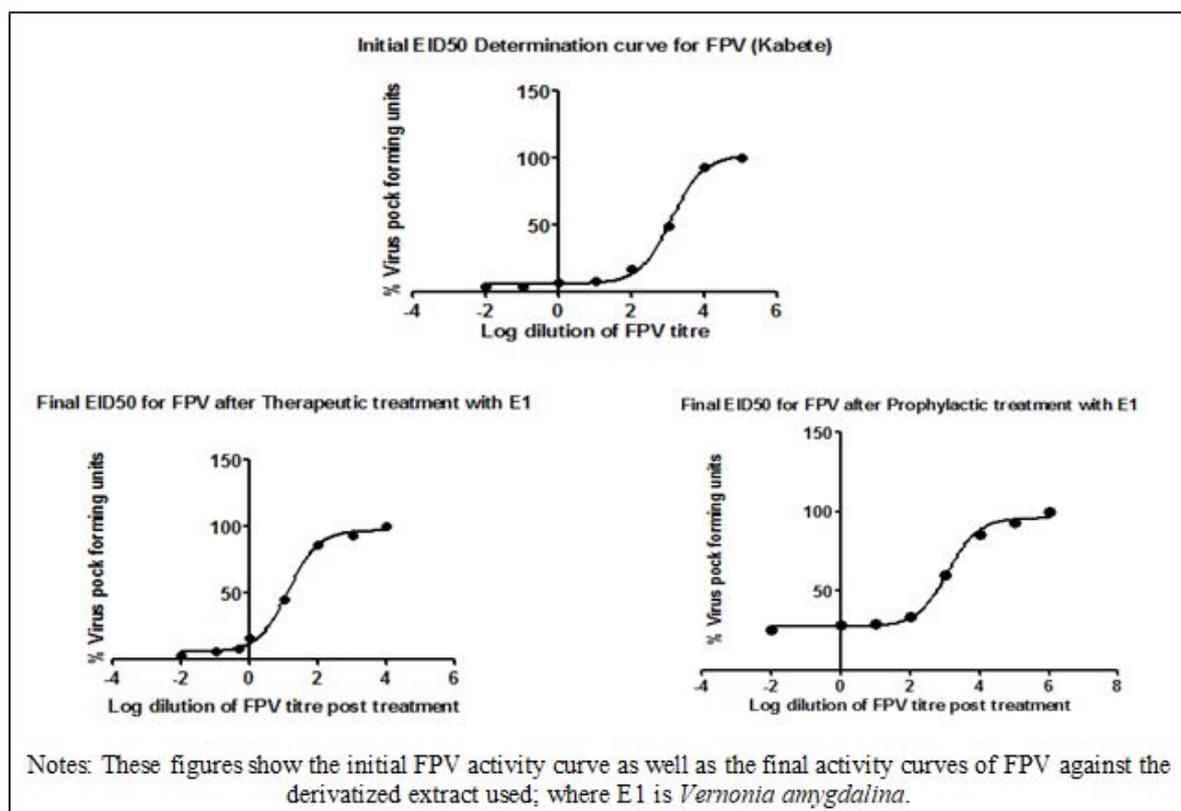


Figure 3: FPV initial activity curve and final viral activity curves for derivatized *V. amygdalina* against FPV.

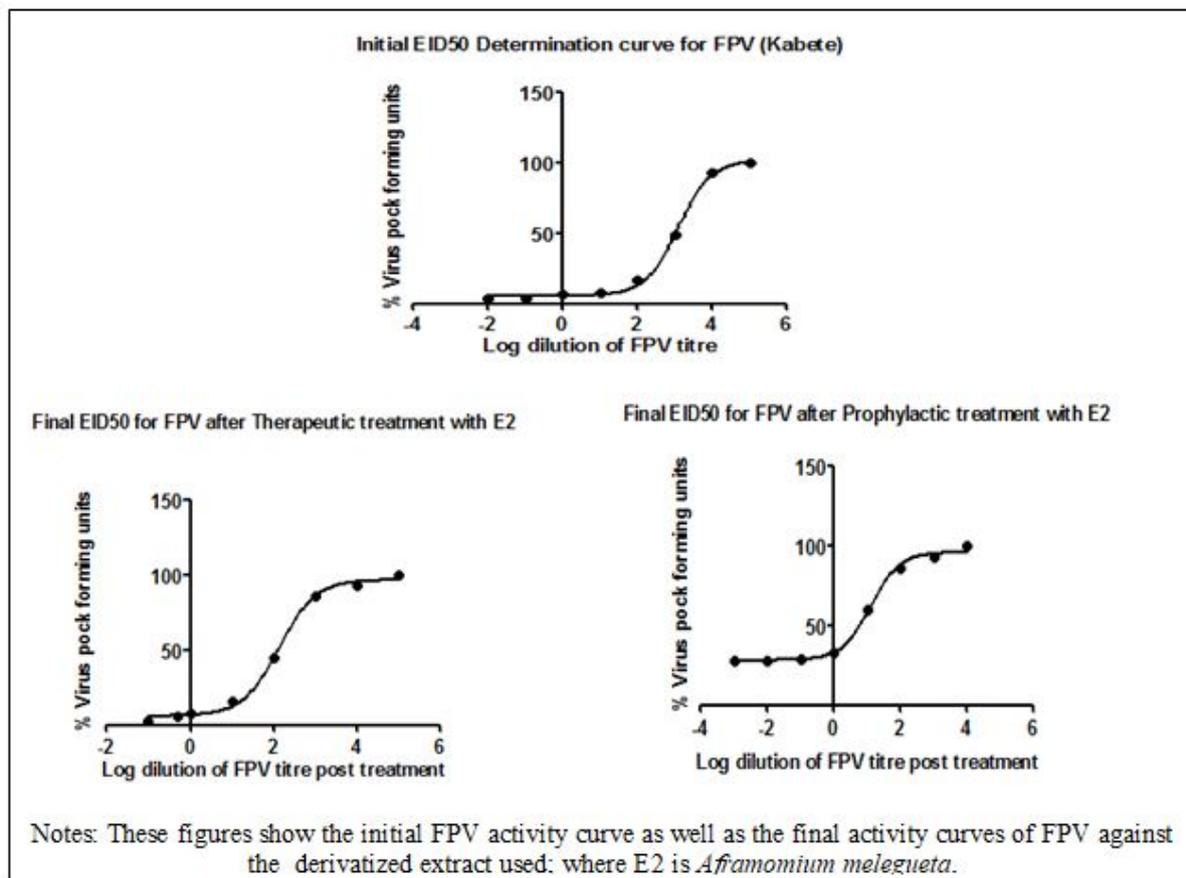


Figure 4: FPV initial activity curve and final viral activity curves for derivatized *A. melegueta* against FPV.

Table 5: Viral Reduction potential and dose dependent index of derivatized extracts from test plants.

Test Plant	Treatment Types	Initial FPV Titre		Inhibition Concentration of extract concentrate (IC50) μm	Final FPV Titre		Reduction Index of extract concentrate (%)
		Log10X	Equivalent volume (ml-1)		Log10X	Equivalent volume (ml-1)	
<i>A. melegueta</i>	Prophylactic	3.86	7244.40	159.49 \pm 2.16	2.78	601.30	91.70
	Therapeutic	3.86	7244.40	216.85 \pm 4.18	3.34	2209.50	69.50
<i>V. amygdalina</i>	Prophylactic	3.86	7244.40	240.50 \pm 4.68	3.45	2818.10	61.10
	Therapeutic	3.86	7244.40	179.90 \pm 2.74	3.08	1202.60	83.40

The results above showed that *A. melegueta* extract was more potent for inhibition of atypical FPV while it was less potent for therapy of FPV infections

These data shows that *V. amygdalina* extract was more potent for therapy of atypical FPV while it was less potent for prophylactic inhibition of FPV infections

DISCUSSION

The most abundant compounds in the derivatized extracts [benzaldehyde-3-hydroxy-4-methoxy and butan-2-one-4-(3-hydroxy-2-methoxyphenyl)] for *A. melegueta* and [furan-based

nucleoside analogue Methyl-2-O-benzyl-d-arabinofuranoside and the long chain alcohol Phytol] for *V. amygdalina* might have some degrees of toxicity on embryos in the ECEs (Table 1). Several reasons may account for the toxic effects of the extracts on the chick embryos. Jena et al. showed that a lethal dose (>1000 μm)

of some nucleoside analogues triggers hepatic steatosis in hepatocytes of Wister albino rats eventually resulting in weight reduction of the rats [11]. The study also attributed tissue genotoxic damage to the overdose of nucleoside analogues since they are weak substrates of host tissue DNA polymerases.

Meanwhile, Islam et al. showed by his findings that phytol at concentrations above 600 µm form nano-emulsions in young tissues (artem salina model) thereby blocking kinase dependent inhibitors in such tissues [12]. Likewise, studies focusing on the dose dependent pharmacology of phenolic compounds have highlighted some of their potential toxic responses in living tissues. A study by Tapeiro et al. showed that some tissue functions become impaired at concentrations above 700 µm of plant phenols [13]. The study also attributed tissue surface pro-oxidation to phenolic overdose in Chick hamster cultures. Furthermore, Manach et al. posited that phenols might form inhibition complexes that hinder chemical factors involved in early prophase stage of mitosis by blocking protease dependent activators, leading to toxicity [14].

The mortalities associated with infections of poxviruses in tissues (ECEs) are generally low given the precedence of recent research. Singh and Tripathy explained this in citing most early reports in the last century of research focused on poxviruses and their lethality in living tissues [2]. However, the same study explained that the atypical FPV first reported in Kabete, Kenya has increased virulence and higher the average mortality than those observed in many earlier findings. As evident in the results (Table 2, Figures 3 and 4), the FPV activity and initial/final FPV EID₅₀ threshold showed higher virulence than what was expected on the average. This affirmed why the prospect of live attenuated vaccine production for the atypical FPV was quite infeasible in Zhao et al. [3]. The results (Figures 3 and 4) also showed how the antiviral efficacy of the extracts was since the replication and infection of FPV starts from the epithelial tissues of the ECEs resulting in virions multiplication and deformities of ECE embryos.

The strong antiviral efficacy of the extracts might be due to several cumulative factors. The phenols in the *A. melegueta* extract reacts directly with FPV lipid envelope and partially alter its recognition molecules [15,16]. However recently described how phenols ionize surface receptors on host tissues to cause reduction in the ability of viruses to make direct attachment to host tissues. This cause ligand induced non-fusogenic dissolution of viral membranes; perforating the FPV lipid bilayer envelope [16]. Since the *A. melegueta* extract contained two phenols in abundance, it follows that the phenols provide enough ions into cell cytoplasm, which will weaken the action of the FPV decapping enzymes D₉ and D₁₀ reducing the ability of the virus to replicate effectively [16]. These mechanisms involved in the antagonistic properties of the compounds in the *A. melegueta* extract ensures that the FPV is deactivated even before it can attach to the host tissues. This strongly explains why in the results, the *A. melegueta* extract had better prophylactic efficacy than the *V. amygdalina* extract.

However a study by Reaves et al. showed that phytol one of the compounds of abundance in the *V. amygdalina* extract cause production of weakened virions since the -OH end of phytol can

de-hydrolyse the loose phosphate complexes of the viral DNA fragments involved in viral genome replication [15]. The phytol also weakens the recognition potential of FPV DNA polymerase, which then causes ineffective replication of virions effectively terminating the viral cycle. Alternatively, the *V. amygdalina* extract contain the furan-based nucleoside analogue Methyl-2-O-benzyl-d-arabinofuranoside; Hofman and Nelson showed that nucleoside analogues act as substrate mimics for early and late transcription promoter (TpV₁ and TpV₅) of the FPV thereby blocking effective viral replication in living tissues [17]. Furthermore, the benzyl-group attached to the furan-based nucleoside in the *V. amygdalina* extract affords it an advantage of mimicking standard purine bases without losing its toxicity after contact with the FPV concoctamer complex during late translation [15]. In synchrony, the ways by which the abundant compounds of the *V. amygdalina* extract exert antiviral effects against the FPV shows that the compounds act better in a late phase of the viral cycle when infection has already been established. This accounts for why the *V. amygdalina* extract showed more therapeutic efficacy than the *A. melegueta* extract in the results. This study is limited majorly to the mechanisms by which the test plants act against the atypical FPV in ECEs alone, hence further studies are recommended [18,19].

CONCLUSION

The findings of this current study indicate that the compounds butan-2-1-4-(3-hydroxy-2-methoxyphenyl), benzaldehyde-3-hydroxy-4-methoxy, phytol and Methyl-2-O-benzyl-d-arabinofuranoside in the derivatized extracts from both plants may serve as molecular leads in subsequent study for development of new anti-poxvirus inhibitors in living tissues.

CONFLICTS OF INTEREST

There authors declared no conflict of interests.

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