Journal of Shahrekord University of Medical Sciences
doi: 10.34172/jsums.1050 2025;27(4):175-185 http://j.skums.ac.ir

Original Article

In ovo Antiviral Screening of Four Nigerian Medicinal Plants (*Artemisia absinthium* L., *Annona senegalensis* Pers., *Erythrina senegalensis* DC., and *Jatropha curcas* L.) Against Newcastle Disease Virus

Sylvester Nefai Mathias[1*], Emmanuel Halilu Mshelia[1,2], Chinenye Jane Ugwah-Oguejiofor[3], Ibrahim Yusuf Alkali[3], Aminu Ahmed Biambo[4], Mansur Lawal[1], Aliyu Hamidu Ahmed[1]

[1]Department of Pharmacognosy and Ethnomedicine, Usmanu Danfodiyo University, Sokoto, Nigeria
[2]Faculty of Pharmacy, Cyprus International University, Haspolat/Nicosia, Mersin, Turkey
[3]Department of Pharmacology and Toxicology, Usmanu Danfodiyo University, Sokoto, Nigeria
[4]Department of Clinical Pharmacy and Pharmacy Practice, Usmanu Danfodiyo University, Sokoto, Nigeria

*Corresponding Author: Sylvester Nefai Mathias, Email: sylver_74ng@yahoo.com

Abstract

Background and aims: Newcastle disease virus (NDV) poses a major threat to global poultry production, driving interest in novel plant-based antiviral agents. This study evaluated the *in ovo* antiviral potential of methanol extracts from *Artemisia absinthium* (AA), *Annona senegalensis* (AS), *Erythrina senegalensis* (ES), and *Jatropha curcas* (JC) against NDV.

Methods: The extracts were screened for phytochemical content, elemental composition, and cytotoxicity in embryonated chicken eggs. Antiviral activity was assessed through embryo survival, hemagglutination assay, and statistical analysis. NDV infectivity was validated using the Reed-Muench method to determine the 50% egg infectious dose (EID_{50}).

Results: All extracts contained flavonoids, saponins, alkaloids, and glycosides. Elemental analysis revealed high levels of calcium, iron, potassium, and zinc. Cytotoxicity studies showed that all extracts were safe at concentrations up to 300 mg/mL. JC demonstrated the strongest antiviral effect, with 0% virus-induced mortality at 200–300 mg/mL (10^{-7}–10^{-8} dilutions), followed by ES. AS represented limited activity, while AA was the least effective. Statistical analysis confirmed significant antiviral actions for JC ($P<0.001$) and ES ($P<0.01$), while AS was borderline ($P=0.072$), and AA was not significant. Finally, a significant overall treatment effect on cytotoxicity was observed as well ($P<0.001$).

Conclusion: JC and ES exhibited promising antiviral activity against NDV *in ovo*, with JC being the most potent. However, its effectiveness was dose-dependent, and embryotoxicity occurred at higher levels. These findings support further investigation into their use as plant-based antiviral agents for poultry disease management.

Keywords: Medicinal plants, Antiviral agent, *Jatropha curcas*, EID_{50}, Embryonated chicken eggs, Phytochemicals

Received: January 18, 2025, **Revised:** July 14, 2025, **Accepted:** July 15, 2025, **ePublished:** December 29, 2025

Introduction

Medicinal plants are widely used in traditional medicine across many cultures, particularly in resource-limited settings. According to the World Health Organization, 170 out of 194 member states report using traditional medicine, often as the first line of treatment (1). In Nigeria, plants like *Artemisia absinthium* (AA), *Annona senegalensis* (AS), *Erythrina senegalensis* (ES), and *Jatropha curcas* (JC) are commonly utilized to manage various ailments due to their diverse pharmacological properties (2–4). However, many of these uses lack rigorous scientific validation (3, 5, 6).

Newcastle disease virus (NDV) remains a major threat to poultry worldwide, especially in developing countries where vaccination is inconsistent (7). The emergence of resistant strains has created an urgent need for alternative antiviral agents. AA is traditionally used in northern Nigeria to treat malaria and other infections (2, 8). In addition, AS is applied in folk medicine for tuberculosis, ulcers, and cancer (9, 10). Further, ES is used for conditions such as malaria and convulsions (11, 12), while JC is employed in managing infections, including human immunodeficiency virus and gonorrhea (13,14). These plants contain bioactive compounds, such as alkaloids, flavonoids, and terpenoids, known for potential antiviral activity (14).

Despite their widespread use, limited data exist on their efficacy and safety against viral infections, including NDV. This study investigates the phytochemical composition and *in ovo* antiviral activity of AA, AS, ES, and JC against

NDV to explore their potential as plant-based antiviral candidates.

Materials and Methods

Collection and Identification of the Plant Material

The whole plant, AA, was obtained from herbal vendors in the Sokoto market, who had previously sourced it from 'Kwani', a border town in the Republic of Niger. The stem bark of AS was collected from Sokoto South LGA, while that of ES was gathered from Tureta LGA. Additionally, the root bark of JC was harvested from the Usmanu Danfodiyo University (UDUS) grounds in Wamako LGA, all located in Sokoto State, Northwestern Nigeria. The collected plants were authenticated at the Herbarium Unit of the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, with voucher numbers assigned as PCG/UDUS/Astr/0001, PCG/UDUS/Ann/0008, PCG/UDUS/Faba/0027, and PCG/UDUS/Euph/007, respectively.

Plant Preparation

The whole plant of AA, the stem bark of AS and ES, as well as the root bark of JC, were air-dried and pulverized into moderately coarse powder and stored in a moisture-free container for further use (12).

Extraction of Plant Materials

The powdered plant material of 1 kg for each of the plants was extracted with 70% methanol by maceration for 7 days. The methanol volume of 4 mL was used for 1 g of the powdered plant (ratio 4:1) (w/v). The extract was concentrated *in vacuo* (reduced pressure at 45°C in the rotary evaporator) and partially freeze-dried to yield the residue of the crude aqueous methanol extract of AA, AS, ES, and JC. These were stored in a desiccator prior to further use. The percentage yield of each extract was estimated using the following formula:

$$\%\ \text{yield} = \frac{\textit{Amount of CAME of Plant } x}{\text{Amount of Powdered } \textit{Plant } x} \times 100\%$$

Phytochemical Screening

The qualitative chemical tests for various phytoconstituents were performed on the crude aqueous methanol extract of all four plant extracts (AA, AS, ES, and JC), using the methods described by Trease and Evans (15).

Elemental Analysis (X-Ray Fluorescence)

The samples (the crude powdered drugs of AS, ES, and JC) were crushed using a Mixer/Mill (8000 M) SPEX sample preparation machine for 5 minutes. The powder was transferred into a pellet preparation sample holder and compressed using a hydraulic press at 35 MPa. This was analyzed on XFF ZSX Primus (Rigaku), following the methods described in previous studies (16, 17). However, the crude powdered sample of AA was not suitably conducted.

Antiviral Assay

Source of the Virus and 9-Day-Old Embryonated Chicken Eggs

A velogenic (KUDU 113 strain) of NDV and embryonated chicken eggs were obtained from the Viral Research Department and the Poultry Division, respectively, both of the National Veterinary Research Institute (NVRI), Vom, Nigeria. The entire antiviral assay was thus performed at NVRI.

Cytotoxicity Test of Extracts

Collection of Eggs

The egg trays were disinfected by soaking in the Virkon solution and thoroughly rinsed with water. A total of 10 crates containing 9-day-old embryonated eggs were used for the experiment. Candling was performed to assess the fertility of the eggs, with non-viable (dead) eggs being removed and the viable ones prepared for inoculation.

Sterility Check of Extracts

The plant extracts underwent a sterility test to ensure that they were free from contamination prior to use (18). The inoculated extracts were incubated at 37°C and room temperature (25°C) for 24–72 hours. After incubation, the extracts were examined for signs of contamination. Any extracts found to be contaminated were plated and treated with an appropriate combination of antibiotics (PSGA) to eliminate the contaminants.

Reconstitution of the Extract

Initially, 5 g of the extract was dissolved in 10 mL of phosphate-buffered saline (PBS), implying that for 2 g of the extract, 4 mL of PBS was required as the stock solution, which was calculated as:

$10 \times 2/5 = 4$ mL of PBS.

Subsequently, the stock solution was adjusted for different extract concentrations as follows:

- For 400 mg of the extract:
  400 mg/500 mg = 4/5, implying 4 mL of the stock solution with 1mL of PBS
- For 300 mg of the extract:
  300 mg/500 mg = 3/5, indicating 3 mL of the stock solution with 2 mL of PBS
- For 200 mg of the extract:
  200 mg/500 mg = 2/5, representing 2 mL of the stock solution with 3mL of PBS
- For 100 mg of the extract:
  100 mg/500 mg = 1/5, implying 1 mL of the stock solution with 4mL of PBS

Inoculation of the Extracts into 9-Day Embryonated Eggs

The eggs were sterilized by wiping with 70% alcohol using cotton wool before being punctured with an egg puncher. A 0.1 mL volume of the plant extracts (AA, AS, ES, and JC) at different concentrations (500 mg, 400 mg, 300 mg, 200 mg, and 100 mg) was inoculated into each embryonated egg. After inoculation, the eggs were sealed

with an egg sealant and incubated at 37°C for 72 hours. Candling was performed every 24 hours to monitor and record any embryonic deaths, with non-viable eggs being removed.

Newcastle Disease Virus Egg Infectious Dose$_{50}$

The EID_{50} for NDV was determined using the method described by Young et al (19). A virus stock concentration of 100 EID_{50}/0.1 mL was prepared for the experiment. Four crates of embryonated eggs were candled to check viability. A 1:10 dilution of PSGA and distilled water was made to prevent contamination. The Kudu virus was also diluted 1:10 in distilled water, after which 0.5 mL of the virus was mixed with 4.5 mL of PBS. This solution was then serially diluted from 10^{-1} to 10^{-10}. In addition, 0.1 mL volume of each dilution (from 10^{-6} to 10^{-10}) was inoculated into five eggs. The eggs were sealed at the inoculation site and incubated at 37°C for 96 hours, with daily candling to monitor viability.

Antiviral Evaluation

Preparation of Extracts and Virus Solution (Serial Dilution of Extract/Virus Mixtures)

The plant extracts (AA, AS, ES, and JC) were prepared at concentrations of 100 mg/mL, 200 mg/mL, and 300 mg/mL. Ten sterile bijou bottles were sequentially labeled from 10^{-1} to 10-^{10}. Each bottle was filled with 4.5 mL of sterile distilled water, and 0.5 mL of each prepared extract was added, bringing the total volume in each bottle to 5 mL. The virus solution was prepared by adding equal volumes (0.5 mL each) of NDV suspension to each extract and mixing, resulting in virus-extract mixtures at concentrations of 100 mg/mL, 200 mg/mL, and 300 mg/mL. The mixtures were incubated at room temperature for 30 minutes to ensure thorough mixing and stabilization. Thus, for the serial dilution, 0.5 mL of each virus-extract mixture was transferred to the bottle labeled 10^{-1} and mixed well. A 2-fold serial dilution was then performed by transferring 0.5 mL from bottle 10^{-1} to bottle 10^{-2}, continuing this process through to bottle 10^{-10}.

Inoculation of Embryonated Chicken Eggs

Nine-day-old embryonated chicken eggs were divided into seven groups, with five eggs in each group. The eggs were cleaned with 70% alcohol, swabbed with cotton wool, and placed in sterilized plastic egg trays that had been disinfected with Virkon®. The eggs were then transferred to a microbiological safety cabinet for inoculation.

The inoculation procedure involved punching the eggs and injecting them via the allantoic cavity. The groups were inoculated with 0.1 mL of the virus-extract mixture as follows:

- Groups 1–3 were inoculated with virus-extract mixtures at concentrations of 300 mg/mL (group 1), 200 mg/mL (group 2), and 100 mg/mL (group 3).
- Group 4 received 0.1 mL of 100 EID_{50} standard NDV as the virus control.
- Group 5 received 0.1 mL of the extract suspension at 300 mg/mL as the extract control.
- Group 6 was inoculated with 0.1 mL of PBS as the diluent control.
- Group 7 was not inoculated with anything (uninoculated control).

Post-Incubation Testing

After inoculation, the holes in the eggs were sealed with molten wax, and the eggs were incubated at 37°C. Embryo survival was monitored daily, and some eggs were allowed to hatch to assess the effects of the treatments. Post-incubation, allantoic fluid was collected from the treated eggs for spot testing and hemagglutination testing to detect the presence of NDV.

Statistical Analysis

The data gathered from the antiviral and cytotoxicity tests were analyzed using IBM SPSS, version 23. Descriptive statistics were used to summarize the percentage of mortality and the rates of cytotoxicity. The chi-square test and Fisher's exact test were applied to assess the significance of differences in mortality and cytotoxicity between the treatment and control groups. Additionally, logistic regression analysis was conducted to examine how factors like extract concentration, exposure time, and extract type affect cytotoxicity and antiviral results, and $P \leq 0.05$ was deemed statistically significant.

Results

Extractive Yield of Extracts

Cold aqueous maceration with 70% methanol yielded four extracts with distinct appearances and efficiencies. AA, a golden-brown powder, had the highest yield (20%), followed by AS (dark brown, 16.75%), JC (reddish-brown, 8.19%), and ES (chocolate-brown, 5.6%). The extractive efficiency ranked as AA > AS > JC > ES, indicating that AA and AS were most effectively extracted under the given conditions. These differences likely reflect variations in phytochemical content and solubility. Table 1 summarizes the physical characteristics and percentage yields of each extract.

Phytochemical Screening of Artemisia absinthium, Annona senegalensis, Erythrina senegalensis, and Jatropha curcas Extracts

The phytochemical profile of the four plants (AA

Table 1. Extractive Yield of the Extracts

Extract Code	Physical Appearance	Weight (g)	Percentage Yield (%)
AA	Golden-brown powder	200.0	20.00
AS	Dark brown powder	167.5	16.75
ES	Chocolate brown powder	56.0	5.60
JC	Reddish-brown powder	81.9	8.19

Note. AA: *Artemisia absinthium*; AS: *Annona senegalensis*; ES: *Erythrina senegalensis*; JC: *Jatropha curcas*.

whole), (AS stem bark), (ES stem bark), and (JC root bark) screened in this study showed a diverse array of phytochemicals across different plant extracts, with alkaloids, anthraquinones, carbohydrates, flavonoids, glycosides, phenolics, proteins, saponins, steroids, tannins, and volatile oils notably detected (Table 2).

Elemental Compositions of Annona senegalensis, Erythrina senegalensis, and Jatropha curcas Plant Powder

Table 3 presents the elemental composition of plants AS, ES, and JC, based on experimental values obtained through X-ray fluorescence analysis. Various elements identified in the plant are stated along with their permissible limits, recommended daily allowances, and tolerable daily intake values for each element in each plant, offering a comparative framework to assess the nutritional and toxicological relevance of these plants. It is important to note that the analysis for AA was not performed in this study.

Figure 1 visually highlights each element's experimental percentage composition across the three plant samples (AS, ES, and JC) relative to their permissible limit, recommended daily allowances, or tolerable daily intake values.

Table 2. Phytochemical Profile of *Artemisia absinthium*, *Annona senegalensis*, *Erythrina senegalensis*, and *Jatropha curcas* Plants

Phytochemicals	*Plant Extracts Tested*			
	AA (wp)	AS (sb)	ES (sb)	JC (rb)
Alkaloid	-	+	+	+
Anthraquinones	-	+	+	-
Carbohydrates	+	+	+	+
Flavonoids	+	+	+	+
Glycosides	+	+	+	+
Phenolics	+	+	-	+
Protein	+	+	+	+
Saponins	+	+	+	+
Steroids	+	+	+	+
Tannins	+	+	+	+
Volatile oil	+	+	-	+

Note. Key: + = detected; - = not detected; Wp: Whole plant; sb: Stem bark; rb: Root bark.

Cytotoxicity of Artemisia absinthium, Annona senegalensis, Erythrina senegalensis, and Jatropha curcas Extracts In ovo

The cytotoxicity profiles of AA, AS, ES, and JC extracts were evaluated *in ovo* at concentrations ranging from 100 mg/mL to 500 mg/mL and monitored over 24 hours, 48 hours, and 72 hours. Table 4 provides the observed embryo mortality data at different concentrations and time intervals.

Graphically, Figure 2 illustrates the results of Table 4 to enhance visual comparison. Minimal toxicity was observed at concentrations of 100–300 mg/mL (0/5 mortality), whereas higher toxicity occurred at 400–500 mg/mL, particularly for JC, demonstrating marked embryo lethality by 72 hours.

However, Table 5 presents the results of logistic regression analysis assessing the relationship between extract concentration, exposure time, and extract type on cytotoxicity *in ovo*. The model revealed that concentration and time significantly influenced embryo mortality ($P < 0.05$), whereas differences among extract types were not statistically significant.

Table 3. Elemental Composition of AS, ES, and JC Plants

Elements	AS			ES			JC			*AA
	Expt Values (%)	PL/RDA/TDI (%)	Ref.	Expt Values (%)	PL/RDA/TDI (%)	Ref.	Expt Values (%)	PL/RDA/TDI (%)	Ref.	was not done
Mg	0.3173	0.2	(20)	0.3173	0.5-0.1	(21)	0.2112	0.2	(20)	
Al	0.1716	0.0007	(22)	0.6085	<0.01	(23)	0.5252	0.0007	(22)	
Si	1.1288	0.25	(21)	3.6761	<0.1	(24)	2.0078	0.25	(21)	
P	1.9393	0.7	(21)	0.5138	0.1-0.5	(21)	1.0766	0.7	(21)	
S	0.4629	0.085	(25)	1.1844	0.2-0.3	(21)	0.2893	0.085	(25)	
Cl	1.5780	0.36	(26)	2.9188	0.2-0.4	(22)	2.5101	0.36	(26)	
K	25.3879	0.25	(22)	26.0408	0.2-0.4	(21)	17.5770	0.25	(22,27)	
Ca	59.8783	0.1	(24)	58.5567	0.1-0.5	(28)	48.9550	0.1	(24)	
Mn	1.6249	0.02	(22)	0.3185	0.01	(23)	0.6475	0.02	(22)	
Fe	3.8007	0.02	(22)	3.3811	0.01-0.1	(21)	23.6830	0.02	(22,27)	
Cu	0.2223	0.002-0.015	(22)	0.1630	0.01-0.05	(21)	0.2751	0.005	(22)	
Zn	1.1474	0.005	(22)	0.0582	0.01	(29)	0.1098	0.0005	(27)	
Rb	0.3028	0.0005	(30)	0.0582	0.01	(29)	0.1098	0.0005	(27)	
Sr	0.4038	0.00015	(31)	1.4564	0.01	(32)	0.5490	0.00015	(31)	
W	1.6340	0.000001 (0.01 mg/TDI)	(21)	0.8042	0.01	(33)	1.0134	0.000001 (0.01 mg/TDI)	(21)	

Note. *AA: Not done; PL: Permissible limit; RDA" Recommended daily allowance; TDI: Tolerable daily intake.AS: *Annona senegalensis*; ES: *Erythrina senegalensis*; JC: *Jatropha curcas*.

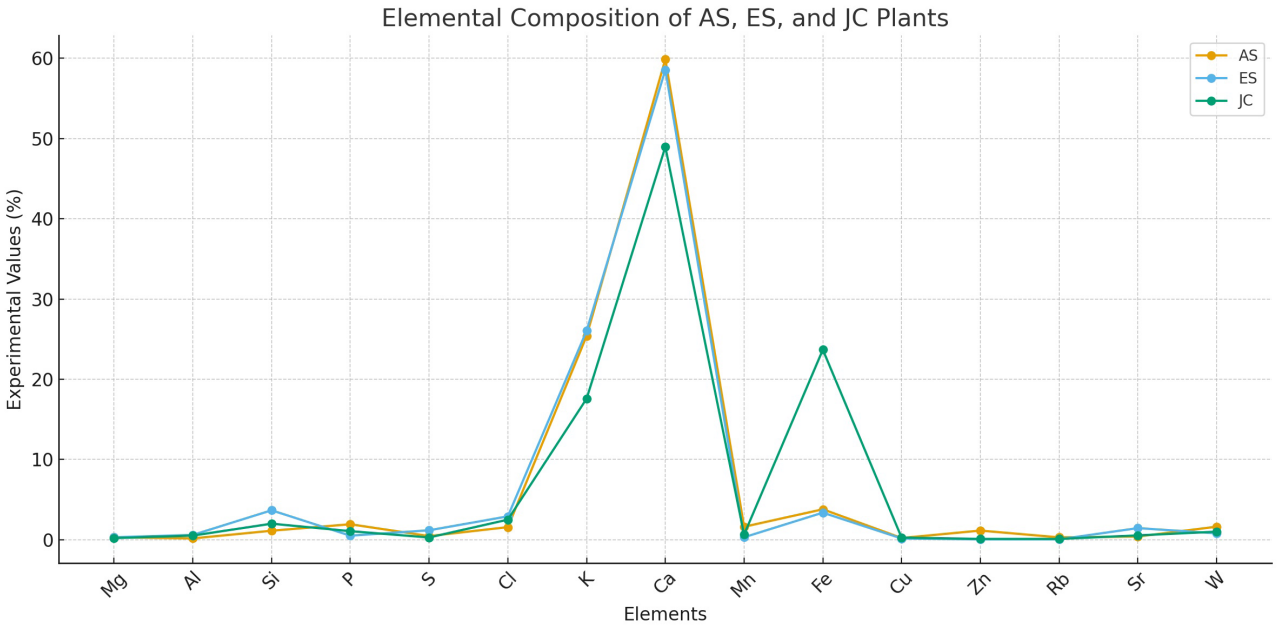

Figure 1. Comparative Elemental Composition of AS, ES, and JC Samples against Recommended Values. *Note*. AS: *Annona senegalensis*; ES: *Erythrina senegalensis*; JC: *Jatropha curcas*.

Table 4. *In ovo* Cytotoxicity Evaluation of AA, AS, ES, and JC Extracts on 9-Day Embryonated Eggs

Extract	Concentration (mg/mL)	24 Hours	48 Hours	72 Hours
AA	500	0/5	1/5	0/4
AS	400	0/5	0/5	3/5
	500	0/5	0/5	3/5
ES	400	0/5	0/5	2/5
	500	0/5	1/5	3/4
JC	400	0/5	0/5	2/5
	500	0/5	0/5	4/5

Note. 1. Extract concentrations (of 100-300 mg/mL) that gave cytotoxicity rating of 0/5 at all-time points are not shown. 2. Control groups are ^{PBS}C: Diluent (PBS) control and ^{EG}C: Non-inoculated egg control.

Table 6 summarizes the cytotoxicity profiles of the extracts based on chi-square and Fisher's exact tests. While individual comparisons between each extract and the control showed no statistically significant differences ($P > 0.05$), the overall Fisher's test indicated a significant treatment effect ($P < 0.001$), suggesting that extract exposure collectively impacted embryo viability.

Based on logistic regression (Tables 5 and 6), the extracts' cytotoxicity was affected by their concentrations and how long they were exposed. Although the individual extracts (AA, AS, ES, JC) did not represent significant differences from the control or among themselves ($P > 0.05$), the Fisher's exact test revealed a noteworthy individual and overall treatment effect ($P < 0.05$), highlighting statistically significant cytotoxicity when compared to the control group. This implies that the cytotoxic effects are influenced by the dose and the duration of exposure.

Newcastle Disease Virus Egg Infectious Dose$_{50}$ (in ovo)

Table 7 presents the NDV infectivity across serial dilutions (10^{-6} to 10^{-10}) and the control group, measured at 24 hours, 48 hours, 72 hours, and 96 hours, plus a final spot test. Thus, it shows the infectivity pattern of NDV in embryonated eggs based on serial dilutions using the Reed-Muench method to estimate the EID_{50}. Higher mortality and positive HA results were observed at lower dilutions (10^{-6}, 10^{-7}), indicating greater viral infectivity, while higher dilutions (10^{-9}, 10^{-10}) demonstrated little to no infection. The calculated EID_{50} of 200 confirms the potency of the NDV strain and underscores the model's reliability for evaluating antiviral efficacy *in ovo*.

The infectivity pattern of NDV (Table 7) is further displayed in Figure 3, which visually represents the dose-response relationship over time. Viral infectivity decreased with an increase in dilution, with the highest rates of embryo mortality and HA positivity occurring at dilutions of 10^{-6} and 10^{-7}. This graphical representation backs up the EID_{50} determination using the Reed-Muench method and emphasizes the model's effectiveness in screening antiviral efficacy.

Antiviral Assay of Artemisia absinthium, Annona senegalensis, Erythrina senegalensis, and Jatropha curcas Extracts against Newcastle Disease Virus (in ovo)

Table 8 lists the *in ovo* antiviral activities of AA, AS, ES, and JC extracts against NDV at concentrations of 100 mg/mL, 200 mg/mL, and 300 mg/mL, tested through a series of dilutions (10^{-5} to 10^{-10}). We kept an eye on mortality rates at 24 hours, 48 hours, 72 hours, and 96 hours after inoculation, with HA tests confirming the presence of the virus. Among the extracts, JC stood out with the most potent antiviral effect, achieving a remarkable 0% virus-induced mortality at 200–300 mg/mL (10^{-7} to 10^{-8}), indicating that it completely suppressed the virus. ES also

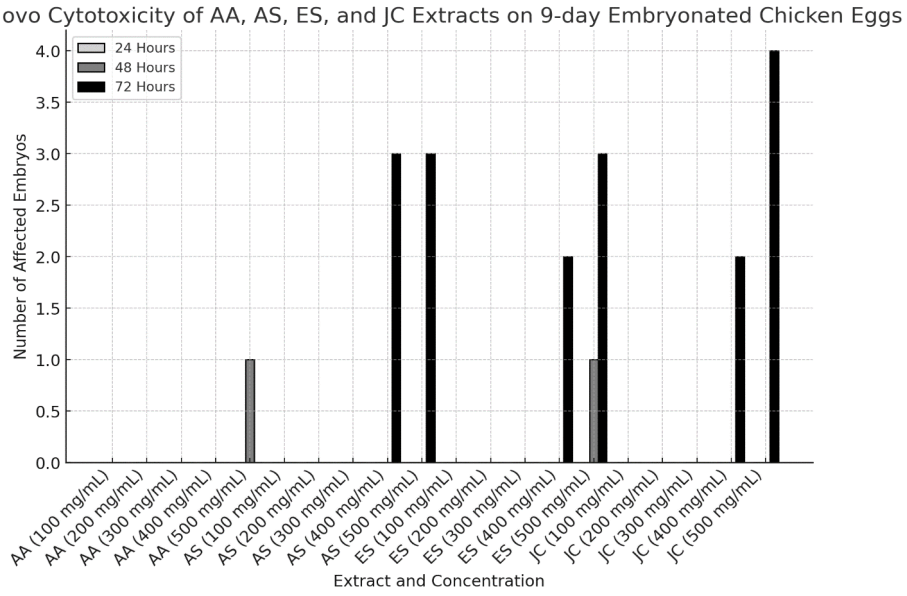

Figure 2. Cytotoxicity Evaluation of AA, AS, ES, and JC Extracts on 9-Day Embryonated Chicken Egg. *Note*. AA: *Artemisia absinthium*; AS: *Annona senegalensis*; ES: *Erythrina senegalensis*; JC: *Jatropha curcas*.

Table 5. Logistic Regression Analysis of Cytotoxicity of AA, AS, ES, and JC Extracts against NDV

Variable	Coefficient	Std. Error	Z-Value	*P*-Value	95% CI_{Lower}	95% CI^{Upper}
AS (vs. AA)	2.01	2.17	0.93	0.3523	-2.23	6.26
Control (vs. AA)	-59.83	4.65×10^{15}	-1.29×10^{-14}	1.000	-9.12×10^{15}	9.12×10^{15}
ES (vs. AA)	3.53	2.36	1.50	0.1344	-1.09	8.15
JC (vs. AA)	2.01	2.17	0.93	0.3523	-2.23	6.26
Concentration	0.030	0.013	2.42	0.0155	0.0058	0.0549
Time	0.153	0.065	2.37	0.0180	0.0263	0.2803

Note. AA: *Artemisia absinthium*; AS: *Annona senegalensis*; ES: *Erythrina senegalensis*; JC: *Jatropha curcas*; NDV: Newcastle disease virus; Std. error: Standard error; CI: Confidence interval.

Table 6. Chi-Square and Fisher's Exact Test Cytotoxicity Analyses of Extracts AA, AS, ES, and JC

Extract	Cytotoxic Frequency (%)	Chi-Square *P*-Value	*Fisher's Exact *P*-Value
AA	1.39 (1.75)	1.000	0.489796
AS	8.00 (6.75)	1.000	0.009625
ES	8.00 (6.75)	1.000	0.009625
JC	8.00 (6.75)	1.000	0.009625
Control	0.00 (0.75)	1.000	N/A
Overall	-	0.99998	0.000002

Note. *N/A (not applicable). AA: *Artemisia absinthium*; AS: *Annona senegalensis*; ES: *Erythrina senegalensis*; JC: *Jatropha curcas*.

Table 7. NDV Egg Infectious $Dose_{50}$

Dilution	24 Hours	48 Hours	72 Hours	96 Hours	Spot Test (HA)
10^{-6}	0/4	1/4	2/3	0/1	4/4
10^{-7}	0/4	0/4	2/2	0/2	4/4
10^{-8}	0/4	1/4	1/3	0/2	3/4
10^{-9}	0/4	1/4	0/3	0/3	0/4
10^{-10}	0/4	0/4	0/4	0/4	0/4
Control	0/4	0/4	0/4	0/4	0/4

Note. NDV: Newcastle disease virus; PBS: Phosphate-buffered saline; HA: Hemagglutination test after 96 hours; Control: Diluent (PBS) control (^{PBS}C) and Non-inoculated egg control (^{EG}C).

had a significant impact, reducing mortality at similar doses, while AS showed a more moderate response. AA, on the other hand, was the least effective overall. Interestingly, at the lowest concentration of 100 mg/mL, AA recorded 0% mortality across all dilutions, which might be due to sub-lethal viral levels or early-stage viral neutralization. Both AS and ES extracts demonstrated partial suppression, bringing virus-related mortality down to as low as 20% at certain dilutions (10^{-6} to 10^{-8}), especially at 100 mg/mL. These results hint at a dose-dependent and dilution-dependent antiviral trend, with JC and ES emerging as the most promising candidates. As anticipated, the virus control group experienced 100% mortality, confirming the virulence of NDV in untreated embryos. Meanwhile, all negative control groups (extract, PBS, and non-inoculated egg controls) represented 0% mortality, thereby validating the safety of the extracts at the tested concentrations and supporting the viability of the model.

The antiviral effects (Table 8) are further supported by the statistical analyses presented in Table 9 and illustrated in Figure 4. Table 9 provides chi-square, Fisher's exact,

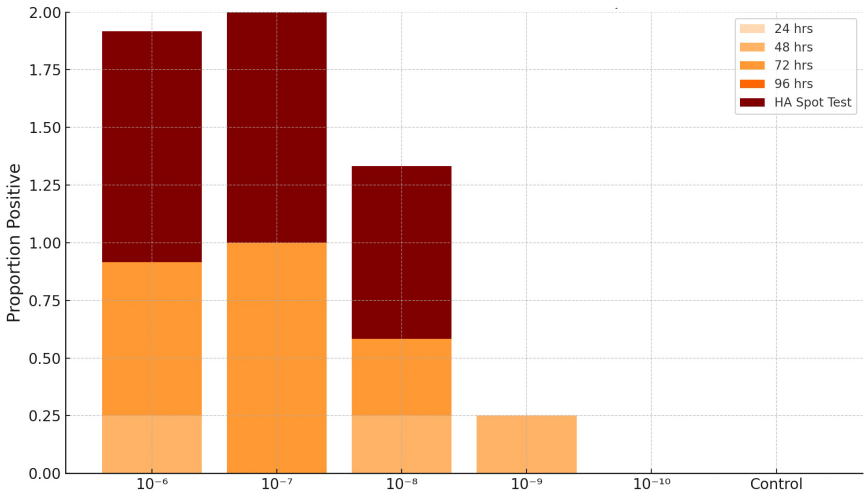

Figure 3. NDV Egg Infectious $Dose_{50}$ Over Time. *Note*. NDV: Newcastle disease virus; HA: Hemagglutination test.

and logistic regression *P*-values, which quantify the significance of the antiviral activity observed for each extract. JC demonstrated the most statistically significant inhibition of NDV ($P < 0.001$ across all tests), followed by ES ($P < 0.01$), while AS approached significance ($P \approx 0.07$). AA showed no significant antiviral effect ($P > 0.05$). The graphical representation in Figure 4 visually reinforces these results, highlighting the consistency and strength of JC's antiviral action compared to the other extracts.

Figure 4 depicts the *P*-values with error bars, highlighting variability. JC and ES demonstrated lower standard errors, suggesting more consistent antiviral effects, while AA and AS had higher variability, indicating that their antiviral responses may be less stable across test conditions.

Discussion

The differential extractive yields observed among plant samples AA > AS > JC > ES (Table 1) highlight variations in their phytochemical profiles and solubility in 70% methanol. The relatively high yield of AA may be attributed to its abundance of polar compounds, such as flavonoids and phenolics, which are efficiently extracted by aqueous methanol (2,23). In contrast, the lower yields from ES and JC may reflect lower concentrations of extractable constituents or higher fiber content impeding solvation.

Phytochemical screening (Table 2) confirmed the presence of key bioactive groups in all extracts, with flavonoids, saponins, glycosides, and steroids consistently detected. These compounds have been widely recognized for their antiviral, anti-inflammatory, and immunomodulatory roles (34,35). While all four plants contained flavonoids, their antiviral efficacy varied considerably. AA, despite containing multiple bioactives, showed the weakest antiviral performance, suggesting that mere presence does not equate to potency. This aligns with the findings of Abad et al (36), emphasizing that extract efficacy is influenced not only by composition but also by bioavailability, concentration, and synergistic interactions.

The elemental analysis (Table 3 and Figure 1) revealed elevated concentrations of essential and trace elements, such as Ca, K, Fe, Mn, and Zn. The high Fe content in JC was particularly notable, suggesting possible contributions to its antiviral effects through enhanced erythropoiesis and immune stimulation. However, as pointed out by Nkuba and Mohammed (20), excessive mineral levels can pose toxicity risks, reinforcing the need for dosage control in therapeutic applications.

The cytotoxicity evaluation (Table 4 and Figure 2) demonstrated that all extracts were non-toxic at lower concentrations (100–300 mg/mL), but JC displayed dose-dependent toxicity at 400–500 mg/mL, which conforms to previous findings on *J. curcas* toxicity, which is attributed to phorbol esters and other irritant (13,37,38). Logistic regression analysis (Table 5) further confirmed the significant effect of concentration ($P = 0.0155$) and exposure time ($P = 0.0180$) on embryo viability, highlighting the importance of optimizing dose levels in future applications. While extract type had no significant influence ($P > 0.05$), Fisher's exact test (Table 6) indicated a statistically significant overall treatment effect ($P < 0.001$), implying that cumulative exposure to plant extracts, regardless of type, impacts embryo survival.

The NDV infectivity model using Reed-Muench calculation yielded an EID_{50} of 200 (Table 7), consistent with previous reports (19,39). The model's sensitivity was validated by the predictable mortality pattern across serial dilutions (Figure 3), establishing a robust foundation for antiviral screening. This model is particularly useful in bridging the gap between *in vitro* and *in vivo* systems, allowing for the early-phase evaluation of antiviral efficacy.

Antiviral screening (Table 8) clearly demonstrated JC as the most effective extract, achieving complete suppression of virus-induced mortality at 200–300 mg/mL (dilutions 10^{-7}–10^{-8}). This is strongly supported by statistical analyses (Table 9), where JC showed highly significant inhibition

Table 8. *In ovo* Antiviral Evaluation of AA, AS, ES, and JC Extracts against NDV

Extract (Code)	Conc. (mg/mL)	Dilution Strength	Mortality							% Mortality (Due to Virus)
			24 Hours	48 Hours	72 Hours	96 Hours	+ve	-ve	HA	
AA	100	10^{-5}- 10^{-10}	0/5	0/5	0/5	0/5	0	5	5/5	0
	200	10^{-5}	0/5	1/5	2/4	0/2	3	2	5/5	60
		10^{-6}	0/5	1/5	1/4	0/3	2	3	5/5	40
		10^{-7}	0/5	1/5	2/4	0/2	3	2	5/5	60
		10^{-8}	0/5	2/5	0/3	0/3	2	3	5/5	40
		10^{-9}	0/5	1/5	0/4	1/4	2	3	5/5	40
	300	10^{-5}	0/5	3/5	0/2	1/2	4	1	5/5	80
		10^{-6}	0/5	1/5	0/4	2/4	3	2	5/5	60
		10^{-7}	0/5	2/5	0/3	1/3	3	2	5/5	60
		10^{-8}	0/5	1/5	0/4	1/4	2	3	5/5	40
		Controls	0/5	0/5	0/5	0/5	0	5	0/5	^{E}C, ^{PBS}C, ^{EGC}, ^{V}C
AS	100	10^{-5}	0/5	1/5	1/4	0/3	2	3	5/5	40
		10^{-6}	0/5	0/5	1/5	0/4	1	4	5/5	20
		10^{-7}	0/5	0/5	1/5	0/4	1	4	5/5	20
		10^{-8}	0/5	0/5	1/5	0/4	1	4	5/5	20
	200	10^{-5}	0/5	0/5	3/5	0/2	3	2	5/5	60
		10^{-6}	0/5	0/5	2/5	0/3	2	3	5/5	40
		10^{-7}	0/5	0/5	2/5	0/3	2	3	5/5	40
	300	10^{-5}	0/5	1/5	2/4	0/2	3	2	5/5	60
		10^{-6}	0/5	0/5	3/5	0/2	3	2	5/5	60
		10^{-7}	0/5	0/5	2/5	0/3	2	3	5/5	40
		10^{-8}	0/5	0/5	1/5	0/4	1	4	5/5	20
		Controls	0/5	0/5	0/5	0/5	0	5	0/5	^{E}C, ^{PBS}C, ^{EGC}, ^{V}C
ES	100	10^{-5}	0/5	1/5	0/4	1/4	2	3	5/5	40
		10^{-6}	0/5	0/5	1/5	0/4	1	4	5/5	20
		10^{-7}	0/5	0/5	0/5	1/5	1	4	5/5	20
	200	10^{-5}	0/5	0/5	2/5	1/3	3	2	5/5	60
		10^{-6}	0/5	0/5	1/5	1/4	2	3	5/5	40
		10^{-7}	0/5	0/5	1/5	1/4	2	3	5/5	40
		10^{-8}	0/5	0/5	0/5	1/5	1	4	5/5	20
	300	10^{-5}	0/5	1/5	2/4	0/2	3	2	5/5	60
		10^{-6}	0/5	0/5	2/5	0/3	2	3	5/5	40
		10^{-7}	0/5	0/5	1/5	0/4	1	4	5/5	20
		Controls	0/5	0/5	0/5	0/5	0	5	0/5	^{E}C, ^{PBS}C, ^{EGC}, ^{V}C
JC	100	10^{-5}	0/5	1/5	1/4	3/3	5	0	5/5	100
		10^{-6}	0/5	0/5	1/5	1/4	2	3	5/5	40
		10^{-7}	0/5	0/5	1/5	1/4	2	3	5/5	40
	200	10^{-7}	0/5	0/5	0/5	0/5	0	5	3/5	0
		10^{-8}	0/5	0/5	0/5	0/5	0	5	1/5	0
	300	10^{-7}	0/5	0/5	0/5	0/5	0	5	4/5	0
		10^{-8}	0/5	0/5	0/5	0/5	0	5	1/5	0
		Controls	0/5	0/5	0/5	0/5	0	5	0/5	^{E}C, ^{PBS}C, ^{EGC}, ^{V}C

Note. PBS: Phosphate-buffered saline. Controls: ^{V}C: Virus control gave 100% mortality; ^{E}C: Extract control gave 0% mortality; ^{PBS}C: Diluent (PBS) control gave 0% mortality; ^{EG}C: Non-inoculated egg control gave 0% mortality; HA: Hemagglutination test after 96 hours.

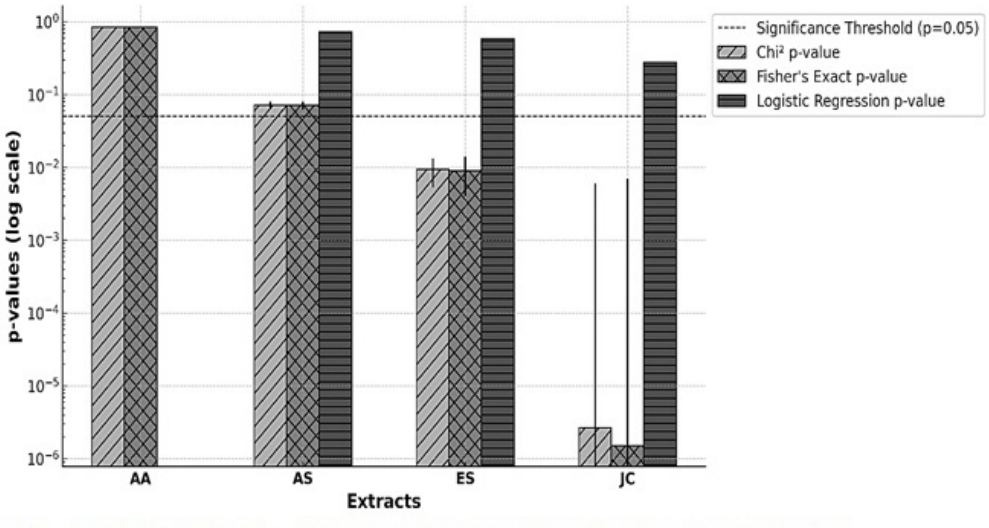

Figure 4. Analysis of *P*-Value Significance of AA, AS, ES, and JC Extracts against NDV. *Note*. AA: *Artemisia absinthium*; AS: *Annona senegalensis*; ES: *Erythrina senegalensis*; JC: *Jatropha curcas*; NDV: Newcastle disease virus.

Table 9. Statistically Significant Inhibition of AA, AS, ES, and JC Extracts against NDV

Extract	Chi² Value	Chi² *P*-value	Fisher's Exact *P*-value	Logistic Regression *P*-value
AA	0.04	0.841	0.842	N/A
AS	3.24	0.072	0.071	0.719
ES	6.76	0.009	0.009	0.588
JC	22.05	0.000003	0.000002	0.274

Note. AA: *Artemisia absinthium*; AS: *Annona senegalensis*; ES: *Erythrina senegalensis*; JC: *Jatropha curcas*; NDV: Newcastle disease virus. JC shows the most significant antiviral activity ($P<0.001$) across all tests. ES also demonstrates significant antiviral potential ($P<0.01$). AS approaches statistical significance ($P \approx 0.07$). However, AA does not demonstrate significant antiviral activity ($P>0.05$).

across all tests (Chi² $P=0.000003$, Fisher $P=0.000002$). These findings corroborate earlier reports of *J. curcas* antiviral activity against diverse viral pathogens (13,40). ES also displayed significant activity ($P=0.009$), with a favorable safety profile, making it a promising candidate for further development. AS, although rich in alkaloids and anthraquinones, represented only borderline activity ($P \approx 0.07$). However, AA was statistically ineffective ($P>0.05$), despite its traditional use in infection treatment.

Figure 4 visually reinforces the statistical trends, with JC and ES exhibiting consistent antiviral efficacy and lower variability in their *P* values. The superior performance of JC may stem from synergistic interactions between its alkaloids, saponins, and flavonoids, as well as possible contributions from its high elemental content, particularly Fe and Zn, known to support immune responses (41,42). Nevertheless, the embryotoxicity observed at higher doses mandates further fractionation studies to isolate the active, non-toxic constituents.

Overall, the study validates the traditional use of JC and ES as potential antiviral agents. Their efficacy in suppressing NDV *in ovo* suggests the presence of compounds capable of inhibiting viral replication or entry. However, translating these findings into safe therapeutic applications requires further toxicological profiling, compound isolation, and in vivo validation. Additionally, AA and AS may still hold potential if evaluated in other viral models or following the purification of specific active principles.

Conclusion

This study assessed the *in ovo* antiviral efficacy of four medicinal plants from Nigeria against NDV, placing JC and ES on the map as candidate natural antiviral medicines. Scientific evidence for their traditional use is confirmed, although safety concerns, such as cytotoxicity, need a complete investigation. It is suggested that subsequent research should seek to isolate active molecules, define mechanisms, and confirm efficacy and safety *in vivo*. These findings justify the continued exploration of plant-based alternatives for managing viral diseases, especially of poultry.

Acknowledgements

We extend our gratitude to the management of Usmanu Danfodiyo University, Sokoto, for facilitating the release of funds through the Tertiary Education Trust Fund and granting approval for the commencement of the project (TETF/ES/DR&D-CE/NRF2020/SETI/110/VOL.1, 27th August 2021). Special thanks also go to the management and staff of the NVRI, Vom, Plateau State, Nigeria, for providing access to their laboratory. We sincerely appreciate Mr. Bature G. A. and Mrs. Kaigama J. A. of the Viral Research Unit, NVRI, for their invaluable technical assistance.

Authors' Contribution

Conceptualization: Sylvester Nefai Mathias, Emmanuel Halilu Mshelia.
Data curation: Aminu Ahmed Biambo, Sylvester Nefai Mathias.
Formal analysis: Sylvester Nefai Mathias, Aminu Ahmed Biambo.
Funding acquisition: Sylvester Nefai Mathias.
Investigation: Sylvester Nefai Mathias, Chinenye Jane Ugwah-Oguejiofor.
Methodology: Chinenye Jane Ugwah-Oguejiofor, Emmanuel Halilu Mshelia.
Project administration: Sylvester Nefai Mathias.
Resources: Sylvester Nefai Mathias.
Software: Sylvester Nefai Mathias, Aminu Ahmed Biambo.

Supervision: Sylvester Nefai Mathias.
Validation: Chinenye Jane Ugwah-Oguejiofor, Aminu Ahmed Biambo.
Visualization: Mansur Lawal, Aliyu Hamidu Ahmed.
Writing–original draft: Sylvester Nefai Mathias, Emmanuel Halilu Mshelia.
Writing–review & editing: Mansur Lawal, Ibrahim Yusuf Alkali, Sylvester Nefai Mathias.

Competing Interests

The authors declare that they have no conflict of interests.

Ethical Approval

This study received full consent approval from the Health Research Ethics Committee of Usmanu Danfodiyo University, Sokoto, Nigeria (reference No. UDUS/UREC/2020/006 dated 20/10/2020).

Funding

This research was financially supported by the Tertiary Education Trust Fund under the National Research Fund Grant Award Scheme (reference No. TETF/ES/DR&D-CE/NRF2020/SETI/110/VOL.1).

References

1. Gidado M, Mitchell EM, Adejumo AO, Levy J, Emperor O, Lawson A, et al. Assessment of TB underreporting by level of reporting system in Lagos, Nigeria. Public Health Action. 2022;12(3):115-20. doi: 10.5588/pha.22.0008
2. Ahamad J, Mir SR, Amin S. A pharmacognostic review on *Artemisia absinthium*. Int Res J Pharm. 2019;10(1):25-31. doi: 10.7897/2230-8407.10015
3. Carmona F, Pereira AM. Herbal medicines: old and new concepts, truths and misunderstandings. Rev Bras Farmacogn. 2013;23(2):379-85. doi: 10.1590/s0102-695x2013005000018
4. Ohemu TL, Ahmed A, Alemika TE, Chollom SC, Yakubu TP, Dafam DG, et al. In-ovo biological activity of *Boswellia dalzielii* stem bark extract and fractions against Newcastle disease virus. J Pharmacogn Phytother. 2020;12(3):62-70. doi: 10.5897/jpp2019.0556
5. Obakiro SB, Kiprop A, Kigondu E, K'Owino I, Kiyimba K, Drago Kato C, et al. Sub-acute toxicity effects of methanolic stem bark extract of *Entada abyssinica* on biochemical, haematological and histopathological parameters in Wistar albino rats. Front Pharmacol. 2021;12:740305. doi: 10.3389/fphar.2021.740305
6. Ugwah-Oguejiofor CJ, Nefai MS, Emmanuel MH, Alkali IY, Biambo AA, Lawal M, et al. Safety assessment of crude aqueous methanol extract of *Annona senegalensis* stem bark: acute and sub-chronic toxicity studies. Drug Chem Toxicol. 2024;47(6):1155-64. doi: 10.1080/01480545.2024.2344640
7. Kapczynski DR, Afonso CL, Miller PJ. Immune responses of poultry to Newcastle disease virus. Dev Comp Immunol. 2013;41(3):447-53. doi: 10.1016/j.dci.2013.04.012
8. Akram M, Zainab R, Daniyal M, Rasul S, Munir N. Phytochemistry and pharmacology of *Artemisia absinthium* Linn: a multipurpose medicinal plant. Plant Biotechnol Persa. 2024;6(1):76-87. doi: 10.61186/pbp.6.1.76
9. Yakubu OF, Metibemu DS, Adelani IB, Adesina GO, Edokwe CB, Oseha OE, et al. *Annona senegalensis* extract demonstrates anticancer properties in N-diethylnitrosamine-induced hepatocellular carcinoma in male Wistar rats. Biomed Pharmacother. 2020;131:110786. doi: 10.1016/j.biopha.2020.110786
10. Fongnzossie Fedoung E, Biwole AB, Nyangono Biyegue CF, Ngansop Tounkam M, Akono Ntonga P, Nguiamba VP, et al. A review of Cameroonian medicinal plants with potentials for the management of the COVID-19 pandemic. Adv Tradit Med. 2023;23(1):59-84. doi: 10.1007/s13596-021-00567-6
11. Gill LS. Ethnomedical Uses of Plants in Nigeria. Uniben Press; 1992.
12. Oliver-Bever BE. Medicinal plants in tropical West Africa. Cambridge: Cambridge University Press; 1985. p. 5-7.
13. Abdelgadir HA, Van Staden J. Ethnobotany, ethnopharmacology and toxicity of *Jatropha curcas* L. (Euphorbiaceae): a review. S Afr J Bot. 2013;88:204-18. doi: 10.1016/j.sajb.2013.07.021
14. Makkar HP, Francis G, Becker K. Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. Animal. 2007;1(9):1371-91. doi: 10.1017/s1751731107000298
15. Evans WC. Trease and Evans' Pharmacognosy. 6th ed. Elsevier Health Sciences; 2009. p. 1-603.
16. Yagi S, Rahman AE, Elhassan GO, Mohammed AM. Elemental analysis of ten Sudanese medicinal plants using X-ray fluorescence. J Appl Ind Sci. 2013;1(1):49-53.
17. Al-Bataina BA, Maslat AO, Al-Kofahil MM. Element analysis and biological studies on ten oriental spices using XRF and Ames test. J Trace Elem Med Biol. 2003;17(2):85-90. doi: 10.1016/s0946-672x(03)80003-2
18. Mukherjee PK. Antiviral evaluation of herbal drugs. In: Quality Control and Evaluation of Herbal Drugs. Elsevier; 2019. p. 599-628. doi: 10.1016/b978-0-12-813374-3.00016-8
19. Alders RG, Spradbrow PB, editors. Controlling Newcastle Disease in Village Chickens: A Field Manual 2001.
20. Nkuba LL, Mohammed NK. Heavy metals and essential elements in selected medicinal plants commonly used for medicine in Tanzania. Chem Sci Int J. 2017 ;19(2):1-11. doi: 10.9734/csij/2017/31963
21. Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington, DC: National Academies Press; 1997.
22. FAO/WHO: Expert Consultation on Human Vitamin and Mineral Requirements 1998: Bangkok, Thailand 21–30 September. Availbale from: https://www.fao.org/4/y2809e/y2809e01.pdf.
23. Lemus R, Venezia CF. An update to the toxicological profile for water-soluble and sparingly soluble tungsten substances. Crit Rev Toxicol. 2015;45(5):388-411. doi: 10.3109/10408444.2014.1003422
24. Rafferty K, Davies KM, Heaney RP. Potassium intake and the calcium economy. J Am Coll Nutr. 2005;24(2):99-106. doi: 10.1080/07315724.2005.10719450
25. Lenntech. Sulphur (S) - Chemical Properties, Health and Environmental Effects [Internet]. Lenntech; 2018. p. 1. Available from: https://www.lenntech.com/periodic/elements/s.htm.
26. Kaur S, Venktaraman G, Jain M, Senapati S, Garg PK, Batra SK. Recent trends in antibody-based oncologic imaging. Cancer Lett. 2012;315(2):97-111. doi: 10.1016/j.canlet.2011.10.017
27. Baba HS, Mohammed MI. Determination of some essential metals in selected medicinal plants. ChemSearch J. 2021;12(1):15-20.
28. Shaw D, Graeme L, Pierre D, Elizabeth W, Kelvin C. Pharmacovigilance of herbal medicine. J Ethnopharmacol. 2012;140(3):513-8. doi: 10.1016/j.jep.2012.01.051
29. National Research Council: Mineral Tolerance of Animals: Second Revised Edition, Committee on Minerals and Toxic Substances in Diets and Water for Animals NRC. National Panel, Society Interactions, Engineering Society, National. National Academies Press; 2005. p. 790. Available from: http://www.nap.edu/catalog/11309.html%0AVisit.
30. Babu NG, Raju TP, Srinivasu CC, Ramanamam V, Ram SS, Sudershan M, et al. Estimation of elemental concentrations of Indian medicinal plants using energy dispersive X-ray fluorescence (EDXRF). Int J Sci Eng Res. 2015;6(7):1379-87.
31. Jyothsna S, Manjula G, Suthari S, Nageswara Rao AS. Qualitative elemental analysis of selected potential anti-asthmatic medicinal plant taxa using EDXRF technique.

Heliyon. 2020;6(2):e03260. doi: 10.1016/j.heliyon.2020.e03260
32. Keith S, Moffett DB, Rosemond Z, Wohlers DW, Amata RJ, Diamond GL, et al. Toxicological Profile for Tungsten. Atlanta, GA: Agency for Toxic Substances and Disease Registry; 2005. p. 153.
33. Mandal M, Misra D, Ghosh NN, Mandal V. Physicochemical and elemental studies of *Hydrocotyle javanica* Thunb. for standardization as herbal drug. Asian Pac J Trop Biomed. 2017;7(11):979-86. doi: 10.1016/j.apjtb.2017.10.001
34. Lalani S, Poh CL. Flavonoids as antiviral agents for enterovirus A71 (EV-A71). Viruses. 2020;12(2):184. doi: 10.3390/v12020184
35. Badshah SL, Faisal S, Muhammad A, Poulson BG, Emwas AH, Jaremko M. Antiviral activities of flavonoids. Biomed Pharmacother. 2021;140:111596. doi: 10.1016/j.biopha.2021.111596
36. Abad MJ, Guerra JA, Bermejo P, Irurzun A, Carrasco L. Search for antiviral activity in higher plant extracts. Phytother Res. 2000;14(8):604-7. doi: 10.1002/1099-1573(200012)14:8<604::aid-ptr678>3.0.co;2-l
37. Devappa RK, Makkar HP, Becker K. *Jatropha* toxicity--a review. J Toxicol Environ Health B Crit Rev. 2010;13(6):476-507. doi: 10.1080/10937404.2010.499736
38. Ndhlala AR, Amoo SO, Ncube B, Moyo M, Nair JJ, Van Staden J. Antibacterial, antifungal, and antiviral activities of African medicinal plants. In: Kuete V, ed. Medicinal Plant Research in Africa: Pharmacology and Chemistry. Oxford: Elsevier; 2013. p. 621-59. doi: 10.1016/b978-0-12-405927-6.00016-3
39. Reed LJ, Muench H. A simple method of estimating fifty per cent endpoints. Am J Epidemiol. 1938;27(3):493-7. doi: 10.1093/oxfordjournals.aje.a118408
40. Utshudi AL, Oleko RO, Kayembe CT, Onautshu DO, Kitete EM, Mbala BM, et al. Antiviral activities of *Jatropha curcas*: a review. Int J Pathog Res. 2022;9(3):33-46. doi: 10.9734/IJPR/2022/v9i330228
41. Makkar HP, Siddhuraju P, Becker K. Plant secondary metabolites. Methods Mol Biol. 2007;393:1-122. doi: 10.1007/978-1-59745-425-4_1
42. Abookleesh FL, Al-Anzi BS, Ullah A. Potential antiviral action of alkaloids. Molecules. 2022;27(3):903. doi: 10.3390/molecules27030903