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Minifying the Effects of Newcastle Disease Virus on Structural Development of Chicken Embryo Using *Curcuma longa* and *Baphia nitida* Extracts

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Abstract

Newcastle disease is an important disease of poultry globally. Its economic impact is severe due to the high mortality rate which may reach 100 % in affected poultry farms. Leaf extracts of *Baphia nitida* and *Curcuma longa* were subjected to phytochemical screening and antiviral assay against Newcastle disease virus using embryonated chicken eggs. Nine (9) day-old embryonated chicken eggs were grouped into 5 which received various treatments. The phytochemical analysis revealed the presence of active and nutritionally relevant compounds. The ability of the extracts to neutralize the infectivity and multiplication of NDV in treated groups was carried out by determining the egg/embryo (EE) weight ratio, the egg/embryo neutralization, mean death time and structural changes. Embryo survival was observed daily. The study showed that the weights of the egg and embryo were significantly ($p < 0.05$) higher in the protected groups and the survival rate increased in the protected group as well. The structural anomalies observed on embryos from infected eggs were fully developed in those eggs protected by individual extracts, and the mixture of the two extracts proved to be most effective. Therefore, this has shown the *Baphia nitida* and *Curcuma longa* extracts have antiviral activity, and the activity was felt most the mixture of the two extracts was mixed.

Keywords: *Newcastle Disease Virus (NDV), Chicken Embryo, Antiviral Activity, Baphia nitida, Curcuma longa*

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1. Introduction

The use of plants as traditional medicine against viral diseases in the production of animals has been described and practiced worldwide. The use of herbs and their extracts as antiviral agents began following World War II in Europe, and the research was later developed worldwide (Ahmad *et al*., 2014). The poultry industry is one of the most important agricultural industries, providing food to almost 7 billion people worldwide. The demand for chicken meat has been steadily increasing and is expected to reach 131,607.3 thousand tonnes in the year 2026 (Abd-Alla *et al*., 2012).

Disease-causing microorganisms in the poultry industry include various viruses, bacteria and protozoa. The most challenging pathogens among these is the virus pathogen which continues to emerge through various genetic modifications such as mutations, recombination or co-evolution with vaccines. The most destructive avian viral diseases are Newcastle disease virus (NDV), avian influenza virus (AIV), infectious bursal disease virus (IBDV), infectious bronchitis virus (IBV), egg drop syndrome avian adenovirus, and fowl pox virus (Yasmin *et al*., 2020). Newcastle disease virus (NDV) is the etiological agent for Newcastle disease (ND), which is a viral disease of birds (Lakshmi, 2014). The virus belongs to the paramyxovirus (PMV) which is of public health importance and it is significant in poultry as it constitutes one of its major threats. Velogenic strains of Newcastle disease virus (NDV) can cause conjunctivitis in humans, usually when the person has been exposed to the virus consistently in large quantities (Bakari *et al*., 2012). Vaccination programmes against these viruses have been applied in many countries worldwide. However, the problems arise from backyard-reared chicken infections, which are normally not vaccinated, but still prevalent, leading to the spread of the virus that eventually causes outbreak in the community (Yasmin *et al*., 2020). Modern treatments of the infected avian species are laborious and expensive. Treatments with medicinal plants have been practiced traditionally to overcome the virus infection.

Baphia nitida (Leguminosae- Papilionoideae) is one of the species of *Baphia,* known locally as 'okazi' in the Igbo tribe of Nigeria. It is a shrub which grows to a height of about 9 m, geographically, it is found in the wetter parts of the coastal regions, the rain and secondary forests and on abandoned farmland from sea level up to 600 m altitude. Various parts of *B. nitida* have been used by indigenes of many West African countries for a wide range of ethno-medicinal purposes and are often also used for ornamental purposes. Turmeric is an herbaceous evergreen plant from the family zingiberaceae. There are many species in the genus *Curcuma*, among which *C. longa* (turmeric) is the most studied. Turmeric is a unique source of various types of chemical compounds, which are responsible for a variety of activities.

2. Materials and Methods

Preparations of Plant Materials: The leaves of *Baphia nitida* were collected from Onitsha, Anambra State, Nigeria. The plant material was authenticated appropriately by Dr B. Garuba, in the Botany Department, Michael Okpara Federal University of Agriculture, Umudike. The plant material was washed and dried under shade at room temperature for 14 days. The dried plant material was ground to powder form using a sterile electric grinder. (Iheukwumere *et al.,* 2020).

Extraction of the essential oil: A 2000 mL Soxhlet extractor that has three main sections: a percolator (boiler and reflux) which circulates the solvent, a thimble (usually made of thick filter paper) which retains the solid to be extracted, and a siphon mechanism, which periodically empties the thimble was used for the process. Twenty grams (100 g) of the plant material to be extracted was placed inside the thimble. The thimble was then loaded into the main chamber of the Soxhlet extractor. Then 1000 mL of ethanol was placed in a 1000 mL distillation flask. The flask was placed on the heating mantle (2000 mL, 220 V, 500 W). The Soxhlet extractor was placed at the top of the flask. A reflux condenser was placed at the top of the extractor. When the ethanol was heated to reflux, the solvent vapour travelled up a distillation arm and flooded into the chamber housing the thimble of solid. The condenser ensured that any solvent vapour cooled, and dripped back down into the chamber housing the solid material. The chamber containing the solid material was slowly filled with warm solvents. When the Soxhlet chamber was almost full, the chamber was emptied by the siphon. The solvent was then returned to the distillation flask. The thimble ensured that the rapid motion of the solvent did not transport any solid material to the still pot. This cycle was allowed to repeat many times for 12 hours. After extraction, the solvent is removed, typically using a rotary evaporator to collect the extract.

Phytochemical analysis of the plant extracts

The phytochemical components (alkaloids, glycosides, flavonoids, phenolics, tannins, steroids and saponins) of the plant extracts were determined quantitatively using the methods described by Iheukwumere *et al*. (2020) and Iheukwumere *et a*l. (2025a)

Alkaloids: Five milliliters of the sample was mixed with 96% ethanol and 20% tetraoxosulphate (VI) acid (1:1). One milliliter of the filtrate from the mixture was added to 5 ml of 60% tertraoxosulphate (VI) acid and allowed to stand for 5 minutes. Then 5 ml of 0.5% formaldehyde was added and allowed to stand for 3 h. The reading was taken at an absorbance of 550 nm.

Glycosides: This was carried out using Buljet's reagent. One gram of the fine powder of the sample was soaked in 10 ml of 70% alcohol for 2 h and then filtered with Whatman No. 1 filter paper. The extract was then purified using lead acetate solution and disodium hydrogen tetraoxosulphate (VI) solution before the addition of freshly prepared Buljet's reagent. The absorbance was taken at 550nm.

Flavonoids: Five milliliters of the extracts were mixed with 5 ml of dilute hydrochloric acid and boiled for 30 minutes. The boiled extract was allowed to cool and then filtered with Whatman No. 1 filter paper. One milliliter of the filtrate was added to 5 ml of ethyl acetate and 5 ml of 1% ammonia solution at 420 nm of the absorbance was taken.

Phenolics: Ten milliliters of the sample was boiled with 50 ml acetone for 15 minutes. Five milliliters of the solution was pipetted into 50 ml flask. The 10 ml of distilled water was added. This was followed by addition of 2 M ammonium hydroxide solution and 5 ml of concentrated amyl alcohol solution. The mixture was left for 30 minutes and absorbance was taken at 550nm.

Tannins: Ten milliliters was pipetted into 50 ml plastic containing 50 ml of distilled water. This was mixed for 1 h on a sterile mechanical shaker. The solution was filtered with

Whatman No. 1 filter paper, and 5 ml of the filtrate was mixed with 2 ml of iron (III) chloride solution in 0.1 N hydrochloric acid. The absorbance was taken at 550nm.

Saponins: Five milliliters of the sample was dissolved in aqueous methanol. The 0.25 ml of aliquot was taken for spectrophotometric determination for total saponins at 544 nm.

Preparation of Extract: The plant extracts (*Curcuma longa* and *Baphia nitida*) were each reconstituted with phosphate buffer saline (PBS). One (1.0) g of the ethanolic plant extracts were each dissolved in 10 ml of PBS to make 0.10 ppm of the extracts using sterile conical flasks. This was evenly homogenized and stored in clean sterile containers for use (Iheukwumere *et al*., 2025 a).

Viral Sample Preparation: The lyophilized viral stock (LaSota strain of the Newcastle virus) was prepared by dissolving each vial in 2.5 ml of phosphate buffer saline (PBS). Each was thoroughly homogenized and used immediately after the preparation.

Embryonated Egg Samples: The embryonated egg samples were purchased from Dr C. Udechukwu's poultry farm at Ojoto in Idemili South L.G.A, Anambra State, Nigeria. The embryonated egg samples were candled using the candling machine to determine the viability of the egg and its suitability for the study. The egg samples that were not viable and suitable for the study were discarded. The selected eggs were packed in egg trays which were properly arranged in a carton (70 cm × 30 cm × 40 cm) and carefully transported to the laboratory for analysis (Iheukwumere *et al.*, 2024a; Iheukwumere *et al* ., 2025b; Iheukwumere *et al*., 2025c)).

Preparation of the Egg Samples: The embryonated egg samples were properly cleaned with a sterile towel moistened with distilled water, and then disinfected with 70% (v/v) ethanol. The disinfected embryonated egg samples were carefully and aseptically placed in a vertical position in a disinfected and sterile incubator prior to egg inoculation (Mansour *et al*., 2016).

Egg Inoculation: The embryonated eggs were grouped into 5 and labelled according to the extract used. The weight of each egg in the group was measured using an electronic weighing balance. Thereafter the inoculation site was swabbed with 70% v/v ethanol and a 2 mm hole borne using an eggshell punch. The first group was inoculated with 0.2 ml of the viral sample. The second and third groups received 0.5 ml of the *Curcuma longa* and *Baphia nitida* respectively. The fourth group was inoculated 0.5 ml of a mixture of both extracts. This was allowed for 1 h on the egg rack, after which 0.2 ml of the viral suspension was inoculated into the eggs. The holes were sealed using candle wax and incubated at 35± 2^0C for 96 h (Chollom *et al*., 2012; Iheukwumere *et al.,* 2024b). The fifth group was uninoculated and served as the normal control.

Post Inoculation: The inoculated eggs were observed daily for a period of 4 days. The daily change in weight of the eggs was recorded. One (1) egg was randomly picked from each group, cracked and the embryonic weight, full length of embryo including head, neck and leg length were recorded. After 4 days of incubation, the remaining eggs were harvested. The weight of the eggs and their respective embryos were recorded. (Mansour *et al*., 2016; Iheukwumere *et al.*, 2024c; Iheukwumere *et al*., 2025d).

Statistical Analysis: The data generated from this study was presented in form of mean ± standard deviation (SD), percentage and also in Tables and figures. The significance of the study was determined using a one-way Analysis of Variance (ANOVA) at 95% confidence limit. The pair-wise comparison was done using the student 't' Test (Iheukwumere *et al*., 2018).

3. Results

The four predominant isolates (M, N, O and P) exhibited similar cultural and morphological characteristics but differed slightly in their appearances on Columbia blood Agar and in sizes as shown in Table 1. Isolates M and P were pale grayish whereas isolates N and O were light grayish on Columbia blood Agar. The isolates were all catalase, oxidase, urease and hydrogen production positive. They fermented glucose but were negative to arabinose, lactose and maltose. They showed varied slight reactions to xylose, inositol, sorbitol and mannitol and these formed the basis of their strain variations. The sequence analysis of the bacterial isolates showed 100% identifies for all four isolates and the identified isolates were: *Helicobacter pylori* strain K154 (HPK154, *Helicobacter pylori* strain BS07 (HPBS07), *Helicobacter pylori* strain K93 (HPK93) and *Helicobacter pylori* strain K115 (HPK115) as shown in Table 2

The susceptibility profile of the *Helicobacter* pylori isolates against selected antibiotics is demonstrated in **Table 3** below. Levofloxacin was the most effective antibiotic, as 62.50% of the *H. pylori* isolates were sensitive to it, followed by clarithromycin (18.75%), and graze (6.25%), while amoxicillin and metronidazole were the least effective antibiotics, as 100.0% of the *H. pylori* isolates were resistant to them. It was also observed that Isolates HPK 115 and HPK 93 were the most resistant strains as they showed 100 % resistance to grazone, amoxicillin and metronidazole. HPK 115 showed 100 % resistance to clarithromycin.

The medical histories of the participants were analysed as possible predictors of *H. pylori* infection among the study participants and are presented in **Table 4**. None of the medical histories were found to be a predictor for *H. pylori* infection among the study participants, as they were all statistically non-significantly associated (p > 0.05) with the infection. The sociodemographic data of the participants were analysed as possible risk factors for *H. pylori* infection among the study participants and are presented in **Table 5**. Only age was found to be a significant risk factor, such that the age groups, 15–24 years and 35–44 years, were statistically significantly associated (p (0.044) and (0.034) > 0.05, respectively) with an increased risk of *H. pylori* infection. The hygiene profiles of the participants were analysed as possible risk factors for *H. pylori* infection among the study participants and are presented in **Table 6**. None of the hygiene profiles were found to be a risk factor for *H. pylori* infection among the study participants, as they were all statistically non-significantly associated (p >

0.05) with the infection. The behavioural attributes of the participants were analysed as possible risk factors for *H. pylori* infection among the study participants and are presented in **Table 7**. None of the behavioural attributes were found to be a risk factor for *H. pylori* infection among the study participants, as they were all statistically non-significantly associated ($p > 0.05$) with the infection.

The phytochemical constituents of ethanolic leaves extract of *Baphia nitida* and root extract of *Curcuma longa* revealed the presence of alkaloids, flavonoids, saponins, terpenoids, glycosides, tannins and phenolics. The study also showed that alkaloids, flavonoids, saponins, glycosides, tannins and phenolics were quantified more in *Baphia nitida* extract than *Curcuma longa* extract (Table 1).

The study also revealed that the weights of embryonated egg samples infected with NDV decreased progressively, and these trends were significantly ($p< 0.05$) increased among the egg samples protected by the extracts (Table 2). It was also observed that the weights of those embryonated chicken eggs protected with the extracts increased progressively but these increases were not statistically significant ($p> 0.05$). The increase observed in *Curcuma longa* extract was not significantly ($p> 0.05$) similar to that of *Baphia nitida* extract. Synergism existed between *Curcuma longa* extract and *Baphia nitida* extract as their combination increased the weights of the eggs more than single administration, and this showed similar increase to that of normal control (Table 2).

The embryonic weights of the protected egg samples increased progressively, and these were significantly reduced among the egg samples infected with NDV (Table 3). It was also observed that the increase was more pronounced among the egg samples protected with the combination of *Curcuma longa* extract (CI) and *Baphia nitida* extract (BN), and these were slightly higher than the control group (Table 3).

The infectivity of the virus was significantly reduced among the egg samples protected by the plant extracts, and this was most pronounced among the embryonated egg samples protected with CI and BN as shown in Table 4. It was also observed that the reduction rate increased daily.

The development of the eggs protected with the extracts where more enhanced on those eggs treated with the plant extracts but there was retardation in the structural features of those eggs infected with the virus as shown in Table 5. The full length, head length, neck length and leg length of the eggs protected with the extracts increased daily and this was statistically non significant ($p > 0.05$) among the different plant materials and a mixture of both. This increase in the structural development was observed daily and with little or no difference among the control groups (Table 5).

Table 1: Phytochemical constituents of ethanolic leaves extract of *Baphia nitida* and *Curcuma longa*

Phytochemical	*Baphia nitida* (%)	*Curcuma longa* (%)
Alkaloids	3.40±0.01	0.78±0.01
Flavonoids	2.60±0.01	0.48±0.01
Saponins	1.74±0.01	0.57±0.01
Terpenoids	0.27±0.00	1.74±0.01
Glycosides	3.28±0.02	0.02±0.00
Tannins	6.68±0.03	1.14±0.01
Phenolics	0.64±0.00	0.12±0.00

Table 2: Weights of the embryonic eggs

Sample	Day 0 (g)	Day 1 (g)	Day 2 (g)	Day 3 (g)
VS (2.5 ml)	63.18±0.12	53.01±0.11	45.11±0.13	44.12±0.12
CI (0.10 ppm)	56.83±0.17	57.46±0.21	58.66±0.19	60.17±0.14
BN (0.10 ppm)	50.69±0.16	58.29±0.12	60.32±0.14	63.96±0.11
CI + BN	50.66±0.11	59.83±0.12	62.45±0.10	64.31±0.14
Control	58.12±0.14	63.18±0.11	64.01±0.14	64.86±0.11

VS = Viral Suspension; CI = *Curcuma longa* Extract; BN = *Baphia nitida* Extract

Table 3: Weights of the chicken embryos

Sample	Day 0 (g)	Day 1 (g)	Day 2 (g)	Day 3 (g)
VS (2.5 ml)	4.75±0.01	4.06±0.01	4.01±0.01	3.86±0.01
CI (0.10 ppm)	4.99±0.01	5.25±0.01	5.46±0.01	5.64±0.01
BN (0.10 ppm)	4.16±0.01	5.08±0.01	5.46±0.01	5.88±0.01
CI + BN	4.52±0.01	5.63±0.02	5.87±0.01	5.98±0.01
Control	4.90±0.01	5.52±0.01	5.76±0.01	5.97±0.01

VS = Viral Suspension; CI = *Curcuma longa* Extract; BN = *Baphia nitida* Extract

Table 4: Effects of the extracts on embryo/ egg ratio

Sample	Day 0	Day 1	Day 2	Day 3
VS (2.5 ml)	0.075	0.077	0.089	0.087
CI (0.10 ppm)	0.088	0.090	0.093	0.094
BN (0.10 ppm)	0.080	0.088	0.093	0.093
CI + BN	0.089	0.094	0.094	0.093
Control	0.084	0.087	0.090	0.092

VS = Viral Suspension; CI = *Curcuma longa* Extract; BN = *Baphia nitida* Extract

Table 5: Structural development of the chicken embryo

Sample	24 h				48 h				72h			
	FL	HL	NL	LL	FL	HL	NL	LL	FL	HL	NL	LL
VS (2.5 ml)	1.40	0.60	0.30	0.80	1.40	0.60	0.20	0.80	1.40	0.60	0.20	0.80
CI (0.10 ppm)	1.70	0.60	0.40	0.90	1.70	0.60	0.60	0.90	2.00	0.70	0.60	1.00
BN (0.10 ppm)	1.70	0.60	0.40	1.00	1.90	0.60	0.60	1.00	2.10	0.70	0.60	1.20
CI + BN	1.80	0.60	0.40	1.00	2.00	0.70	0.60	1.10	2.30	0.80	0.60	1.40
Control	1.90	0.60	0.50	1.00	2.10	0.70	0.60	1.20	2.40	0.80	0.60	1.40

4. Discussion

The phytochemical constituents of *Curcuma longa* and *Baphia nitida* is in line with the findings of Gupta *et al*. (2015), Ndukwe *et al*. (2020) and Juliet *et al*. (2021). But partially agrees with the findings of Agwa *et al*. (2011) who reported the absence of saponins and tannins in *B. nitida*. Flavonoids elicit inhibitory effects against viruses including HIV and respiratory syncytial virus. Terpenoids have been reported to be active against bacteria, fungi, viruses and protozoa. It is believed to be active against viruses by disruption of the viral envelope by the lipophilic compounds. Alkaloids have been commonly found to have antimicrobial properties. It is also useful against HIV infection as well as intestinal infections associated with AIDS. Tannins are found in almost every plant part; wood, leaves, bark, roots and fruits and tannin-containing beverages can cure or prevent a variety of viral infections. Studies have shown tannins to be inhibitory to viral reverse transcriptases.

The decrease in weight of the Newcastle disease virus-infected eggs and embryos agrees with the findings of Qosimah *et al*. (2018). The increase in weight of those eggs and embryos that received the extract agrees with the findings of Mabiki *et al*. (2013) who conducted a similar study using *S. glauscencens* plant extract. The ability of *Curcuma longa* and *Baphia nitida* to reduce the infectivity of NDV in embryonated chicken eggs agrees with the findings of Taman (2010), Madbouly *et al*. (2011); Mabiki *et al*. (2013) and Agwa *et al*. (2011), who conducted similar studies. This could be as a result of the bioactive components which possess the antimicrobial activity exhibited by these extracts. The structural development of the embryo is in agreement with the reports of Alabi *et al*. (2018) who stated that an embryonated egg is rich in high quantity of quality and highly-bioavailable nutrients that support the 21-day embryonic developmental stages. The daily progressive increase in embryonic length also agrees with the findings of Alabi *et al*. (2018).

Conclusion

Ethanolic extract of *Curcuma longa* and *Baphia nitida* have shown promising efficacy as an antiviral agent against Newcastle disease virus which is accompanied by a decrease in virus infectivity and weight gain in protected embryos. Hence could be used in the management of this virus in the poultry industry.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Ethical approval: Not applicable

Authors Contributions

All contributed towards the study design, experiment execution, data analysis, and manuscript drafting.

Availability of Data and Materials

All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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