

# Assessment of the effects of *Cucumis metuliferus* fruits alkaloids against Newcastle disease virus-LaSota

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## Abstract

**Objective:** The objective of this study is to investigate the antiviral activity of the *Cucumis metuliferus* alkaloids against Newcastle disease virus-LaSota (NDV-L).

**Methods:** Hemagglutination (HA) test method using embryonated eggs for study.

**Results:** The result showed “nonlethality” of the alkaloids in 10 days old embryonated eggs. Exposure of the alkaloids to NDV-L infected embryonated eggs showed negative HA test result from varying 2-fold diluted concentration between 50 and 3.125 mg/ml of the alkaloid, while the concentration between 1.563 and 0.195 mg/ml gave positive HA test. Negative HA response means the alkaloids at that concentration have antiviral activities while positive HA test implies the presence of antibodies production in response to an antigen as seen with the control, meaning that viral replication was not inhibited when the embryonated eggs were exposed to lower doses of the alkaloids.

**Conclusion:** The study showed that the alkaloids of *C. metuliferus* fruit pulp extract have good margin of safety with a high level of antiviral properties as confirmed by the result of the HA test.

**Key Words:** Alkaloids, antiviral, *Cucumis metuliferus*, hemagglutination test, Newcastle disease virus

## INTRODUCTION

The use of herbs for medicinal purposes is on the increased, and it has been shown that the renewed interest in the use of herbal product has been attributed to its abundance, cheapness, and ready accessibility by the local populace; in addition to increased availability of documented information on the safety and efficacy of herbs for health care.<sup>[1,2]</sup>

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*Cucumis metuliferus* belongs to the family *Cucurbitaceae* and is endemic to the semi-arid region of southern and central Africa.<sup>[3,4]</sup> The detail characteristic features of the plant are as described by Jimam *et al.*<sup>[5]</sup> the claimed medicinal activities of the fruit pulp by a traditional healer in some part of Plateau state of Nigeria and beyond have been documented.<sup>[6]</sup> The fruit of the plant was also used during the bird flu (avian influenza) outbreak by some local farmers on the Plateau with some level of success.<sup>[7]</sup> These authors have also reported on the antiviral properties of the ethanol extract of the fruit using the method of hemagglutination (HA) test on embryonated eggs against Newcastle disease virus (NDV-Komarov and NDV-Lasota).

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Alkaloids isolated from some plants have been proven to have antiviral properties.<sup>[8]</sup> Not much has been documented on the antiviral properties of the alkaloids isolated from *C. metuliferus* fruits. The aim of this study was therefore to investigate the antiviral properties of the alkaloids of the fruit pulp extract using HA inhibition test in embryonated eggs.

## MATERIALS AND METHODS

### Plant collection and identification

The ripe fruit of *C. metuliferus* was harvested from Chong'openg of Jos South Local Government Area of Plateau state, Nigeria. The plant was identified and authenticated by Prof. C. O. Akueshi of the Department of Plant Science of the University of Jos, Nigeria.

### Preparation of *Cucumis metuliferus*

The mesocarp of the fruits and seeds were carefully scooped out of the pericarp using a spatula and was well stirred after which the yellowish fibrous portion was sun-dried. Sieves of different sizes were used to separate the seeds from the greenish fluid portion, after which the fluid portion was spread on trays and placed in an oven set at 55°C until it was dried. The yellowish and the greenish dried portions were then mixed and reduced to fine particle size using mortar and pestle.

### Extraction of alkaloids of the *Cucumis metuliferus* fruit pulp

The alkaloids from *C. metuliferus* fruit pulp were isolated according to the method described by Agrawal and Paridhavi.<sup>[9]</sup> The pure alkaloid was stored in an air-tight container at room temperature before use.

### Toxicity test

One thousand milligrams of the alkaloids was dissolved in 2 ml of sterile distilled water. Two-fold dilutions of the alkaloids were made from the ratios 1:2 to 1:64. One milliliter of each dilution was inoculated into a set of five eggs each (10-day-old embryonated eggs) and incubated for 72 h at 37°C. The eggs were candled every 24 h to check for viability and mortality. Dead eggs were recorded and discarded appropriately according to the method of Cardoso *et al.*<sup>[10]</sup>

### Effect of the extract on Newcastle disease virus-LaSota

500 mg/ml of the alkaloid was reconstituted, and 2-fold dilutions of the alkaloids were made from the ratio of 1:2 to 1:64 dilutions. One milliliter of NDV-L (with median embryo infective dose of 10<sup>9.7</sup>/0.1 ml and HA titer of 2<sup>9</sup>) was then added to 1 ml each of the various dilutions of the alkaloids and incubated at + 4°C and 37°C for 30 min, respectively. 0.2 ml of the alkaloid-virus suspension of each dilution was inoculated into a set of five eggs each (10 days old embryonated eggs) and incubated at 37°C for 72 h. The

embryonated eggs were candled every 24 h to check for viability and mortality (dead embryonated eggs were chilled at +4°C and the allantoic fluid harvested for rapid HA test (to check the presence or absence of viral activity). After 72 h incubation, the embryonated eggs were chilled at + 4°C for 24 h. The eggs were cut open with a pair of scissors and the allantoic fluid aspirated into bijou bottles. A rapid HA test was carried out on a glass slide using 10% washed chicken red blood cells on the harvested allantoic fluid. The presence of HA confirmed viral activity, and its absence confirmed antiviral activity of the test extract as described by Palmieri and Bennett.<sup>[11]</sup>

## RESULTS

### Toxicity of alkaloids isolated from *Cucumis metuliferus* fruit pulp

Table 1 shows that the toxicity of the alkaloids in the embryonated eggs was time and concentration dependent. The five embryonated eggs exposed to 100 mg/ml of the alkaloids of *C. metuliferus* fruit after 24 h died, while those exposed to 50 mg/ml of the alkaloids died after 48 h. There was no any mortality recorded under the remaining concentrations at varying time as well as the control.

### Effects of isolated alkaloids of *Cucumis metuliferus* fruit on Newcastle disease virus-LaSota suspension

Table 2 shows the impact of varying temperature on the effects of the alkaloids on preventing the mortality of the embryonated eggs exposed to NDV-L at various time and concentrations. Four of the embryonated eggs exposed to both NDV-L and 50 mg/ml concentration of the alkaloids died under the temperature of +4°C, while two out of the five died under 37°C after 48 h incubation period. Only one embryonated egg exposed to the NDV-L and 25 mg/ml of the alkaloid died under a temperature of +4°C after an incubation period of 72 h.

### Alkaloid of *Cucumis metuliferus* fruit pulp on the pathogenicity of Newcastle disease virus-LaSota in embryonated eggs

The result of the rapid HA test showed no antigen-antibody reaction for the groups exposed to varying concentrations of between 50 and 3.125 mg/ml of the alkaloid, while those exposed to 1.563–0.781 mg/ml produced HA reactions under the temperatures of + 4 and 37°C [Table 3].

Table 1: Toxic effects of alkaloids isolated from *Cucumis metuliferus* fruit pulp on embryonated eggs

Concentration (mg/ml)	100	50	25	12.5	6.25	3.125	1.563
Incubation period, h (mortality/viability)							
24	5/0	0/5	0/5	0/5	0/5	0/5	0/5
48	-	5/0	0/5	0/5	0/5	0/5	0/5
72	-	-	0/5	0/5	0/5	0/5	0/5
Total	5/0	5/0	0/5	0/5	0/5	0/5	0/5
Control	0/5	-	-	-	-	-	-

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Table 2: Effects of temperature variation on activity of *Cucumis metuliferus* fruit alkaloids on Newcastle disease virus-LaSota suspension (n=5)

Concentration (mg/ml)	50		25		12.5		6.25		3.125		1.563		0.781	
	+4	37	+4	37	+4	37	+4	37	+4	37	+4	37	+4	37
Temperature (°C)														
Incubation period, h (mortality/viability)														
24	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
48	4/1	2/3	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
72	-	-	1/4	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Total	4/1	2/3	1/4	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

Table 3: Effect of the isolated alkaloid of *Cucumis metuliferus* fruit pulp on the pathogenicity of Newcastle disease virus-LaSota in embryonated eggs (n=5)

Concentration (mg/ml)	50		25		12.5		6.25		3.125		1.563		0.781		Control	
	+4	37	+4	37	+4	37	+4	37	+4	37	+4	37	+4	37	+4	37
Temperature (°C)																
Positive/negative for virus	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	5/5	5/5	5/5	5/5	5/5	5/5

**DISCUSSION**

The result of the toxicity studies of the alkaloids *C. metuliferus* on the embryonated eggs did not reveal any cytopathic effects on the cells between the concentrations of 1.563 mg/ml and 50 mg/ml [Table 1], which similar to other herbs on embryonated eggs;<sup>[12]</sup> this was in accordance with the safety margin of the crude extract of the plant which had been shown to be safe in laboratory rats at oral dose concentrations of up to 5000 mg/kg.<sup>[13]</sup> The death recorded in the 5 embryonated eggs after 24 h could be due to the fact that the concentration administered was beyond the maximum safety levels of the fruit alkaloids; because the wild plant have been seen to contain some levels of toxic chemical known as cucurbitacins which are absent or at suppressed levels in the cultivated species, and makes it safe.<sup>[13,14]</sup> On the other hand, the recorded death on the 48<sup>th</sup> hours of the study in those administered 50 mg/ml showed a time-depended effect of the alkaloids.<sup>[5]</sup>

Antiviral testing for plant is generally aims at determining the inhibitory activities of the virus-induced cytotoxicity of appropriate host cells.<sup>[15]</sup> The result of this study showed a temperature and time-dependent activities of the alkaloid extract of *C. metuliferus* fruit [Table 2]. There was an increased protective activity of the alkaloid on the embryonated eggs against attacked by the virus with increasing temperature, and that was why the number of death from 4 at +4°C was reduced to 2 at 37°C on the 48<sup>th</sup> hours of the study. This result was similar to those some studies on the antiviral activities of some plant extracts on NDV at various temperatures.<sup>[16-18]</sup>

HA test is used for definitive detection of viral antibody<sup>[19]</sup> and the test is based on the principle of adsorbing out the cross-reacting antibodies to NDV-L antigen. Studies have shown that negative HA response observed with the higher dose of the extract means that there was no viral replication in the embryonated eggs suggesting the absence of antibodies production in response to the viral antigen and hence antiviral activity of the extract; whereas positive HA response imply

the presence of antibodies production in response to antigen as is seen with the control, where viral replication was not inhibited.<sup>[7,19]</sup> This result confirmed the safety and widely use of the plant in animals and human,<sup>[7]</sup> hence need for further work on the extract to ascertain the type of the alkaloids responsible for activities and the mechanism of action.

**CONCLUSION**

The result of the study showed that the *C. metuliferus* fruit pulp alkaloid extracts have good margin of safety on the embryonated eggs which was concentrations and time-dependent; with increased protective activities on the embryonated eggs against attacked by the NDV-L at a higher temperature.

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**Conflicts of interest**

There are no conflicts of interest.

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