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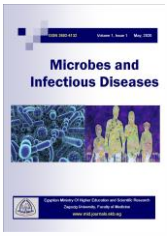

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**Original article**

# Antiviral screening of crude methanolic extract of *Harungana madagascariensis* (Lam. ex Poir) roots against Newcastle disease virus in embryonated hens' eggs

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**ABSTRACT**

**Background:** Newcastle Disease Virus (NDV) causes Newcastle disease in avian species, especially poultry, and has remained a perennial problem plaguing the poultry industry in both developing and developed countries. Despite the challenge posed by this disease, a curative treatment modality has not been developed and prophylactic vaccines available are not potent enough to eliminate the virus. Thus, efforts to develop novel and more effective preventive strategies remain a priority. The present study was conducted to determine the antiviral activity of the methanolic extract of *Harungana madagascariensis* roots on Newcastle disease virus in embryonated hens' eggs. **Method:** Seventy-five (75) embryonated hens' eggs were arranged in fifteen groups of five. A toxicological assay was carried out with an extract concentration of 250 mg/ml on two groups, and another two groups served as negative and diluent controls. The antiviral assay was carried out by inoculating 0.2 ml of the 100 $LD_{50}$ of the virus in four groups, followed by oral administration of graded concentrations of the extract (250 mg/ml, 200 mg/ml, 100 mg/ml and 50 mg/ml) to four (4) groups. Virus control and uninoculated or egg control (negative) were also set up. Rapid haemagglutination assay was used to determine virus clearance by the extract. **Result:** The extract exhibited mild toxic potentiality ($p > 0.05$) on the embryonated hens' eggs as well as had antiviral properties on Newcastle Disease Virus at all used doses. Significant viral clearing was observed ($p<0.05$) at all concentration of the extract. **Conclusion**: The study indicated a strong inhibitory effect of the plant extract on Newcastle Disease Virus and thus we call for further investigation on the safety profiles of the plant to shed light on the toxicological effects and potential benefits as anti-Newcastle Disease Virus agents.

## Introduction

Interestingly, approximately 33% of total modern drugs produced by industrialized countries are of plant origin [1]. The modern pharmaceutical industry itself still relies largely on the diversity of secondary metabolites in plants, of which ≥12,000 have been isolated and characterized [2]. *Harungana madagascariensis* (Lam ex Poir), which grows at medium to low altitudes in evergreen forests at forest margins and along river and stream banks, is commonly found in the tropical part of Madagascar, Sudan, South Africa, Tanzania, Namibia, Uganda, and West Africa [3]. The *H. madagascariensis* plant belongs to the family Hyperiaceae and grows as a tress or shrub generally forming a brownish-yellow appearance in the field [4]. The plant has shown a broad spectrum of

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antimicrobial potential including antibacterial, antifungal, and antiviral [5]. In the rural and urban areas of most African countries, the leaves, bark, and roots of *H. madagascariensis* are used traditionally for the treatment of different ailments. For instance, the extract from the bark is used to treat river blindness, toothache, parasitic infection, and as a child's purgative; the fresh roots and buds are used to treat stomachache and the bark decoction is used to treat asthma, hepatitis, ulcer and dysmenorrhoea [6]. Furthermore, the leaves of *H. madagascariensis* are used to treat chest pain while the root is used to treat ringworm, diarrhea, and dysentery [7].

Newcastle Disease Virus (NDV) is the etiologic agent of Newcastle disease, one of the most devastating infectious bird diseases, with a high potential for rapid spread among thousands of native and newly introduced bird species [3, 8]. The virus is a negative sense, single-stranded RNA virus, characterized into three different pathotypes according to the mean death time in chicken embryos, namely; lentogenic (40–60 hrs), mesogenic (60–90 hrs), and velogenic (90–150 hrs) [3].

Newcastle disease is a common disease found in poultry and other birds globally. It is a highly contagious disease caused by the *Paramyxovirus* of the family Paramyxoviridae through direct contact of faeces and nasal discharges of infected birds, also indirectly through drinking and feeding or contact with cloth etc.; affecting the respiratory, nervous and digestive systems of birds and poultry resulted in death or paralysis and convulsion [3]. Poultry farmers or those working in slaughterhouses are occupationally at risk due to their frequent exposure to the high dose of the virus [3].

There is currently no licensed antiviral against Newcastle disease, though vaccines are available that can confer immunity on birds [9]. Of concern, the available vaccines cannot provide sterilizing immunity and vaccinated birds may become infected with NDV and equally shed the virulent virus [3, 10]. Therefore, studies to discover vegetal drug leads with potential anti-NDV and/ or highlight the scientific basis for the efficacy of plants employed in herbal medicine have become a priority globally. Newcastle disease remains a common health problem for humans and livestock in developing countries. Therefore, this study determined the antiviral activities of crude methanolic extract of *H.madagascariensis* root against Newcastle disease virus in embryonated hens' eggs.

## Material and methods

### Experimental site

The extraction process was carried out in both the Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University Awka, and Biochemistry Laboratory, Federal College of Animal and Medical Technology. The antiviral assay of crude methanolic extract of *H. madagascariensis* **(Figure 1)** on Newcastle Disease Virus (Kuru Isolate) was carried out in the Regional Laboratory for Avian Influenza and other Trans-boundary Avian Viral diseases, National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria.

### Collection and preparation of the plant extracts

The roots of *H. madagascariensis* were collected from their natural habitat in Ugwolawo, in Ofu Local Government Area of Kogi State, by up-rooting with hoes and cutlasses. The identification and authentication of the plant was done by Prof. P.C. Okeke, a plant Taxonomist in the Department of Botany, Nnamdi Azikiwe University Awka, Anambra State. Accordingly, a voucher specimen was kept in the herbarium of Nnamdi Azikiwe University, Awka.

The roots of *H. madagascariensis* were washed and air-dried for about seven days at room temperature in the Biology Laboratory of the Department of Science Laboratory Technology, Federal Polytechnic Idah, Kogi State. The roots were then pulverized by pounding in a clean wooden mortar with a pestle to increase its surface area. The extraction was carried out using the modified methods of **Samson et al.** [11] and **Edegbo et al.** [12]. Briefly, about 30g of the pulverized roots of *H. madagascariensis* were weighed, wrapped in filter paper (Wattman No 24-filter paper), and placed in the thimble of the Soxhlet extractor which was fitted to a round bottom flask containing the extraction solvent (methanol). The flask was subjected to heat on a heating mantle. The sample extract was removed, and the solvent was evaporated using a rotary evaporator thus concentrating the extract. The powder was collected, weighed, and stored in a black polythene bag in the cupboard until used. The quality control of the extract was carried out for possible microbial contamination by plating the extract on blood agar and incubating it at 37 $^{o}$C for

24 hrs in the presence of PSGA (Penicillin, Streptomycin, Gentamycin, and Amphotericin B) to clear the contaminations where available.

**Preparation of eggs**

Nine to eleven-day-old embryonated or fertile hens' eggs were obtained from the Poultry Production Department of National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria, and candled to determine their viability and to mark out the allantoic cavity position for virus inoculation.

**Source of the virus**

The Newcastle disease Virus (very virulent NDV, Kuru strain) was obtained from the Regional Laboratory for Avian Influenza Virus and other Transboundary Avian Viral Diseases, National Veterinary Research Institute (NVRI), Vom, Plateau State.

**Virus titration**

The stock of the virus isolate/strain was serially diluted from $10^{-1}$ - $10^{-9}$. Approximately, 0.1ml of each dilution from $10^{-3}$ to $10^{-9}$ was inoculated into the allantoic cavity of each of the disinfected 5 eggs. About 0.1 ml of phosphate-buffered saline (PBS) was inoculated into each of 5 eggs which was used as negative control. The eggs were incubated at 37ºC and observed for viral activity (mortality and survival) every 24 hrs for 96 hrs (100 $LD_{50}$ of the virus suspension was used). In the virus titration stage, seventy-five (75) embryonated hens' eggs in fifteen groups of five were used.

**Antiviral determination of the extract**

Forty eggs were used for this experiment. Approximately 0.2 ml of the 100 $LD_{50}$ of the virus was inoculated into the allantoic cavity of each egg in the six groups and incubated at 37ºC for 1 hr. 0.1 ml of the extract concentration (250 mg/ml, 200, 150, 100, and 50 mg/ml) was inoculated into each egg in each group of five eggs, respectively. The controls consisted of 250 mg/ml of the extract concentration alone as extract control, virus control, and Buffered PSGA as diluent control. The eggs were incubated at 37ºC and were observed for embryo mortality and survival after each 24 hr for 4 days.

**Spot agglutination test**

A drop of 10% washed chicken red blood cell of specific pathogen free chicken was placed on a clean and sterile white tile. A drop of allantoic fluid from the egg was placed on the spot, rocked gently to mix, and then observed for agglutination between 1-3 minutes.

**Statistical analysis**

Data were analyzed using Statistical Packages for Social Sciences (SPSS) and qualitative variables were computed with a Chi-square test at a confidence interval of 95%. A probability (***p***) value < 0.05 was taken as the level of statistical significance.

**Results**

The virus titration for determination of $LD_{50}$ shows that within 24 hrs, no mortality was observed in the embryo in all the dilutions. Two and three embryos died at 48 hrs and 72 hrs respectively at dilution $10^{-6}$ step **(Table 1)**. Against dilution $10^{-7}$ step, one and two embryonic deaths were observed at 48hrs and 72hrs, respectively. There was no mortality at the dilution $10^{-8}$ step **(Table 1)**. At the $10^{-9}$ dilution step, one embryonic death was observed within 48 hrs and 72 hrs. The $LD_{50}$ was found to be $10^{-7.31}$. For the first cytotoxicity assay, no embryonic death was recorded at the different time intervals in both the test and control groups. For the repeated experiment, only one embryo died in 24 hrs, and no death was observed in the second control group setup **(Table 2)**. After 24 hrs, there were 3 deaths in the virus control, one in the negative control, and none in the extract. After 48 hrs, two deaths occurred in the virus control while in the extract and negative control, no embryonic death was observed. In general, the extracts at concentrations 50 mg/ml, 100 mg/ml, and 250 mg/ml only produced one embryonic death after 24 hrs and zero death at higher time intervals. Extract concentration of 200 mg/ml with complete absence of embryonic death displayed more antiviral activity **(Table 3)**. Results of the study revealed that samples of two embryonated eggs agglutinated chicken red blood cells from 100 mg/ml, while five samples each haemagglutinated chicken red blood cells (cRBC) from 50mg/ml and virus control **(Table 4)**. Samples of 250, and 200 mg/ml, extract control, and negative control were all negative for agglutination **(Table 4)**.

**Table 1.** Virus titration using embryo death

| Virus dilution | Mortality | Ratio/Time (hrs) | | | Total dead |
|---|---|---|---|---|---|
| | 24 | 48 | 72 | 96 | |
| $10^{-5}$ | 0/5 | 0/5 | 5/5 | 0/0 | 05 |
| $10^{-6}$ | 0/5 | 2/5 | 3/3 | 0/0 | 05 |
| $10^{-7}$ | 0/5 | 1/5 | 2/4 | 0/2 | 03 |
| $10^{-8}$ | 0/5 | 0/5 | 0/5 | 0/5 | 00 |
| $10^{-9}$ | 0/5 | 1/5 | 1/4 | 0/3 | 02 |
| Total Dead | 0/5 | 04 | 11 | 00 | 15 |

Route: Allantoic cavity, Age of Eggs: 11- days old

**Table 2.** Toxicological effect of crude methanolic extract of *H. madagascariensis* on embryonated eggs

| Sample conc. (mg/ml) | Mortality/24 hrs | Time (hrs) 48 hrs | 72 hrs | 96 hrs | Total no dead |
|---|---|---|---|---|---|
| 250 | 0/5 | 0/5 | 0/5 | 0/5 | 00 |
| CCI | 0/5 | 0/5 | 0/5 | 0/5 | 00 |
| 250 | 1/5 | 0/4 | 0/4 | 0/4 | 01 |
| CC2 | 0/5 | 0/5 | 0/5 | 0/5 | 00 |

**Note:**CCI = First control i.e. uninoculated eggs, CC2 = Second control i.e. repeated uninoculated eggs

**Table 3.** Antiviral effect of the crude methanolic extract of *H. madagascariensis* roots on NDV embryonated eggs

| Concentrations of extract (mg/ml) | Mortality/Time (hrs) | | | | Total dead |
|---|---|---|---|---|---|
| | 24 | 48 | 72 | 96 | |
| 250 | 1/5 | 0/4 | 0/4 | 0/4 | 01 |
| 200 | 0/5 | 0/5 | 0/5 | 0/5 | 01 |
| 100 | 1/5 | 0/4 | 0/4 | 0/4 | 01 |
| 50 | 1/5 | 0/4 | 0/4 | 0/4 | 01 |
| | 3/5 | 2/2 | 0/0 | 0/0 | 05 |
| | 0/5 | 0/5 | 0/5 | 0/5 | 00 |
| | 1/5 | 0/4 | 0/4 | 0/4 | 01 |

**Table 4.** Antiviral determination using Spot haemagglutination test

| Sample /Conc (mg/m1) | No. of Eggs | No. positive | No. negative |
|---|---|---|---|
| 250 | 05 | 00 | 05 |
| 200 | 05 | 00 | 05 |
| 100 | 05 | 02 | 03 |
| 50 | 05 | 05 | 00 |
| Extract control | 05 | 00 | 05 |
| Virus Control | 05 | 05 | 00 |
| Negative control | 05 | 00 | 05 |
| Total | 35 | 12 | 23 |

**Discussion**

Medicinal plants are a huge reservoir of potential novel bioactive agents against viral and non-viral diseases [13]. Since a relatively large population of humans in low and middle-income countries locally use medicinal plants as remedies for virally-associated infectious diseases, it is therefore important to assess the antiviral potential of indigenous medicinal plants with a view to highlight the scientific basis for their use and further provide a reliable option in the discovery of potent chemotherapeutic agents. Therefore, this study investigated the antiviral activity of *H. madagascariensis* commonly used by most traditional medicine in Nigeria for the treatment of viral infections.

In the current study, an $ELD_{50}$ of $10^{7.31}$ was obtained and its agreement with the potency of the virus claimed by the source of the virus (NVRI, Vom), indicates that the virus is potent or virulent and thus appropriate for the study. In contrary to the findings of **Ukwubile** [14], the crude methanolic root extract of *H. madagascariensis* produced no

significant toxicological effects on the 11 – day old chicken embryo ($p > 0.05$) at 250 mg/ml extract concentration that killed all NDV. This finding suggests that the root extract of the plant may be a potential source of anti-NDV diseases in poultry and an opportunity for a new drug discovery. The toxicological results obtained at 250 mg/ml extract concentration could be compared to similar work done by **Amagon et al.** [15] to investigate the antiviral property of flavonoids from *Cucumis metuliferus* fruit pulp in chicken embryo fibroblast (CEF) cells and embryonated chicken eggs (ECE) induced with infectious bursal disease virus (IBDV). They observed that flavonoids (100 to 0.195 mg/ml concentrations) were not cytopathic when exposed to CEF cells. After 24 and 48 hours, all the embryonated eggs died at 100 and 50 mg/ml of the flavonoids respectively, but mortalities were not recorded at other concentrations of the flavonoids.

In the current study, the crude methanolic extract of *H. madagascariensis* roots significantly prevented the death of the chick's embryo by NDV at the 50 mg/mL and 250 mg/ml concentrations, an observation suggesting inhibitory or antiviral potential of the crude root extract of the plant against NDV at those concentrations. The magnitude of viral clearance from the allantoic fluid of the embryo as revealed by the haemagglutination assay indicated that the extract could destroy the viral particles, thereby having the capacity to save the embryo and chicken hosts. Interestingly, the potent antiviral activity of *H. madagascariensis* against NDV has been credited to the presence of phenol, one of the phytochemicals found in the root [16]. Notwithstanding, the embryonic death occurring at 200 mg/mL and 100 mg/mL is not clear, though this observation could be explained, in part, by the influence of non-specific sources or errors not captured during the assay.

In conclusion, our study has demonstrated that *Harungana madagascariensis* root extracts have potent antiviral activity against the Newcastle Disease Virus at all extract concentrations used. The overall findings suggest that the plant extract may be a potential source of novel molecules for the development of new chemotherapeutic agents for the treatment of Newcastle disease and probably other viral diseases. The lack of clear potential for toxicity at high extract concentration (250) mg/mL) depicts the extract of *H. madagascariensis* as promising drug leads, which further warrant investigation.

**Ethical consideration**

The study was carried out in accordance with declaration of Helsinki's code of conduct for biomedical research involving animals.

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The study was not in receipt of any funding.

**Competing Interests**

Authors declare that there was no conflict of interest.

**References**

1- **Raskin I, Ribnicky DM, Komarnytsky S, Ilic N, Poulev A, Borisjuk N, et al .** Plants and human health in the twenty-first century. Trends Biotechnol 2002; 20(12):522-31.

2- **Akharaiyi FC, Boboye B, Adetuyi FC.** Antibacterial, Phytochemical and Antioxidant Properties of *Cnestis ferruginea* DC (Connaraceae) Extracts. J Microbiol Biotech Food Sci 2012; 2(2): 592-609

3- **Brown VR, Bevins SN.** A review of virulent Newcastle disease viruses in the United States and the role of wild birds in viral persistence and spread. Vet Res 2017; 48:68

4- **Humphries SE, Stanton JP.** Weed Assessment in the wet tropic. World Heritage area of North Queensland 2003:75 – 80.

5- **Madubbunyi I, Obi S, Nwebube N, Chine A.** Antimicrobial and antihepatotoxic activities of *H. madagascariensis* leaf extracts. Intl J Pharmacol 2007; 33:129 – 134.

6- **Abbiw T.** Study of tropical Shrubs and plants. Biogeorge J Accra 2000; 213: 591 – 602.

7- **Moulari B, Lboutounne H, Chaumont JP, Guillaume Y, Millet J, Pellequer Y.** Potentiation of the Bactericidal Activity of *Harungana madagascariensis* Lam. ex Poir. (Hypericaceae) Leaf Extract Against Oral Bacteria Using Poly (D, L-lactide-co-glycolide) Nanoparticles: In Vitro Study. Acta Odontol Scand 2006; 64(3):153-8.

8- **Absalón AE, Cortés-Espinosa DV, Lucio E, Miller PJ, Afonso CL.** Epidemiology, control, and prevention of Newcastle disease in endemic regions: Latin America. Trop Anim Health Prod 2019; 51: 1033–1048

9- **FAO Corporate Document Repository: Newcastle disease vaccines—an overview.** Available at: http://www.fao.org/ docrep/ 005/ ac802e/ac802e04.htm. Accessed on 18 November 2023

10- **Kapczynski DR, King DJ.** Protection of chickens against overt clinical disease and determination of viral shedding following vaccination with commercially available Newcastle disease virus vaccines upon challenge with highly virulent virus from the California 2002 exotic Newcastle disease outbreak. Vaccine 2005; 23:3424–3433

11- **Samson SO, Edegbo E, Omatola CA.** In-vitro assessment of antibacterial activity of crude methanolic and aqueous extracts of *Mitracarpus villosus*. Afri J Microbiol Res 2021; 15(1):62-68.

12- **Edegbo E, Okolo M-LO, Adegoke AS, Omatola CA, Idache BM, Abraham JO, et al .** Phytochemical screening and antifungal activity of *Cassia alata* (Linn.) crude leaf extracts. Afri J Microbiol Res 2023; 17(8):176-183,

13- **Abubakar IB, Kankara SS, Malami I, Danjuma JB, Muhammad YZ, Yahaya H, et al**. Traditional medicinal plants used for treating emerging and re-emerging viral diseases in northern Nigeria. Europ J Int Med 2022; 49: 102094

14- **Ukwubile CA.** Anti-viral screening of crude methanol extract of *Harungana madagascariensis* Lam. Ex Poir. (Clusiaceae) roots on new-castle disease virus in embryonated eggs. Adv in Agric, Sci Enginr Res 2012; 2 (11): 516 – 518

15- **Amagon KI, Wannang NN, Iliya HA, Ior LD, Chris-Otubor GO.** Flavonoids Extracted from Fruit Pulp of *Cucumis metuliferus* Have Antiviral Properties. British J Pharm Res 2012; 2(4): 249-258.

16- **World Organization for Animal Health.** Aetiology and epidemiology of Newcastle Disease, Paris, 2009; 12