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AUGMENTING THE ANTIVIRAL POTENCY OF *BAPHIA NITIDA* EXTRACT AGAINST NEWCASTLE DISEASE VIRUS USING VITAMIN C USING EMBROYONATED CHICKEN EGGS

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ABSTRACT

The global cases of Newcastle disease among poutry farming in Nigeria has severely encroached the economic standard of poutry farmers. This study was undertaken in order to assess the activity of *Baphia nitida* leave extract and vitamin C against Newcastle Disease Virus (NDV). The phytochemical constituent of ethanolic extract of the plant was determined quantitatively using spectrophotometric and gravimetric methods. The activity of the extract and mixtures against NDV was determined using *in vivo* technique in embryonated chicken eggs. The photochemical constituents of the plant extract revealed the presence of alkaloids, tannins, flavonoids, phenolics, terpenoids, saponins and glycosides. There was decreased in egg weights from 63.18g to 44.12 and embryonic weights from 4.75g to 3.86g of the infected eggs after72h and these were significantly ($P < 0.05$) increased in these eggs protected with the extract of which the mixture of the extract and vitamin C recorded the highest weight. There was also significant ($P< 0.05$) reduction in infectivity of NDV as the embryo – egg weight ratio (EE) and neutralization values increased among the protected eggs, and the mixture of the extract and vitamin C showed the most pronounced activity. Therefore the extract exhibited protective activity against NDV and the activity was most pronounced for the mixture of the extract and vitamin C.

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Antiviral-potency, Phytochemical, *Baphia nitida*, Newcastle Disease Virus, Vitamin C

INTRODUCTION

The use of plants as traditional medicine against viral diseases in the production of animals have 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 includes various virus, bacteria and protozoa. The most challenging pathogens among these, is the virus pathogen which continue to emerge through various genetic modification such as mutations, recombinations 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. Vaccination programmes against these viruses has 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* has been used by indigenes of many West African countries for a wide range of ethno-medicinal purposes and often also used for ornamental purposes. *B. nitida* is used to treat

constipation, ringworm, sprains and swollen joints, parasitic skin diseases, wounds, ulcers, boils, venereal diseases, and gastrointestinal problems. This study was thus aimed at evaluating the neutralizing potentials of *Baphia indica* augmented with vitamin C against this virus.

MATERIALS AND METHODS

Preparations of Plant Materials: The leaves *Baphia nitida* were collected from Onitsha, Anambra State, Nigeria. The plant material was authenticated appropriately Dr B. Garuba, in 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 sterile electric grinder. (Iheukwumere *et al.*, 2020).

Extraction of the Extract: 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 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 slowly filled with warm solvent. When the Soxhlet chamber was almost full, the chamber was emptied by the siphon. The solvent 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 h. After extraction, the solvent is removed, typically by means of 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)

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 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 of 550nm.

Flavonoids: Five milliliters of the extract was 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 extract and vitamin C were each reconstituted with phosphate buffer saline (PBS). One (1.0) g of each respectively was separately dissolved in 10 ml of PBS to make 100 ppm of both using sterile conical flasks. They were evenly homogenized and stored in clean sterile containers for use.

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 were thoroughly homogenized and used immediately after the preparation.

Embryonated Egg Samples: The embryonated egg samples were purchased from Dr C. Udechukwu poultry farm at Ojoto in Idemili South L.G.A, Anambra State, Nigeria. The embryonated egg samples were candled using candling machine in order to determine the viability of the egg and their 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 tray which were properly arranged in a carton (70 cm × 30 cm × 40 cm) and carefully transported to the laboratory for analysis.

Preparation of the Egg Samples: The embryonated egg samples were properly cleaned with 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 vertical position in disinfected and sterile incubator prior to egg inoculation (Mansour *et al*., 2016).

Egg Inoculation to Ascertain the Antiviral Strength of the Extract and Vitamin: The embryonated eggs were grouped into 5 and labelled accordingly. 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 egg shell punch. Group 1 was inoculated with 0.2 ml of the viral suspension only. Group 2 was inoculated with 0.5 ml of *Baphia nitida* and group 3 received 0.5 ml of vitamin C respectively. Group 4 received 0.5 ml of a mixture of the extract and vitamin C. They were placed vertically on the egg rack for

1 h, 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). The last 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 recorded. After 4 days of incubation, the remaining eggs were harvested. The weight of the eggs and their respective embryos were recorded. The egg: embryo weight (EE) ratio and EE neutralization was determined (Mansour *et al*., 2016, Raj *et al*., 2004).

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

RESULTS

The phytochemical constituent of ethanolic leaves extract of *Baphia nitida* revealed the presence of alkaloids, flavonoids, saponins, terpenoids, glycosides, tannins and phenolics. The study also revealed that tannin was most quantified among the phytochemicals whereas terpenoids recorded the least value (Table 1).

The weights of the embryonated eggs increased progressively in every 24 h and these were significantly ($p < 0.05$) increased among the eggs protected with extracts and vitamin C. The increase in weight was more pronounced among the eggs protected with the mixture of the extract and vitamin C as shown in Table 2, and there was a significant difference ($p > 0.05$) when compared with the trends exhibited by the control group. Similar trends were also seen in Table 3 for weights of the chicken embryos. The infected eggs showed progressive

reduction in weight every 24 h interval whereas the eggs protected with plant extract and vitamin C showed progressive increase in weights every 24 h but these daily increase were statistically non significant ($p > 0.05$). The daily increase in weight was statistically significant when compared to the infected eggs but not significant ($p > 0.05$) when compared to the normal control as shown in Table 3.

The daily increase in the vital infectivity index observed among the infected eggs were significantly reduced among the eggs protected by the plant extract and vitamin C, and the reduction was most pronounced among the eggs protected with the mixture of the plant extract and vitamin C (Table 4). The neutralizing potential of the plant extract and vitamin C was shown in Table 5. The study revealed that the plant extract showed higher neutralization potential than vitamin C, and the neutralization potential of the plant extract was then elevated when mixed with vitamin C after 72 h but after 24 h and 48 h, the neutralization potential of the plant extract was retarded as shown in Table 5.

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

Phytochemical	Amount (%)
Alkaloids	3.40±0.01
Flavonoids	2.60±0.01
Saponins	1.74±0.01
Terpenoids	0.27±0.00
Glycosides	3.28±0.02
Tannins	6.68±0.03
Phenolics	0.64±0.00

Table 2: Weights of the embryonated eggs

Sample	Day 1 (g)	Day 2 (g)	Day 3 (g)	Day 4 (g)
VS (2.5 ml/200 dose vial)	63.18±0.12	53.01±0.11	45.11±0.13	44.12±0.12
BN (100 ppm)	50.69±0.16	58.29±0.12	60.32±0.14	63.96±0.11
Vit C (100 ppm)	54.12±0.12	58.32±0.14	60.52±0.21	61.46±0.11
BN + Vit C	53.10±0.17	58.55±0.11	60.92±0.13	64.19±0.10
Control	58.12±0.14	63.18±0.11	64.01±0.14	64.86±0.11

VS = Viral Suspension

BN = *Baphia nitida* extract

Table 3: Weights of the chicken embryos

Sample	Day 1 (g)	Day 2 (g)	Day 3 (g)	Day 4 (g)
VS (2.5 ml/200 dose vial)	4.75±0.01	4.06±0.01	4.01±0.01	3.86±0.01
BN (100 ppm)	4.16±0.01	5.08±0.01	5.46±0.01	5.88±0.01
Vit C (100 ppm)	4.47±0.01	4.88±0.01	5.14±0.01	5.38±0.01
BN+ Vit C	4.25±0.01	5.17±0.02	5.68±0.01	5.97±0.03
Control	4.90±0.01	5.52±0.01	5.76±0.01	5.97±0.01

VS = Viral Suspension

BN = *Baphia nitida* Extract

Table 4: Effects of the extract on the infectivity of the virus

Sample	Day 1	Day 2	Day 3	Day 4
VS (2.5 ml/200 dose vial)	0.075	0.077	0.089	0.087
BN (100 ppm)	0.082	0.087	0.091	0.092
Vit C (100 ppm)	0.083	0.084	0.085	0.088
BN + Vit C	0.080	0.088	0.093	0.093
Control	0.084	0.087	0.090	0.092

Table 5: Neutralizing effects of the extracts and the mixture against NDV

Sample	Day 1	Day 2	Day 3	Day 4
BN (100 ppm)	1.093	1.130	1.011	1.057
Vit C (100 ppm)	1.107	1.091	0.956	1.011
BN + Vit C	1.067	1.143	1.045	1.069
Control	1.120	1.130	1.011	1.057

DISCUSSION

The phytochemical constituents of *Baphia nitida* agrees with the findings of Ndukwe *et al*., (2020), but partly agrees with the findings of Agwa *et al*., 2011 who reported the absence of saponins and tannins in *B. nitida*. The decrease in weight of the Newcastle disease virus infected eggs and embryos agree with the findings of Qosimah *et al*., 2018 who observed similar trend. The increase in weight of those eggs and embryo that received the extract is in collaboration with the findings of Mabiki *et al*. (2013) who conducted a similar study using *S. glauscencens* plant extract and Alabi *et al*., 2018 who stated that embryonated eggs contains high amount of quality and highly bioavailable nutrients that supports the embryonic

developmental stage. The positive effect of augmenting with vitamin C on the weights of the eggs and its embryos agrees with the reports of Hieu *et al*. (2022) who reported that vitamin c has the ability to improve performance in chicken such as weight gain. The ability of *Baphia nitida* to reduce the infectivity of NDV and further neutralization of the virus in embryonated chicken eggs agrees with the findings of Mabiki *et al*., 2013 who conducted a similar study and Agwa *et al*., 2011 reported that *B. nitida* possessed antimicrobial activity against bacteria due to the presence of rich arrays of bioactive compounds. The potency of vitamin C to improve the effectiveness of the extracts is in line with the reports of El-Senousey *et al*. (2017) and Neethu *et al*. (2022) who stated that vitamin C possesses antioxidant and anti-inflammatory activity which makes it suitable in cases of infection in chickens and as well its inhibitory activity in viral infection. Chambial *et al*. (2020) further reported that vitamin C protects the immune system, reduces severity of allergic reactions and helps fight off infections.

CONCLUSION

This study has shown that ethanolic extract of *Baphia nitida* possess antiviral effect against Newcastle disease virus which is accompanied by a decrease in virus infectivity and weight gain in the examined eggs and embryo. These effects are most pronounced when the extract is supplemented with vitamin C. Hence could be used in the management of this virus by the poultry farmers.

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