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## Antiviral activities of *Cucumis metuliferus* fruits alkaloids on Infectious Bursal Disease Virus (IBDV)

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### ABSTRACT

**Objective:** The toxicity of the alkaloids isolated from the fruit pulp of *Cucumis metuliferus* was investigated in chicken fibroblast cells. **Materials and Methods:** The alkaloids from *C. metuliferus* fruit pulp were isolated and their effects assayed on infectious bursal disease virus (IBDV) using chicken embryo fibroblast cells. **Results:** There was no observed structural change on the chicken fibroblast cells when exposed to concentrations of 0.195 and 100 mg/ml of the alkaloids; and there was observed antiviral activities on IBDV at concentration between 6.125 and 100 mg/ml, while concentrations of the alkaloids between 0.195 to 3.125 mg/ml showed no antiviral activities compared to the control. **Conclusion:** The alkaloid showed good margin of safety on chicken fibroblast cells, with antiviral activities on infectious bursal disease virus (IBDV).

**Keywords:** Alkaloids, Chicken fibroblast cells, Cytotoxicity, *C. metuliferus*, Infectious bursal disease virus.

### INTRODUCTION

Infectious bursal disease virus (IBDV) is a viral disease that affects young birds of between two to six weeks of age with highly active bursa of fabricius.<sup>[1,2]</sup> Human immuno-deficiency virus (HIV), Newcastle disease virus (NDV) and infectious bursal disease virus (IBDV) have been shown to be similar as well as having similar pathways of entry and replication in host cells, as they all belong to the class of retroviruses.<sup>[3,4]</sup>

The increase public interest in the use of alternative medicine as source of medication in human and veterinary medicine has led to good levels of interest and acceptance among health professionals.<sup>[5-8]</sup> Alternative antiviral obtained from plants have been documented traditionally and scientifically to have protective activities against viruses.<sup>[9-13]</sup>

*Cucumis metuliferus* plant belongs to the family of cucurbitaceae, and is a monoecious, climbing annual herb, with staminate flowers typically appearing several days before pistillate flowers. The fruit is bright yellow-orange in colour when mature, consisting of greenish juicy bland tasting tissues.<sup>[14,15]</sup> The widely use of the plant as food and for medicinal purposes have been documented.<sup>[16,17]</sup> The fruit of the non-bitter variety of *C. metuliferus* has been found to be less toxic and cultivated on large scale for consumption by the local populace.<sup>[15]</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; and the antiviral properties of the ethanolic fruit extract had been reported on Newcastle Disease Virus (NDV-K and NDV-I).<sup>[18]</sup>

The main purpose of this study was to investigate the antiviral properties of the isolated alkaloids of this fruit pulp extract in chicken fibroblast cell using infectious bursal disease virus.

### MATERIALS AND METHODS

#### Plant collection and authentication

The ripe fruit of *C. metuliferus* were harvested from Chong' Openg village of Jos south Local Government Area of Plateau State, Nigeria. The plant was identified and authenticated by Professor C. O. Akueshi of the department of plant science of the University of Jos, Nigeria.

#### Preparation of *C. metuliferus*

The mesocarp content of the ripened fruit of *C. metuliferus* was carefully scooped out from the pericarp with the aid of a spatula. The fleshy content was blended using electric blender and the fluidy product of blending was passed through a sieve size of 0.25mm to separate the seeds from the juicy contents.

The smooth filtrate was evenly spread on an aluminium tray and dries in a drying cabinet, at about 55 °C until the liquid content had been evaporated. The resultant product was air dried for several hours and then pounded to powder using mortar and pestle and appropriately stored in an air-tight container.

**Isolation of alkaloids of the *C. metuliferus* fruit pulp**

The alkaloids from *C. metuliferus* fruit pulp were isolated according to the method described by Agrawal and Paridhavi.<sup>[19]</sup> The pure alkaloid was stored in an air-tight container at room temperature prior to use.

**Cytotoxicity assay for *C. metuliferus* fruit pulp alkaloid on chicken embryo fibroblast cells**

Chicken embryo fibroblast cells were cultured in 24-well tissue culture tray (1ml per well using suspended cells at a concentration of 10<sup>4</sup> cells per ml). The cells were grown using Hanks Minimum Essential Medium (HMEM) plus 10% foetal bovine serum. The cultured cells were incubated under a humidified CO<sub>2</sub> atmosphere (5% CO<sub>2</sub>/95% filtered air at 37<sup>0</sup>C until a confluent monolayer was obtained. Doubling dilutions of the alkaloids in serum-free medium (HMEM) from a concentration of 100 mg/ml to 0.195mg/ml were prepared. The alkaloids were tested for cytotoxicity by exposing the monolayer of the chicken embryo fibroblast cells cultures to the dilutions of the alkaloids. The medium in the cells were first aspirated using a multi-channel pipette before the introduction of the various concentrations of the alkaloids which were done in four replicates as

described by Cardoso *et al.*<sup>[20]</sup> The cells were monitored daily, visually for seven days using an inverted microscope for any evidence of structural changes in the chicken embryo fibroblast cells as a result of its exposure to the alkaloid isolates.

**Antiviral assay for *C. metuliferus* fruit pulp alkaloids on infectious bursal disease virus (IBDV)**

The chicken embryo fibroblast cells were cultured and incubated at 37<sup>0</sup>C until a confluent monolayer was obtained as described above. After one hour incubation, 1ml of IBDV in HMEM plus 0.1% foetal bovine serum containing 100pfu (plaque forming units) were added to each well containing the various concentration of the alkaloids, these were repeated in replicates of four. Control cultures were included cells with alkaloids but without virus (negative control) and cells without alkaloids but with virus (positive control) were all set up. When the virus- induced cell cultures started showing the characteristic pathological changes induced in normal infected cells known as virus-induced cytopathic effects (CPE), all other cultures were examined microscopically and assessed for CPE.

**RESULTS**

**Toxicity study of *C. metuliferus* fruit pulp alkaloids on chicken embryo fibroblast**

The cell cultures exposed to varying concentrations of *C. metuliferus* fruit pulp alkaloids (100mg/ml to 0.195mg/ml) showed no cytopathic effects on the chicken embryo fibroblast cells compare to the control (Table 1).

**Table 1:** Effect of isolated alkaloid of *C. metuliferus* fruit pulp on chicken embryo fibroblast cells

Concentration											
(Mg/ml)	100	50	25	12.5	6.25	3.125	1.562	0.781	0.391	0.195	Control
Treatment: NCPE NCPE NCPE NCPE NCPE NCPE NCPE NCPE NCPE NCPE NCPE											
n = 4											
Keys: NCPE = No Cytopathic effect											
Control = Cell alone											

**Table 2:** Effect of Alkaloids Isolated from *C. metuliferus* Fruit Pulp on IBDV using Chicken Embryo Fibroblast Cells

Concentration											
(mg/ml)	100	50	25	12.5	6.25	3.125	1.562	0.781	0.391	0.195	+ve -ve
Treatment: NCPE NCPE NCPE NCPE NCPE CPE CPE CPE CPE CPE NCPE											
n = 4											
Keys: NCPE = No Cytopathic effect,											
CPE = Cytopathic effect,											
+ve = Positive control (cell + IBDV)											
-ve = Negative control (cell + alkaloids)											

## Antiviral activities of *C. metuliferus* fruit pulp alkaloids on IBDV

On the other hand, cell cultures that were exposed to IBDV and then treated with alkaloids of *C. metuliferus* fruits showed no cytopathic effects at higher concentrations of between 100 to 6.25mg/ml, while lower concentrations of between 3.12 to 0.195mg/ml showed cytopathic effects (Table 2). The positive control group that was administered IBDV alone showed presence of cytopathic effects while the negative group that was administered only alkaloids showed no cytopathic effects.

## DISCUSSION

The non cytopathic effects of the alkaloids on the egg fibroblast observed in Table 1 was in accordance to previously established safety margin of the oral dose of 5000 mg/kg of the crude extract in laboratory animals.<sup>[21]</sup> The safety and widely used of the fruit of this plant for its antiviral activities have been documented.<sup>[18]</sup> The toxicity result of this study was similar to toxicity study of the flavonoids extract of the plant's fruits conducted by Amagon *et al.*,<sup>[22]</sup> and also reports on other herbs using embryonated eggs.<sup>[23]</sup>

Absence of cytopathic effect on the fibroblast cell of the eggs when exposed to the virus and the alkaloids at doses of between 6.25 and 100 mg/kg (Table 2) was an indication of the activity of the alkaloids at these doses against the known structural changes that occurs in host cells due to viral invasion. It is a known fact that when virus invade host cells, the infecting virus either impact cytopathic effects by lysis of the host cells or killing the cell without lysis through inhibiting its reproductive activities.<sup>[24,25]</sup>

The cytotoxic and growth inhibitory effects of many plant extract have been investigated using different approaches to testing the antiviral activities of new products including herbs;<sup>[26,27]</sup> with the ultimate purpose of determining the inhibition of the virus-induced cytotoxicity of appropriate host cells.<sup>[28]</sup>

This result implies that the alkaloids of *C. metuliferus* was able to inhibit the replication of IBDV and hence its' damaging effects on the chicken embryo fibroblast cells at higher concentration, while at lower concentration, the effect of the extract does not inhibit the growth of the virus thereby increasing its damaging effects on the chicken embryo cells. In cases where no CPE were evident, the virus was assumed to be completely inactivated or inhibited.<sup>[22]</sup>

## CONCLUSION

The result of the study showed that the alkaloids extracted from *C. metuliferus* fruit have some good margin of safety and antiviral activities on IBDV compared to the control.

## Conflict of interest

Authors declare that there is no conflict of interest to reveal.

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