

# Effect of the Pulp Extract of Balsam Fruit (*Momordica balsamina*) on Avian Newcastle Disease Virus

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**Abstract:** One hundred and twenty (120) fertile eggs were incubated, embryonation confirmed and grouped into three (A, B, C) of 40 each. To A and B were introduced 0.1 µl velogenic Newcastle virus strain, with the plant extract mixed into group B; only the plant extract was introduced into group C. The eggs were incubated and candled variously; the number that survived after 7 days were recorded. Dilutions 10<sup>-3</sup>, 10<sup>-4</sup> and 10<sup>-5</sup>, all had 100% mortality, and 10<sup>-7</sup> and 10<sup>-6</sup> had 25% and 75% mortality in Group A. Groups B and C both had 100% survival. 160 day-old chicks were brooded until the 21<sup>st</sup> day and divided into 4 groups (A, B, C and D) of 40. Group A and B were vaccinated with Lasota vaccine on the 22<sup>nd</sup> day. Group B and C were treated with the plant extract from the 23<sup>nd</sup> to 28<sup>th</sup> day at a dose of 200mg/kg body weight orally. Group D was treated with a mixed of the Lasota vaccine and plant extract on the 28<sup>th</sup> day. They were all bled on the 13<sup>th</sup> and 18<sup>th</sup> day post treatment. Haemagglutination Inhibition test was carried out on the serum extracted from the blood samples. Result showed a significant lowering in antibodies on both the 13<sup>th</sup> and 18<sup>th</sup> day post treatment for Group B and only 18<sup>th</sup> in Group D (P < 0.05). In the Third phase, 120 birds were divided into two groups with three replicates of twenty birds each. Group I was challenged with the velogenic strain Newcastle virus and not treated. Group II was challenged with the virus and treated with the extract at a dose of 200mg/kg body weight daily for 14 days. Result showed a 100% morbidity; mortality was 100% across all replicates in Group I, while in Group II, mortality was 10%, 5% and 20% in replicates R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub>, respectively. Post mortem findings showed presence of petechial haemorrhages on the internal mucosa of the proventriculus of birds that died in Group I. Haemorrhages were absent in the dead and recovered birds in Group II. Phytochemistry and mineral element analysis of the plant showed the presence of Alkaloid, vohimbin; Saponins, hyperoside and desglucoruscin; Flavonoids, rutin, Isoquercitrin and chlorogenic acid; Tannin, catechin were identified along with copper, zinc calcium, iron, magnesium, phosphorus, potassium and sodium. Some of the active ingredients have been recorded to have antiviral properties.

Keywords: Momordic abalsamina, Alkaloids, Saponins, Flavonoids, Tannins, Resins, Glycosides

## 1. Introduction

African Balsam fruit (*Momordica balsamina*), Cucurbitaceae is a creeping or climbing vine that grows wild in the drier parts of the tropics. The plant flowers in September in Nigeria and the fruit ripens in the harmattan period of November to December [1]. Another genus of the plant which is usually cultivated in India and China is *Momordica charantia*. The whole plant is used as a bitter stomachic, an infusion, as a wash for fever and for yaws [1]. Medicinal properties ascribed to it as reported by [1] include

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the use of the pulp (freed from seeds) as liniment; the root used as an abortifacient as well as a remedy for urethral discharges. Extensive research has been carried out with *Momordica charantia* especially on its use in the treatment of diabetes mellitus and HIV infection [2].

Momordica balsamina contains a wide spectrum of medicinal and nutritional values and has been used as a traditional folk medicine in many countries in the management of viral diseases [3]. This study is aimed at verifying that claim by monitoring its effect against avian Newcastle disease virus- a pleomorphic, negative sense RNA-containing virus in the genus paramyxovirus [4]. This disease still poses a serious economic challenge to all segments of the poultry industry because of its highly contagious and mortality records [5]. The only control method so far still remains vaccination, which in some cases fail [6]. The importance of an environment-friendly, easily reproducible, cheap and effective viricidal plant product cannot be over-looked. The overall aim of the study is to investigate the effect of the pulp extract of Momordica balsamina on Newcasttle disease virus with a view to increase production by poultry farmers.

## 2. Methodology

## 2.1. Plant Material

The ripe fruit of *Momordica balsamina* were harvested from Bukuru in Jos South Local Government Area of Plateau state. The plant and fruit were identified according to description given by [1]. Further identification was carried out by comparison to a voucher specimen (ECN/02F/FCF, Jos) kept in the Herbarium of the Federal College of Forestry, Jos. The fruit was cut open with a sharp knife, the pulp removed and sieved with a 0.25mm mesh into a clean beaker to separate the seed. This was freeze-dried (EKAT2000) and stored in a desiccator until use.

## 2.2. Phytochemical Screening of fruit Pulp Extract of *Momordica balsamina*

Phytochemicals such as Alkaloid, Tannin, Glycoside, Resin, Flavonoid and Saponin were analysed for based on standard acceptable scientific method [7].

## 2.2.1. Test for Alkaloids

0.5g of the fruit pulp extract of *Momordica balsamina* was dissolved 5mls of 1% aqueous HCl on a steam bath. This was filtered and 1ml of the filtrate treated with a few drops of Draggendorffs reagent and 1ml of a second portion of the filtrate treated with Wagner's reagent; the formation of precipitates indicated the presence of alkaloid.

## 2.2.2. Test for Tannins

0.5g of the fruit pulp extract of *Momordica balsamina* was dissolved in 10ml of distilled water. This was filtered and 2ml of 5% FeCl<sub>3</sub> added to the filtrate. A deep green colouration showed the presence of tannins. A second portion of the filtrate was treated with a few millilitres of iodine

solution. A faint bluish colouration confirmed the presence of tannins.

## 2.2.3. Test for Saponins

0.5g of fruit pulp extract of *Momordica balsamina* was measured placed into a test tube, 5mls of distilled water added and shaken thoroughly. The formation of froth which persists on warming indicated the presence of saponins.

## 2.2.4. Test for Flavonoids

0.5g of the fruit pulp extract of *Momordica balsamina* was measured, placed in a test tube and dissolved in 2mls of dilute NaOH solution. A few drops of concentrated H<sub>2</sub>SO<sub>4</sub> were then added. The appearance of a colourless solution indicated the presence of flavonoids.

## 2.2.5. Test for Resins

0.5g of the fruit pulp extract of *Momordica balsamina* was measured and placed in a test tube and 5mls of boiling ethanol added. The solution was then filtered using a Whatman's No. 1 filter paper and the filtrate diluted with 4mls of 1% aqueous HCl. The formation of a heavy resinous precipitate indicated the presence of resins.

## 2.2.6. Test for Glycosides

0.5g of the fruit pulp extract of *Momordica balsamina* was dissolved in 10mls of distilled water. The solution was filtered and 2mls of the filtrate hydrolyzed with a few drops of concentrated HCl. The mixture was then alkalized with a few drops Ammonia solution. Five (5) drops of this solution was added to 2mls of Benedict's qualitative reagent and boiled. A reddish brown precipitate showed the presence of glycosides.

## 2.2.7. Test for Tannins

2g of the fruit pulp extract of *Momordica balsamina* was treated with Potassium ferric cyanide and ammonia solution. A deep red colour indicated the presence of tannins.

#### 2.3. Chromatographic Screening of Phytochemicals in Powdered Plant Parts of *Momordica balsamina*

The phytochemicals that were found present qualitatively were extracted and assayed chromatographically. The solvent system used was ethylacetate-methanol-water in the ratio of 100:13.5:10 [8].

## 2.3.1. Preparation of Chromatoplates for Thin Layer Chromatographic Analysis

Five (5) chromatoplates were thoroughly washed with water and allowed to dry. The plates were further cleaned with acetone and set on the plate holder. 50g of silica gel powder was dissolved in 110mls of deionized water, the mixture corked and fixed on an Action Wrist Shaker, balanced with 50mls of water and shaken for 30 minutes. The mixture was then applied on the plates and drawn from one to the other with a spreader at a thickness of 0.25mm and allowed to dry for 30 minutes.

#### 2.3.2. Extraction of Active Principles from Dried Pulverized Leaves of *Momordica balsamina*

The phytochemicals identified were extracted and prepared for chromatographic Screening using a standard acceptable scientific method [8].

I. Alkaloids: 1g of the fruit pulp extract of *Momordica* balsamina was mixed thoroughly with 1ml of 10% ammonia solution and extracted by shaking for 5 minutes with 5mls of methanol at 60°C on a water bath, filtered and the filtrate used for the chromatography.

II. Saponins: 1g of the fruit pulp extract of *Momordica* balsamina was shaken for 15 minutes with 20mls of chloroform and filtered. The filtrate was then evaporated to dryness and residue dissolved 2mls of chloroform/methanol (1:1). The mixture was then used for the chromatography.

III. Flavonoids: 1g of the fruit pulp extract of *Momordica* balsamina was extracted with 10mls of methanol for 5 minutes on a water bath at 60°C and filtered. The filtrate was used for the chromatographic screening.

IV. Tannins: Samples were prepared by diluting the crude extracts of chloroform; acetone, methanol and water with 1g of the fruit pulp extract of *Momordica balsamina* and then applied usually 1-10 $\mu$ l volumes to the origins of a TLC plate 2cm above its bottom with the help of capillary tubes.

#### 2.3.3. Activation of Chromatoplates

The chromatoplates were set in the hot air oven and activated at 110°C for 30 minutes, removed and was allowed to cool.

#### 2.3.4. Application of the Extracts on the Chromatoplates

Each of the extracts was spotted on the chromatoplates on a line from the lower edge (origin) of the plates with a spacing of 2-3cm in-between.

#### 2.3.5. Saturation of the Chromatographic Tank

The chromatographic tank was saturated for 1 hour with the developing solvent chroloform/ methanol (85:15) for alkaloids; chloroform/methanol/water (64:50:10) for saponins; n-butanol/glacia-acetic acid/water (40:10:50) for flavonoids and chloroform/methanol (95:5) for the tannins, respectively. The spotted plate is then placed in the chromatographic tank containing the solvent, petroleum jelly (Vaseline) applied on the edges, covered with the lid and allowed to stand. When the solvents reached the score line or solvent front, the plate was removed and reactivated in an oven at a temperature of 110°C for 10 minutes.

#### 2.3.6. Visualization and Identification of Separated Components

The chromatographic film was set in the ultra violet light machine and viewed under the short wave length. The individual components were identified by their characteristic colours and their  $R_f$  values. The  $R_f$  values obtained were compared with those of known standard in a standard atlas.

 $R_f = \frac{\text{Distant moved by the solute}}{\text{Distant moved by the solvent}}$ 

#### 2.4. Mineral Element Analysis

Various vitamins and some macro and micro elements were determined after ashing and digestion, using Atomic Absorption Spectrometer according to the method described by the Association of the Official Analytical Chemist [9].

#### 2.4.1. Ashing and Digestion

2g of the fruit pulp extract of *Momordica balsamina* was weighed into a crucible and ashed in a murfle furnace preheated to  $600^{\circ}$ C for 4 hours. The crucible was then transferred directly to a desicator and allowed to cool. The various ash from different parts of the plant were then separated treated with a few milliliters of HCl and a few drops of concentrated HNO<sub>3</sub> and boiled. These were then cooled and filtered, and the filtrate made up to 10mls in a standard volumetric flask with deionized water. These solutions were used for the determination of cations.

#### 2.4.2. Determination of Cations

Seven (7) cations; sodium, calcium, magnesium, potassium, phosphorus, selenium and lead were determined with the aid of Atomic Absorption Spectrometer. The results were converted from ppm to mg per 100g using the formular:

$$\frac{\text{mg}}{100\text{g}} = \frac{\text{conc. (ppm)}}{10} \times \frac{\text{Soln. Vol}}{\text{wt. (g)}^2} \times \frac{100}{1}$$

#### 2.5. Pathogenicity Test

#### 2.5.1. Embryonated Eggs

One hundred and twenty (120) embryonated eggs (obtained from the Poultry Division of National Veterinary Research Institute, Vom) were incubated and candled on the 9<sup>th</sup> day. They were grouped into three (3), (A-C). Group A served as the control where only the Velogenic strain Newcastle virus dissolved in Phosphate Buffered Saline (PBS) and treated with antibiotic was inoculated into the egg. Various dilutions of the concentration of VGF<sup>-1</sup> that gave EID<sub>50</sub> were used. Group B was treated with various concentrations of the plant extract mixed with an equal volume of the virus. While group C was treated with only the plant extract. The eggs were incubated at 37°C and candled every 24 hours for a maximum period of 10 days post treatment. The number of eggs that survived over the experimental period was expressed in percentage [4].

#### 2.5.2. Grouping of Birds

One hundred and sixty (160) day-old cockerels were purchased and brooded until the  $21^{st}$  day. They were then divided into 4 groups (A, B, C and D) of 40 birds.

(i). Test of Immune Response Vaccination and Treatment with the Fruit Pulp Extract of *Momordica balsamina*.

Treatment with the fruit pulp extract of *Momordica* balsamina was done at a dose of 200mg/kg body weight by oral administration.

- a. Group A: vaccinated with Lasota (Lentogenic strain) on the 29<sup>th</sup> day of age and bled 13 and 18 days later.
- b. Group B: treated with aqueous *Momordica balsamina* extract from the 23<sup>rd</sup> to 28<sup>th</sup> day, after having been vaccinated with Lasota on the 22<sup>nd</sup> day and bled as in Group A.
- c. Group C: treated with aqueous *Momordica balsamina* extract only from the 23<sup>rd</sup> to 28<sup>th</sup> day and bled as in Group A.
- d. Group D: the Lasota vaccine was mixed with aqueous fruit pulp extract of *Momordica balsamina* and administered orally to the birds on the 29<sup>th</sup> day and bled as in Group A.

Serum was extracted from all the blood samples and Haemagglutination Inhibition (HI) test carried out, according to an acceptable scientific method [10].

(ii). Challenge of birds with Velogenic strain Newcastle Virus

One hundred and twenty birds (120) were divided into two groups (I, II) with three replicates of twenty (20) birds each were used in this phase:

- a. Group I: challenged with Velogenic strain Newcastle virus and not treated.
- b. Group II: Challenged with Velogenic strain Newcastle virus and treated with aqueous *Momordica balsamina* fruit extract at a dose of 200mg/kg body weight daily for 14 days.

#### 2.5.3. Post Mortem Examination

Post mortem examination was carried out on dead birds and at the end of 14 days on the recovered ones.

#### 2.5.4. Statistics

The mean and standard deviation of the serum antibody levels were recorded. The difference between the control (Group A) and the test values were evaluated with student t-test [11].

## **3. Results and Discussion**

#### 3.1. Phytochemicals and Minerals Identified in *Momordica balsamina* Fruit Pulp Extract

The phytochemical analysis of the fruit pulp extract of *Momordica balsamina* showed the presence of alkaloids and tannins in little quantities, saponins and flavonoids in large quantities; resins and glycosides are absent. The mineral element analysis of the plant showed the presence of calcium, magnesium and phosphorus in large quantities; iron, copper, potassium and sodium moderately present and zinc in little quantity (Table 1).

Tannins is used in styptic preparations which produce contractions of blood vessels; stopping bleeding having the quality of retaining hemorrhages when applied to the bleeding part. Tannins may be employed medicinally in antidiarrheal, haemostatic, and antihemorrhoidal compounds. The anti-inflammatory effects of tannins help control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders [12]. Diarrhea is also treated with an effective astringent medicine that does not stop the flow of the disturbing substance in the stomach; rather, it controls the irritation in the small intestine. Tannins not only heal burns and stop bleeding, but they also stop infection while they continue to heal the wound internally. The ability of tannins to form a protective layer over the exposed tissue keeps the wound from being infected even more [12]. Tannins are also beneficial when applied to the mucosal lining of the mouth. Tannins can also be effective in protecting the kidneys. Tannins have been used for immediate relief of sore throats, diarrhea, dysentery, hemorrhaging, fatigue, skin ulcers. Tannins can cause regression of tumors that are already present in tissue, but if used excessively over time, they can cause tumors in healthy tissue. They have been also reported to have anti-viral antibacterial and antiparasitic effects [12].

Saponins have the property of causing haemolysis of cells even at low dilution, tends to be deposited on the surface of cells with which they come in contact and are not absorbed by the normal epithelium of the alimentary canal. The presence of saponin in the plant is a demonstration of the fact that the plant may have expectorant actions which are a very useful in the management of inflammation of the upper respiratory tract in addition to its cardio-tonic properties [13].

Alkaloid represents the active principle of vegetable drugs. They are alkaline in reaction and richly combine with acid forming salts soluble in water. All contain nitrogen. Some drugs may contain more than alkaloid and the actions of these may be antagonistic. Alkaloid produces analgesic, antiinflammatory and adaptogenic effects which help to develop resistance against disease and endurance against stress [14].

Glycosides have a tendency to block the conduction of the electrical impulse that causes contraction as it passes from the atria to the ventricles of the heart. Cardiac gylcosides also have a tendency to produce an abnormal cardiac rhythm by causing electrical impulses to be generated at points in the heart other than the normal pace marker region, the cells that rhythmically maintain the heartbeat [15].

Flavonoids are used as a supplement which reduces the symptoms of hemorrhoids. A number of flavonoids have been shown to have anti-inflammatory effects and to strengthen blood vessels. Flavonoids have been investigated for possible anti-inflammatory effects and anti-viral property. Chlorogenic acid is one of the most important flavonoid in the field of pharmacology- has been shown to have antibacterial, antimutagenic, antitumor, and antiviral activities, plus antioxidant and clastogenic activities. The transisomer acts as an insect oviposition stimulant, and it may also reduce larval growth [16].

Diverse functional roles of terpenoids have been critically studied and well-accepted now. Some of them include natural flavor additives for food or fragrances in perfumery and in traditional and alternate medicines as aromatherapy [17]. Most comprehensively studied of which is the effect of terpenes in prevention and treatment of cancer. Illustratively, Taxol derivative (paclitaxel and docetaxel) are among the widely used drugs in cancer chemotherapy. Other important therapeutic uses of terpenoids include antimicrobial, antifungal, antiviral, antihyperglycemic, anti-inflammatory, antioxidants, antiparasitic, immunomodulatory, and as skin permeation enhancer [17].

 Table 1. Phytochemicals and Mineral element composition of Momordica balsamina fruit pulp extract.

	Concentration
Phytochemical	
Resin	-
Alkaloid	++
Saponin	+++
Tannin	++
Flavonoid	+++
Glycoside	-
Mineral	
Cupper	++
Zinc	+
Calcium	++
Iron	++
Magnesium	+++
Phosphorus	+++
Potassium	++
Sodium	++

Key: +++ Highly Present, ++ Moderately Present, + Slightly Present, - Absent.

#### 3.2. Identified Active Principles from the Fruit Pulp Extract of *Momordica balsamina*

Result from the chromatographic screening of the fruit pulp extract of *Momordica balsamina* showed the presence of alkaloid with  $R_f$  values 0.75 (Yohimbine); saponins with  $R_f$  values 0.55 and 0.65 (Hyperoside and Desglucoruscin, respectively); flavonoid with  $R_f$  values 0.22, 0.61 and 0.75 (Rutin, Isoquercitrin and Chlorogenic acid, respectively) and tannin with  $R_f$  value 0.20 (Catechin) (Table 2).

Alkaloid Yohimbine is a preterential presynaptic  $\alpha$ -Adrenolytic agent. Yohimbin and its stereoisomers are used as tools for study of different adrenoceptor sites. It is antidepressant used clinically and shows hypertensive activity - causes vasopressin release and antidiuretic activity. It is also alleged to have aphrodisiac capabilities [18].

Desglucoruscin possesses properties of anti-inflammatory and cytostatic. It is also used in the treatment of chronic venous insufficiency, it is used for hemorrhoids, gallstones, hardening of arteries and or symptoms of poor blood circulation. Isochlorogenic acid which is widely present in fruits, vegetables, and herbal medicines and its characterized by its anti-inflammatory and anti-viral property [19].

Table 2. Identification of the Possible Active Principles in the Phytochemicals.

Phytochemical	R <sub>f</sub> Value	Colour	Solvent System	Detection	Possible identity
Alkaloid	0.75	Very light brown	Ethylacetate-methanol-water (100:13:10) Draggendorf Reagent		Yohimbine
Saponin	0.55	Yellow	Ethylacetate-methanol-water (100:13:10)	thylacetate-methanol-water (100:13:10) Under UV light 354nm	
	0.65	Milky	Ethylacetate-methanol-water (100:13:10)	Under UV light 354nm	Desglucoruscin
	0.22	Light blue	Ethylacetate-methanol-water (100:13:10)	Under UV light 354nm	Rutin
Flavonoid	0.61	Pale blue	Ethylacetate-methanol-water (100:13:10)	Under UV light 354nm	Isoquercitrin
	0.75	Deep blue	Ethylacetate-methanol-water (100:13:10)	Under UV light 354nm	Chlorogenic acid
Tannin	0.20	Very light brown	Ethylacetate-methanol-water (100:13:10)	Sulphuric acid/Vannilin reagent	Catechin

#### 3.3. Pathogenicity Test of Velogenic Strain Newcastle Virus on Embryonated Eggs Treated with Different Concentrations of the Fruit Pulp Extract of *Momordica balsamina*

Result from the pathogenicity test showed that by the  $10^{\text{th}}$  day post treatment, sample A (virus alone) with dilutions  $10^{-7}$  and  $10^{-6}$  had 25% live and 75% dead in two of the samples A, dilutions  $10^{-5}$ ,  $10^{-4}$  and  $10^{-3}$  however, all had 0% live and 100% dead. Sample C (plant alone) with dilutions  $10^{-7}$ ,  $10^{-6}$ ,  $10^{-5}$ ,  $10^{-4}$  and  $10^{-3}$  both had 100% live and 0% dead. Sample B (mixture of plant and virus), all had 100% live and 0% dead in all the dilutions (Table 3).

**Table 3.** Effect of Serial Dilution of the Different Samples on Embryonated Eggs.

Concentration Sample	Live (%)	Dead (%)	
10-7			
А	25	75	
В	100	0	
С	100	0	
10-6			

Concentration Sample	Live (%)	Dead (%)
А	25	75
В	100	0
С	100	0
10-5		
А	0	100
В	100	0
С	100	0
10-4		
А	0	100
В	100	0
С	100	0
10-3		
А	0	100
В	100	0
С	100	0

Sample A = Virulent Newcastle Virus, Sample B = Virulent Newcastle Virus + Plant extract, Sample C = Plant extract.

#### 3.4. Immune Response of Birds Vaccinated with Lasota Vaccine and Treated with the Fruit Pulp Extract of *Momordica balsamina*

Result from the mean and standard deviation (SD) of the

serum antibody levels of vaccinated birds treated with the aqueous fruit pulp extract of *Momordica balsamina* after 13 and 18 days showed a significant lowering in antibodies on both the 13<sup>th</sup> and 18<sup>th</sup> day post treatment for Group B (vaccinated with Lasota and treated with plant extract) on comparism with Group A (control) (P < 0.05)). Group D, however showed only a significant lowering in antibodies only on the 18<sup>th</sup> day post treatment (Table 4).

There was a lowering of the serum antibody levels of birds treated after 13 and 18 days with Group D, which was treated with a mixture of the aqueous fruit extract of Momordica balsamina and the Lentogenic strain Newcastle virus (Lasota) which was significant  $(4.50 \pm 1.52^{a})$  on the  $18^{th}$  day as well Group B which showed significant lowering on both the 13<sup>th</sup>  $(3.50 \pm 0.34^{a})$  and  $18^{th}$   $(4.00 \pm 0.80^{a})$  day post treatment. Based on the above, it is suggested that Momordica balsamina fruit have potentials in being used to immunize birds against Newcastle disease. Optimum immunity was achieved in Group A with the lasota vaccine. The lowering of antibody levels in groups B and D was not total which might be related to the dosage of extracts used. The lowering effect it had may be as a result of individual or combined efforts of the phytochemicals. It is equally possible that the extracts acted as contaminants in Groups D inhibiting the function of the vaccine but it is important to note that there was immunity build up after 13 days which later dropped by the 18<sup>th</sup> day. Another thing to note is there is a possibility that if the dosage of the plant extracts was increased it might have greatly reduced the serum antibody levels, if not completely.

**Table 4.** Effect of Aqueous Momordica balsamina fruit pulp extracts treatment on the serum antibody levels of vaccinated birds after 13 and 18 days.

Group	13 <sup>th</sup>	18 <sup>th</sup>	
А	$7.00 \pm 2.00$	$26.67 \pm 5.24$	
В	$4.50 \pm 1.52^{a}$	$3.50\pm0.34^{a}$	
С	$0.00 \pm 0.00$	$0.00\pm0.00$	
D	$10.50 \pm 2.68$	$4.00\pm0.80^{\text{a}}$	

Values are mean  $\pm$  SD for 40 chicks (a = P < 0.05).

#### 3.5. Challenge of Birds with Velogenic Strain Newcastle Virus and Treated with Velogenic Strain Newcastle Virus

Result from performance of birds infected with the velogenic strain Newcastle virus and treated with the fruit pulp extract of *Momordica balsamina* showed that morbidity was 100% across all replicates in Group I and II. Mortality was 100% across all replicates in Group I (not treated), while in Group II mortality was 10%, 5% and 20% in replicates  $R_1$ ,  $R_2$  and  $R_3$ , respectively. Post mortem findings showed presence of petechial haemorrhages on the internal mucosa of the trachea, cloaca, proventriculus and on the surface of the crop of the dead ones in Group I (not treated). Haemorrhages were absent in the dead and recovered ones in Group II (Table 5).

This suggests that *Momordica balsamina* fruit have great potentials in being used in the treatment of birds already

infected with Newcastle disease. The high morbidity in Groups I and II and high mortality in Group I as well as those recorded in Group II may be related to the fact that the birds were not given antistress before and after they were infected with the velogenic strain Newcastle virus or it may be that the dose of 200mg/kg body weight of the extracts daily was not enough.

It is suggested that Tannins may be involved as was recorded by [12] that tannins have styptic properties producing contractions of the blood vessels; stopping bleeding; having the quality of retaining haemorrhages when applied to the bleeding part - this might be the reason why haemorrhagic lesions were absent in the dead birds (Table 5) as was observed from the post mortem findings because [20] reported that there is characteristic petechial haemorrhagic lesions in the mucosa of digestive and respiratory tracts of dead birds during outbreaks of Newcastle disease infections. Stress could also be another factor as to why the birds died as antistress was not administered during the experimental process which may have resulted to their death as they may have been shed of the virus but failed to survive due to stress and weakened immune system.

 Table 5. Performance of Birds infected with Newcastle virus and treated with Momordica balsamina extract after 14 days.

	Group I		Group	Group II		
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>1</sub>	$\mathbf{R}_2$	R <sub>3</sub>
Morbidity %	100	100	100	100	100	100
Mortality %	100	100	100	10	5	20
Post Mortem Lesions						
Dead	+	+	+	-	-	-
Recovered	*	*	*	-	-	-

Key: + Presence of Petechiae; - Absence of Petechiae; \* Not Applicable.

## 4. Conclusion

*Momordica balsamina* fruit pulp extract have great potentials of being used in the control of Newcastle disease virus of birds. The active principles in *Momordica balsamina* could be Alkaloid (Yohimbine), Saponin (Triterpenoid saponin), flavonoid (rutin, chlorogenic acid and isoquecitrin) and tannin. The mode of action of *Momordica balsamina* in controlling Newcastle disease virus might be the synergistic action of the phytochemicals and minerals.

#### References

- Burkhill, H. M. (1985). In: The Useful Plants of West Tropical Africa. 2<sup>nd</sup> edn., Royal Botanic Garden Kew. Pp 430-437.
- [2] Zhang Q. C. (1992). Preliminary report on the use of Momordica Spp Extract by HIV patients. J. Naturopath Med. 3: 65.
- [3] Gulab, S. T., Manoranjan, B., Bhagwan, S. S., Pratiksha, B., Mousumi, D., Prasad, G. B. K. S and Bisen, P. S. (2009): *Momordica balsamina*: A Medicinal and Neutraceutical Plant for Health Care Management. Journal of Current Pharmaceutical Biotechnology. Vol. 10 Issue 7. https://www.ncbi.nlm.nih.gov/pubmed/20383879#.

7

- [4] Rangit, R. and Richard, C. (1990). In: Immunochemistry of Viruses II. The Basis for Serodiagnosis and vaccines. Elsevier Sci. Publ., U.S.A. pp 217-236.
- [5] Ogali, I. N., Wamuyu, L. W., Lichoti, J. K., Mungube, E. O., Agwanda, B. and Ommeh, S. C. (2018). Molecular Characterization of Newcastle Disease Virus from Backyard Poultry Farms and Live Bird Markets in Kenya. International Journal of Microbiology. Volume 2018, Article ID 2368597, 11 pages. https://doi.org/10.1155/2018/2368597.
- [6] Useh, N. M. (2000). Prevalence of Newcastle Disease and its Control in Zaria, Nigeria. A Postgraduate term paper presented on Advance Clinics. Department of Pathology and Microbiology Faculty of Veterinary Medicine. Ahmadu Bello University, Zaria, Nigeria. Pp 7.
- [7] Trease, G. E. and Evans, W. C. (2005): In: A Textbook of Pharmacognosy, 13<sup>th</sup> Edn. Bailliere Tindall Ltd., London. 795 p.
- [8] Wagner, H., Bladt, S. and Zgainski, E. M. (1984). In: Plant Drug Analysis. A Thin Layer Chromatography Atlas. Berlin Heidelberg Springer-Verlag. 319 pp.
- [9] AOAC (1975): Official Methods of Analysis. Association of official analytical Chemists. Washington, D. C, USA, PP 450.
- [10] Hsiung, G. D. and Caroline, K. Y. F. (1989). In: Diagnostic Virology. Yale University Press, New Haven. Pp 154-155.
- [11] Snedecor, C. W. and Cochran, W. G. (1987). In: Statistic Methods. Oxford and IBH Publishing Company Ltd., Bombay. Pp 4-7.

- [12] Praveen, K. A. and Kumud, U. (2012): Tannins are Astringent. Journal of Pharmacognosy and Phytochemistry. Vol. 1 No. 3 Pp 45. http://www.phytojournal.com/vol11ssue3/.
- [13] Frantisek, S. S. (2002): The natural guide to medicinal herbs and plants. Tiger Barks Institute Twickenham, U.K. Pp. 21-70.
- [14] Gupta, S. S. (2007): Prospects and prospective of natural products in Medicine Indian Journal of Pharmacology, 26: 1-12.
- [15] Clark A. M. (2009). National products as resources for new drugs, Pharm. Res., 3, 1133-11411.
- [16] Jeffery, B. H. and Herbert, B. (1993). Phytochemical Dictionary- A Hand book of Bioactive Compounds from plants. Taylor and Francis Limited Pp. 475.
- [17] Priyanka, P. B. and Pathik S. B. (2013). Terpenes: Chemistry, Biological Role, and Therapeutic Applications. Natural Products pp 2665-2691.
- [18] Chapman and Hall (1994). Dictionary of Natural Products First Edition 3 Page 5001-5002, 6145.
- [19] Sabanda, C. W., Ahmat, N., Jaafar, F. M. and Sahidin, I. (2013): Medicinal Property, Phytochemistry and Pharmacology of several Jatropha species (Euphorbiaceace) a review, Phytochemistry, vol, 85, pp 7-29.
- [20] Errington, H (2017): Newcastle Disease. NADIS Animal Health Skills. St David's Game Bird Services. Reviewed: Published: 2017. https://www.nadis.org.uk/disease-a-z/gamebirds/newcastle-disease/.