

Full Length Research Paper

***In-ovo* biological activity of *Boswellia dalzielii* stem bark extract and fractions against Newcastle disease virus**

**Temitayo Lucia Ohemu¹ Abubakar Ahmed², Taiwo Emmanuel Alemika³,
Solomon Chuwang Chollom⁴, Thomas Philip Yakubu¹, Dalen Gwatau Dafam¹,
Victoria Akpidi Okwori¹ and Nanle Josephine Damos¹**

¹Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria.

²Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Nigeria.

³Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria.

⁴Viral Research Division, National Veterinary Research Institute, Vom, Nigeria.

Received 2 August, 2019; Accepted 24 June, 2020

Newcastle disease remains a disease of global concern and serious economic challenge to the poultry industry because of its high mortality rate, in spite of the various intervention programs including vaccination. This research is aimed at studying the antiviral activity of the extract and fractions of *Boswellia dalzielii* against Newcastle disease virus (NDV) using chicken embryonated eggs. Phytochemical analysis was conducted using standard procedure. The methanol extract of *Boswellia dalzielii* was subjected to solvent- solvent fractionation using solvents of varying polarity. This process generated four fractions namely hexane fraction, ethyl acetate fraction, n-butanol fraction and aqueous fraction. Nine to eleven day-old viable embryonated chicken eggs (ECE) were used for the antiviral assay; these were divided into seven groups of five eggs each. The methanol extract was also screened for its cytotoxicity, prophylactic, therapeutic and neutralization effects against NDV, while the fractions were screened for their cytotoxicity and neutralization effects. The phytochemical screening of the stem bark extract and fractions of *B. dalzielii* showed the presence of tannins, flavonoids, saponin, terpenoids and steroids. It was observed from the results that the extract was toxic to the embryo at a concentration above 100 mg/ml. At the end of the bioassay, methanol extract and fractions of *B. dalzielii* showed antiviral activity against NDV. However, the extract seems to possess the most significant anti-NDV activity than the fractions. The results of the study are very promising and support the use of *B. dalzielii* in the treatment of viral infections in animals and humans.

Key words: Antiviral, *Boswellia dalzielii*, Newcastle disease virus (NDV), embryonated chicken eggs (ECE).

INTRODUCTION

Newcastle disease (ND) is endemic in several developing countries and has the greatest impact on villages where poultry farming serves as source of livelihood (Absalón et al., 2019). ND is an economically important disease and

also a major threat to poultry industry (Belgrad et al., 2018; Rehan et al., 2019). According to variation in strains of Newcastle disease virus (NDV), the rate of mortality and morbidity in a flock varies from 90- 100% in

poorly vaccinated chickens, as well as drops in egg production in well-vaccinated layers (Absalón et al., 2019; Bessell et al., 2020). The disease is highly contagious and remains one of the leading poultry diseases worldwide causing high economic losses in both commercial and backyard poultry in developed and developing countries (Khatun et al., 2018).

ND like every other viral infection is one of the world's most transmissible diseases (Rehan et al., 2019). The absence of drugs for the treatment of ND and the unreliability of the vaccines used especially in developing countries remain a major and continuous burden for researchers. Hence, the need for search for antiviral compounds from plants that is safe and effective in the treatment of ND.

It is important to note that, like other plant based products, antivirals of natural origin have proved to possess satisfactory pharmacological and pharmaceutical activities, against a wide variety of viral diseases by inhibiting the replication cycle of various DNA and RNA viruses (Ogbole et al., 2018). Studies has shown antiviral potential of plant extract against viral strains resistant to conventional antiviral agents; this has challenged modern drug discovery practices, and research now tends towards exploring medicinal plants with antiviral constituents (Mukhtar et al., 2008).

For many years, medicinal plants have been an important source of novel and new chemical substances with potential therapeutic effects against viruses (Iyare et al., 2017; Liu and Du, 2012). Although their safety and mechanism of action have not been tested scientifically in most cases, they often mediate beneficial response due to their active chemical constituents which determines their medicinal value. The most important of these are alkaloids, tannins, glycosides, saponins, steroids, terpenoids, flavonoids, phlotannins, resins, balsams, volatile oils and cardiac glycosides (Akhtar et al., 2018).

B. dalzielii Hutch (Burseraceae) also known as frankincense tree is a tree of the Savanna forest recognizable by its papery bark peeling off in a ragged manner. It is locally abundant in Northern parts of Ivory coast and may sometimes be planted as a village stockade on the vocal peak massive of Northern Nigeria as a live fence to bring prosperity ('Ba-samu') or to prevent ('hannu') bad luck, hence the Hausa names (Goje et al., 2013). The methanol extract of the stem bark was reported to be useful in treating those bitten by *Echis carinatus* (a saw scaled viper) (Sandeep and Dilip, 2012). The plant has also been extensively studied for hepatoprotective activity (Onoriose et al., 2012). Extracts of the stem bark of *B. dalzielii* were also reported to possess antibacterial, antifungal, anti-inflammatory, cytotoxic and hypoglycemic effects (Nas and Ali, 2017;

Alemika et al., 2018).

The aim of this research was to study the effects of the extracts and fractions of *B. dalzielii* on NDV using embryonated eggs.

MATERIALS AND METHODS

Plant collection and identification

The fresh stem-bark of *B. dalzielii* was collected from Garkawa, Mikang Local Government Area, Plateau State, Nigeria. The botanical identity of *B. dalzielii* was also confirmed and authenticated at the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, where voucher no 1314 is available for reference.

Extraction

The stem-bark of *B. dalzielii* were sorted to eliminate unwanted particles and dead matter, after which they were air dried under shade, then ground to powder and preserved according to the methods described in Evans (2009). The dried powdered bark of the plant was extracted successively by maceration using petroleum ether, ethyl acetate, methanol and distilled water in that order. The extracts were concentrated to dryness in vacuo on a rotary evaporator. This extraction process yielded petroleum ether, ethyl acetate, methanol and aqueous extracts.

Phytochemical screening

The plant extract and its fractions were screened for the presence or absence of secondary metabolites such as carbohydrates, anthraquinone glycosides, saponins, cardiac glycosides, flavonoids, steroids and terpenoids, tannins and alkaloids using standard methods (Sofowora, 2008; Evans, 2009).

Fractionation

Twenty grams of the methanol extract was dissolved in 50 ml methanol/ water at the ratio 1:1. The mixture was allowed to dissolve properly and then filtered using the Whatman filter paper. The filtrate which is the aqueous portion was used for the liquid-liquid partitioning. The solvents used for this separation are hexane, ethyl acetate, n-butanol in that order.

Ethical approval

The research was carried out in accordance with the US guidelines (NIH publication #85-23, revised in 1985) for laboratory animal use and care. Ethical clearance was obtained from the ethical committee of the Faculty of Pharmaceutical Sciences, University of Jos.

Antiviral assay

This assay was carried out at the Viral Research Division of the

*Corresponding author. E-mail: tayogb17@gmail.com.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

National Veterinary Research Institute, (NVRI), Vom, Nigeria.

Source of virus and 9-day old embryonated chicken eggs (ECE)

The velogenic strain of NDV was obtained from the Viral Research Department, NVRI and embryonated chicken eggs (ECE) aged nine day-old were obtained from poultry division, NVRI.

Estimation of the virus

The haemagglutination assay and end point assay were used to determine the presence and quantity of virus/viral particle in the allantoic fluid of the ECE. The haemagglutination assay was performed in V microtitre plates using 25 μ l each of phosphate buffered saline (PBS) allantoic fluid and 1% chick red blood cells (OIE, 2012). The haemagglutination titre (HA) was taken as the highest virus dilution showing complete haemagglutination of the chicken red blood cells.

The end point assay was performed in 9-day-old ECE according to the method of Reed and Muench (1938) using 50% egg infectious dose (EID₅₀). From this, 100 EID₅₀/0.1 ml of the virus stock was made for this study.

Preparation of plant extract

The methanol extract and the three fractions (ethyl acetate, n-butanol and aqueous) were reconstituted with PBS. Five concentrations of 100, 50, 12.5, 6.25 and 3.125 mg/ml were prepared.

Acute toxicity assay of plant extract and fractions

This was done to estimate the minimum toxic concentration of the extract and fractions in 9-days-old ECE using 0.2 ml of each concentration to inoculate five viable ECE per extract and fractions concentration of 200, 100, 50, 25, 12.5, 6.25 and 3.125 mg/ml; the control group received 0.2 ml PBS. Inoculated eggs were incubated for 2 days at 37°C and monitored daily for mortality by candling. The toxicity of the extract and fractions was determined by examination of embryo for lesions and haemorrhages and by the percentage mortality of embryos.

Assay of the stem bark extract and fractions

The procedure was made according to the method described by Faeji et al. (2017) with modifications. This experiment was carried out in two phases. The first phase involved the methanol extract and the second phase involved the fractions (aqueous, n-butanol and ethyl acetate). All the extracts and fractions were treated with antibiotics (penicillin, streptomycin, gentamicin, amphotericin B (5x PSGA).

Phase one: extract assay

The ECE were candled to select viable egg and thereafter properly labelled according to the extract concentrations and groups used. Sets of plastic egg crates/trays were thoroughly disinfected with Virkon and the embryonated chicken eggs were adequately swabbed with 70% alcohol before transferring into the disinfected trays to avoid contamination. Under a properly sterilized bio-safety cabinet, nine-day-old embryonated chicken eggs were divided into

groups of eight of five ECE each; the eggs were punched and inoculated with the extract and virus according to the grouping through the allantoic route.

The concentrations studied were 100, 50, 25, 10 and 5 mg/ml. In the first group designed to test the prophylactic effect, each predetermined concentration of extract at 0.2 ml was inoculated into the ECE first and incubated for 60 min before the inoculation of 100 EID₅₀/0.1 ml of the virus. In the second group, the therapeutic effect was tested by inoculating 100 EID₅₀/0.1 ml of virus first and incubated for 60 min before inoculation of the extracts after the 60 min at the different concentrations studied. The third group, a 1:1 v/v dilution of the 100 EID₅₀/0.2 ml of virus with predetermined extract concentrations was made to put extract final concentration in the virus/extract mixture at 100, 50, 25, 12.5 and 6.25 mg/ml. The virus/extract mixtures were kept at 4°C for 1 h to react before inoculation into the ECE to determine the neutralization potentials.

Phase two: Fraction assay

The ECE were prepared as described above. Then, a 1:1 v/v dilution of the 100 EID₅₀/0.2 ml of virus with fractions concentrations was made to put final concentration in the virus/fraction mixture at 100, 50, 25, 12.5 and 6.25 mg/ml. The virus/fraction mixtures were kept at 4°C for 1 h to react before inoculation into the ECE to determine the inhibitory potentials. For both extract and fraction assays, a group inoculated with standard NDV 100 EID₅₀/0.2 ml was used as virus control, another group inoculated with 0.2 ml DMSO served as diluent control and another group of eggs also was not inoculated nor punched and this serves as non-inoculated control. The ECE were sealed with nail polish and incubated at 37°C in a humidified incubator. The ECE were candled daily and embryo survival was observed. The experiment was finished by chilling at +4°C after 48 h when the virus control group have all died. Bacteria-free-allantoic fluid from the different tested groups was harvested for spot test and haemagglutination assay to detect the presence of NDV in the ECE.

Harvesting and spot haemagglutination test

The harvesting was done after chilling eggs overnight in a 4°C refrigerator. Embryos that had been chilled were brought out of the refrigerator and kept at room temperature for about 30 min to thaw. A drop of 10% washed chicken red blood cells was placed on a clean white tile and using a sterile rubber wire a drop of the allantoic fluid was mixed with the drop of blood. The tile was gently rocked and observed for visible agglutination to indicate viral presence (OIE, 2012).

Haemagglutination titration assay

The haemagglutination assay was used to determine the presence and quantity of virus/viral particle in the allantoic fluid of the tested eggs. The haemagglutination assay was performed in V bottom shaped micro titre plates using 25 μ l each of PBS, allantoic fluid and 1% chicken red blood cells (OIE, 2012). This was performed in replicates and the mean titre value was recorded.

Data analysis

The obtained data were analysed using the SPSS version 21 (IBM, USA). Descriptive statistical tools such as tables and multiple bar charts were also used.

Table 1. Qualitative phytochemical properties of *B. dalzielii* extract and fraction.

Metabolites	Methanol extract	Ethyl acetate fraction	Butanol fraction	Aqueous fraction
Carbohydrate	++	+++	++	+++
Alkaloid	-	-	-	-
Saponin	+++	++	+++	-
Tannin	+++	+++	++	+
Flavonoids	+++	++	+++	+
Steroid	+	++	+	-
Terpenes	+	-	-	-
Anthraquinone	+	++	-	-
Cardiac glycoside	+	++	+	+

-, Not detected; +, slightly present; ++, moderately present; +++, strongly present.

Table 2. Acute toxicity for Methanol Extract and Fractions of *Boswellia dalzielii*.

Dilution (mg/ml)	% Mortality after 48 h			
	Methanol extract	Aqueous fraction	N-butanol fraction	Ethyl acetate fraction
200	100	0	0	60
100	40	0	0	0
50	0	0	0	0
25	0	0	0	0
12.5	0	0	0	0
6.25	0	0	0	0
Control	0	0	0	0

RESULTS AND DISCUSSION

Phytochemical constituents of *B. dalzielii*

Phytochemical analysis of *B. dalzielii* extract and fractions showed the presence of carbohydrate, tannins, saponins, flavonoid, steroids, terpenes and cardiac glycosides (Table 1). These different bioactive compounds may be attributed to the bioactivity seen in the antiviral assay against NDV. Several medicinal plants used in traditional medicine to treat viral diseases have been found to contain high levels of compounds such as alkaloids, terpenes, flavonoids, naphthoquinones, coumarins and anthraquinones (Rezatofighi et al., 2014).

Acute toxicity assay for methanol extract and fractions of *B. dalzielii*

Table 2 shows the maximum toxic concentration of the methanol extract, aqueous, n-butanol and ethyl acetate fractions of the stem bark of *B. dalzielii* against the embryonated chicken egg. It was observed that the maximum tolerable concentration of extract as measured by the embryonic death in comparison with the controls was 100 mg/ml, therefore test were subjected to a

concentration of 100 mg/ml and below. The diluent was safe for the embryo as no mortality was recorded. The extracts and fractions were safe to the inoculated ECE at the studied concentrations.

Prophylactic assay of methanol extract of *B. dalzielii*

The presence of the extract in the embryonated egg before virus inoculation resulted in the complete inhibition of virus growth and activity at 50, 25, 12.5 and 6.25 mg/ml with 0% mortality seen. However, 100% mortality was observed at 100 mg/ml. The prophylactic effect of the extract was further confirmed by 0% agglutination as observed for all concentrations studied and as such virus titres were also not detectable. The un-inoculated and diluent controls showed 0 and 20% mortality. 100% mortality was observed in the virus control as well as 100% agglutination (Table 3). This finding suggests that this plant extract when introduced into ECE before NDV inoculation could alienate the virus and serve as a prophylaxis method (Faeji et al., 2017).

Therapeutic assay of methanol extract of *B. dalzielii*

Table 4 shows the therapeutic effect of methanol extract

Table 3. Prophylactic effect of methanol extract of *B. dalzielii*.

Extract dilutions (mg/ml)	No. of Death/48 h	% Mortality	% Agglutination due to virus	Virus HA Titre
100	5/5	100	0	ND
50	0/5	0	0	ND
25	0/5	0	0	ND
12.5	0/5	0	0	ND
6.25	0/5	0	0	ND
Control	0/5	0	0	ND
Diluent control	1/5	20	0	ND
Virus infected	5/5	100	100	2 ¹²

ND, Not detectable.

Table 4. Therapeutic effect of methanol extracts of *B. dalzielii*

Extract dilutions (mg/ml)	No. of Death/48 h	% Mortality	% Agglutination due to virus
100	0/5	0	80
50	0/5	0	60
25	1/5	20	60
12.5	0/5	0	60
6.25	1/5	20	40
Control	0/5	0	0
Diluent control	1/5	20	0
Virus infected	5/5	100	100

Table 5. Neutralization potential of methanol extract of *B. dalzielii*.

Extract dilutions(mg/ml)	No. of death/48 h	% Mortality	% Agglutination due to virus
100	0/5	0	0
50	0/5	0	0
25	0/5	0	0
12.5	0/5	0	80
6.25	0/5	0	80
Control	0/5	0	0
Diluent control	0/5	0	0
Virus infected	5/5	100	100

of *B. dalzielii*. Even with the establishment of the virus activity in the embryonated egg before the extract inoculation. The extract was capable of inhibiting viral activity with 0% mortality at concentrations 100, 50 and 12.5 mg/ml. 20% mortality was also seen at 25 and 6.25 mg/ml. 100, 50, 25, 12.5 and 6.25 mg/ml showed agglutinations of 80, 60, 60, 60 and 40% respectively. The un-inoculated and diluent controls showed 0 and 20% mortality. 100% mortality was observed in the virus control as well as 100% agglutination. This assay revealed decreasing HA viral titre with increase in concentration of the extracts (Figure 1).

Neutralization assay of methanol extract of *B. dalzielii*

Table 5 shows the neutralization activity of methanol extract of *B. dalzielii*. The methanol extract of *B. dalzielii* was found to completely inhibit virus growth and activity because no mortality was observed at all the concentration studied. The extract also possesses significant anti-NDV property to the extent that no virus agglutination was observed at 100, 50 and 25 mg/ml respectively. However, 80% agglutination was observed at 12.5 and 6.25 mg/ml. The un-inoculated and diluent

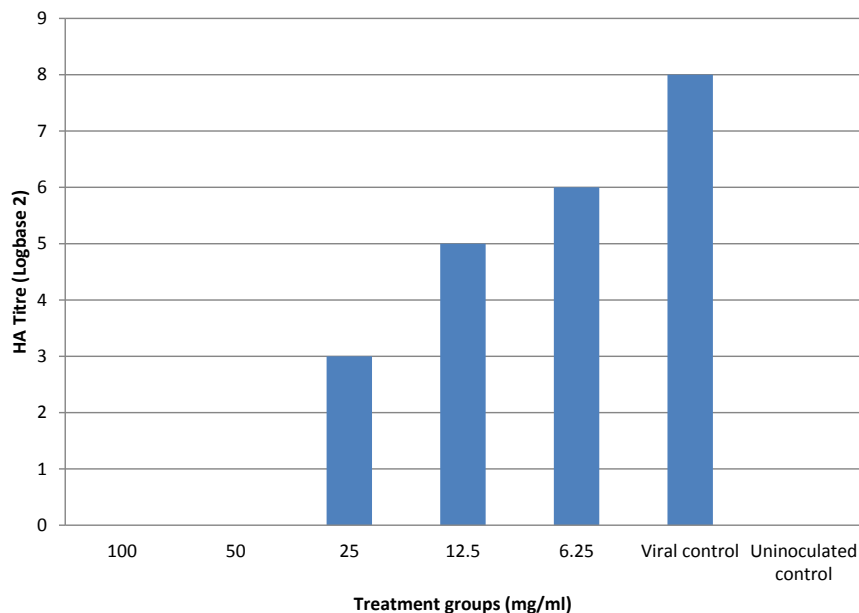


Figure 1. HA titre for therapeutic potential of methanol extract of *B. dalzielii*.

Table 6. Neutralization effect of ethyl acetate fraction of *B. dalzielii*.

Extract dilutions (mg/ml)	No of Death/48 h	% Mortality	% Agglutination due to virus
100	0/5	0	20
50	0/5	0	20
25	0/5	0	20
12.5	1/5	20	60
6.25	3/5	60	60
Control	0/5	0	0
Diluent control	0/5	0	0
Virus infected	5/5	100	100

controls showed 0% mortality and 0% agglutination. 100% mortality was observed in the virus control as well as 100% agglutination. This implies that the methanol extract of *B. dalzielii* may possess significant anti-NDV activity, especially at 100, 50 and 25 mg/ml concentrations.

Neutralization assay of ethyl acetate fraction of *B. dalzielii*

Table 6 shows the neutralization effect of ethyl acetate fraction of *B. dalzielii*. 0% mortality was observed at higher concentrations of 100, 50 and 25 mg/ml; mortality increased with decrease in concentration, hence 20 and 60% mortality with 12.5 and 6.25 mg/ml respectively. This implies that the fraction is capable of inhibiting viral activity in the egg, and its ability to do so reduce with

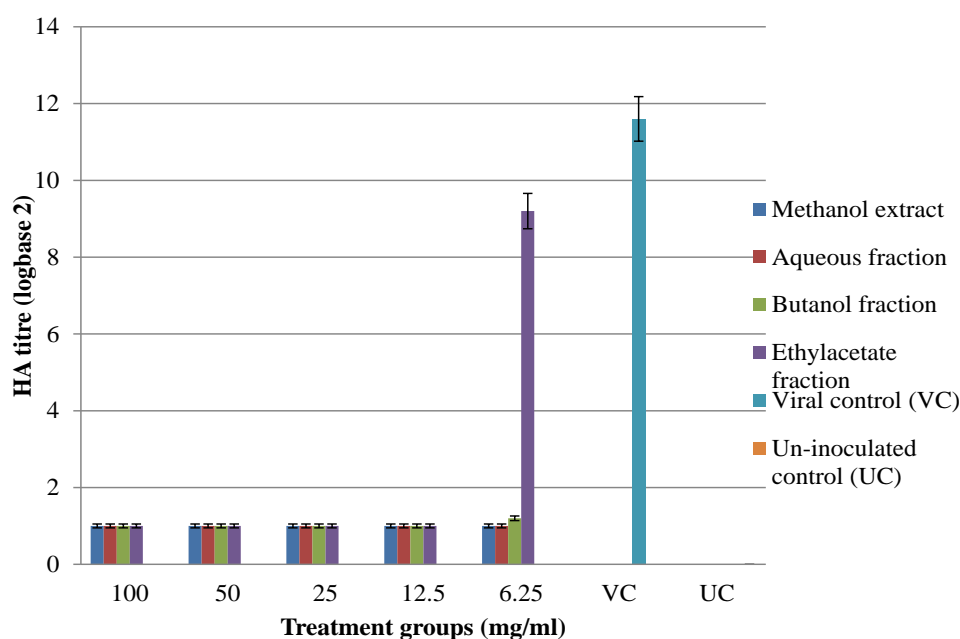
decrease in concentration. This result was further confirmed by results of spot agglutination test and virus titration test. The un-inoculated and diluent controls showed 0 mortality and 0% agglutination. 100% mortality was observed in the virus control as well as 100% agglutination.

Neutralization assay of n-butanol fraction of *B. dalzielii*

The neutralization effect of the n-butanol fraction of *B. dalzielii* against NDV is shown on Table 7. Complete inhibition of virus growth and activity was observed at all concentrations studied with embryo mortalities of 0%. The % agglutination due to virus for concentrations 100, 50, 25, 12.5 and 6.25 mg/ml were 20, 40, 20, 60 and 60% respectively.

Table 7. Neutralization effect of N-butanol fraction of *B. dalzielii*.

Extract dilutions (mg/ml)	No. of Death/48 h	% Mortality	% Agglutination due to virus
100	0/5	0	20
50	0/5	0	40
25	0/5	0	20
12.5	0/5	0	60
6.25	0/5	0	60
Control	0/5	0	0
Diluent control	0/5	0	0
Virus infected	5/5	100	100

**Figure 2.** HA titre for neutralization potentials of extract and fractions of *B. dalzielii*.

Neutralization assay of aqueous fraction of *B. dalzielii*

Table 8 shows the neutralization potential of aqueous fraction of *B. dalzielii*. 0% mortality was observed at concentrations of 100, 50, 25 and 6.5 mg/ml, which implies inhibition of viral activity in the egg. However, 20% mortality was observed at 12.5 mg/ml; this could be due to mechanical injury during inoculation. Agglutination due to virus was 40% for 100, 50, 25 and 12.5 mg/ml respectively, 80% agglutination was observed at 6.26 mg/ml. This result was further confirmed by results of virus titration test. The un-inoculated and diluent controls showed 0% mortality and 0% agglutination. 100% mortality was observed in the virus control as well as 100% agglutination.

HA titre for neutralization potentials of extract and fractions of *B. dalzielii*

The virus titre for methanol extract, ethyl acetate, n-butanol and aqueous fractions of *B. dalzielii* stem bark were capable of neutralizing the virus, hence diminishing virus titre to almost undetectable values of 2^1 , when compared to the virus control (2^{12}), as seen in extract and fraction concentrations of 100, 50, 25, 12.5, and 6.25 mg/ml respectively. However, ethyl acetate fraction had a titre value of 2^9 at 6.25 mg/ml (Figure 2). This implies that the methanol extract and all fractions of *B. dalzielii* can inhibit the replication of NDV under experimental conditions.

This study has revealed that the extract and the fractions possess antiviral activities against NDV at

Table 8. Neutralization potential of aqueous fraction of *B. dalzielii*.

Extract dilutions (mg/ml)	No. of Death/48 h	% Mortality	% Agglutination due to virus
100	0/5	0	40
50	0/5	0	40
25	0/5	0	40
12.5	1/5	20	40
6.25	0/5	0	80
Control	0/5	0	0
Diluent control	0/5	0	0
Virus infected	5/5	100	100

different concentrations. A plant extract is said to possess antiviral activity against NDV if it is capable of inhibiting viral replication by allowing chicken embryo growth, or by reducing viral titre thus preventing embryonic death and viral growth (Mabiki et al., 2013; Yasmin et al., 2020). Previous studies have earlier revealed the possibility of virus inactivation when a potent antiviral candidate is incubated with the virus at controlled conditions. This research is in agreement with studies conducted by Bakari et al. (2012) and Faeji et al. (2017) where extracts of *Commiphora swynnertonii* and *Phyllanthus amarus* respectively, showed prophylactic and therapeutic activities against Newcastle disease. The possibility that the extract blocks viral receptor and also interferes with the neuraminidase-haemagglutinin sites necessary for attachment and penetration of the virion into the living cell is very likely. The blockage of viral receptor results in viral neutralization and ultimately prevention of infection (Obi et al., 2006).

The goal of antiviral search is the discovery of antiviral agents that are specific for the inhibition of viral multiplication without affecting normal cell division. The extract and fractions of *B. dalzielii* as presented in this study have demonstrated the ability to inhibit the viral multiplication without significantly affecting the inoculated ECE. The continuation of the chicken embryo growth unveiled by increase in organ formation in NDV challenged ECE implies that the extracts could potentially interfere with the viral replication cycle either by blocking one point of propagation mechanisms inside the cells, prevent the invasion mechanism or kill the virus in the inoculate.

This biological exhibited by *B. dalzielii* could be attributed to the phytochemicals revealed in the plant extracts, however the varying degrees of the presence of this phytochemicals could be due to the ability of the solvents to extract some of the active ingredient or substances from the plant stem bark based on its polarity (Faeji et al., 2017; Mohammed, 2020). Although the extract and fractions have potentials for antiviral activity against NDV, the extract seem to possess the most significant anti-NDV activity than the fractions. This implies that the chemical compounds in the extract work

in synergy to exhibit the maximum effect observed as compared to the diminished effect observed in the fractions due to fractionation. The results of this study are very promising and support further uses of *B. dalzielii* in the prevention and/or treatment of viral infections in poultry.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors appreciate the Africa Centre for Excellence for Phytomedicine Research and Development (ACEPRD) for supporting this research financially as well as, the staff of Virology Division, National Veterinary Research Institute, Vom, Nigeria for their technical support.

REFERENCES

- Absalón AE, Cortés-Espinosa DV, Lucio E, Miller PJ, Afonso CL (2019). Epidemiology, control, and prevention of Newcastle disease in endemic regions: Latin America. *Tropical Animal Health Production* 51:1033-1048. <https://doi.org/10.1007/s11250-019-01843-z>.
- Akhtar N, Haq I, Mirza B (2018). Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. *Arabian Journal of Chemistry* 11(8):1223-1235
- Alemika TE, Ojerinde OS, Balogun O, Kafuti YS (2018). Potential Application of the West African frankincense, *Boswellia dalzielii* Hutch, for Drug and Perfumery Products. *Journal of Anesthesia and Pain Medicine* 3(3).
- Bakari GG, Max RA, Mdegela RH, Phiri EC, Mtambo MM (2012). Antiviral activity of crude extracts from *Commiphora swynnertonii* against Newcastle disease virus in ovo. *Tropical Animal Health and Production* 44(7):1389-1393.
- Belgrad JP, Rahman MA, Abdullah MS, Rashid MH, Sayeed MA, Anwer MS, Hoque MA (2018). Newcastle disease sero and viro-prevalence in rural poultry in Chittagong, Bangladesh. *Preventive Veterinary Medicine* 160:18-25. <https://doi.org/10.1016/j.prevetmed.2018.09.015>
- Bessell PR, Woolley R, Stevenson S, Al-Riyami L, Opondo P, Lai L, Gammon N (2020). An analysis of the impact of Newcastle disease vaccination and husbandry practice on smallholder chicken

- productivity in Uganda. Preventive Veterinary Medicine 177:104975. <https://doi.org/10.1016/j.prevetmed.2020.104975>.
- Evans WC (2009). *Trease and Evans' Pharmacognosy*, 16th Edition. Saunders Elsevier Toronto, Canada. 1-9, 26, 117-121, 225, 252, 304, 356, 437-440, 541-570.
- Faeji CO, Oladunmoye MK, Adebayo IA, Adebolu TT (2017). *In-ovo* biological activities of *Phyllanthus amarus* leaf extracts against Newcastle disease virus. Journal of Medicinal Plants Research 11(26):419-425.
- Goje LJ, Ghamba PE, Bukbuk DN, Lai I (2013). Toxicological assessments of the aqueous extract of *Boswellia dalzielii* stem bark on liver and kidney of male mice. Journal of Toxicology and Environmental Sciences 5(1):17-22.
- Iyare GI, Omorodion NT, Erameh TO, Achukwu PU, Ogochukwu, AG (2017). The effects of *Anacardium occidentale* leaves extract on histology of selected organs of Wistar rats. MOJ Biology and Medicine 2(2):216-221.
- Khatun M, Islam S, Ershaduzzaman M, Islam HMS, Yasmin S, Hossen A, Hasan, M (2018). Economic Impact of Newcastle Disease on Village Chickens-A Case of Bangladesh. Journal of Economics and Business 1:358-367. 10.31014/aior.1992.01.03.33.
- Liu AL, Du GH (2012). Antiviral Properties of Phytochemicals. In: Patra A. (eds) Dietary Phytochemicals and Microbes. Springer, Dordrecht.
- Mabiki FP, Mdegela RH, Mosha RD, Joseph J, Magadula JJ (2013). *In ovo* antiviral activity of *Synadenium glaucescens* (pax) crude extracts on Newcastle disease virus. Journal of Medicinal Plants Research 7(14):863-870.
- Mohammed HAS (2020). Ethnobotanical, Phytochemical, and Biological Study of *Tamarix aphylla* and *Aerva javanica* Medicinal Plants Growing in the Asir Region, Saudi Arabia. Tropical Conservation Science 12(1).
- Mukhtar M, Arshad M, Ahmad M, Pomerantz RJ, Wigdahl B, Parveen Z (2008). Review of antiviral potentials of medicinal plants. Virus Research 131:111-120.
- Nas FS, Ali M (2017). Antibacterial activity of *Boswellia dalzielii* leaves extracts against some pathogenic bacterial isolates. Journal of Advances in Microbiology 7(1):1-8.
- Obi RK, Iroagba II and Ojiako OA (2006). Virucidal potential of some edible Nigerian vegetables. African Journal of Biotechnology 5(19): 1785-1788.
- Office International des Epizooties (OIE) (2012). Manual of diagnostic tests and vaccines for terrestrial animals: mammals, birds and bees. Biological Standards Commission. World Organization for Animal Health. Paris, pp. 1-19.
- Ogbole OO, Akinleye TE, Segun PA, Faleye TC, Adeniji AJ (2018). *In vitro* antiviral activity of twenty-seven medicinal plant extracts from Southwest Nigeria against three serotypes of echoviruses. Virology Journal 15:110. <https://doi.org/10.1186/s12985-018-1022-7>
- Onoriose DA, Uwakwe AA, Monago CC (2012). Hepatoprotective effect of the methanolic leaf extract of *Boswellia dalzielii* Hutch on carbon tetrachloride induced hepatotoxicity in wistar rats. Indian Journal of Medicine and Healthcare 1(3):55-63.
- Reed LJ, Muench H (1938). A Simple Method of Estimating Fifty Percent End Points. American Journal of Hygiene 27:403.
- Rehan M, Aslam A, Khan MR, Abid M, Hussain S, Umer J, Anjum A, Hussain A (2019). Potential Economic Impact of Newcastle Disease Virus Isolated from Wild Birds on Commercial Poultry Industry of Pakistan: A Review. Hosts Viruses 6:1-15. 10.17582/journal.hv/2019/6.1.1.15.
- Rezatofighi SE, Seydabadi A, Seyyed MSN (2014). Evaluating the efficacy of *Achillea millefolium* and *Thymus vulgaris* extracts against Newcastle Disease Virus *in Ovo*. Jundishapur Journal of Microbiology 7(2):e9016.
- Sandeep VB, Dilip KJ (2012). Profile of medical plant with anti-ophidian property. Journal of Pharmaceutical and Scientific Innovation 1(5):13-20.
- Sofowora A (2008). *Medicinal Plants and Traditional Medicine in Africa*, Spectrum Books Limited, Ibadan, Nigeria. 3rd edition, pp. 70-74, 175-180, 201-202.
- Yasmin AR, Chia SL, Looi QH, Omar AR, Noordin MM, Ideris A (2020). Herbal extracts as antiviral agents. Feed Additives pp. 115-132.