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Short Communication

In-ovo evaluation of the antiviral activity of methanolic root-bark extract of the African Baobab (*Adansonia digitata Lin*)

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Application of ethnoveterinary medicine in the control of poultry diseases is being embraced in many parts of the world for more profitable production. This study investigated the antiviral property of the root-bark extract of the African Baobab tree (*Adansonia digitata* Lin) against Newcastle disease virus. One hundred and seventy five specific antibody negative embryonated chicken eggs and a field strain of Newcastle disease virus were used to test for the antiviral activity of the methanolic root bark extract of the tree. Following a 2-h exposure of the virus to eight graded concentrations of the extract, it was incubated at 37°C and observed 12-hourly for mortality. Dead embryos were spot tested for haemagglutinating activity. The 100EID_{50} concentration of the virus and the highest concentration of the extract were inoculated as positive and negative controls, respectively. All eggs inoculated with the virus alone as well as 5 and 2 mg/ml extract/virus suspensions, died by 72 h post inoculation, while no mortality was observed amongst those inoculated with 250 and 200 mg/ml virus/extract suspensions as well as those inoculated with the pure extract. Mortalities of 40, 40 and 20%, due to viral activity were recorded for 25, 50 and 100 mg/ml suspensions, respectively. This study showed that methanolic root-bark extract of *A. digitata* has antiviral activity against Newcastle disease virus *in ovo*, particularly when used at dose rates of 200 and 250 mg/ml.

Key words: Ethnoveterinary, African Baobab, antiviral activity, Newcastle disease virus.

INTRODUCTION

A major constraint to profitable broiler production is disease outbreaks. The tropical environment provides optimum conditions like high environmental temperature and relative humidity as well as poor biosecurity for disease agents to thrive. These are major challenges being faced by the poultry industry in Nigeria. In the face of persistent disease outbreaks and in spite of the use of conventional chemicals as disinfectants and drugs, it has become imperative to seek means of achieving effective biosecurity and control measures which ethnoveterinary preparations could offer (Gueye, 1999; Musa et al., 2008).

The Baobab tree (*Adansonia digitata* Lin) is indigenous in many African countries (Wickens, 1982; Sidibe and Williams, 2002). Many parts of the plant, especially leaves, fruit pulp, seeds and bark fibers, have been used traditionally for medicinal and nutritional purposes (Sidibe and Williams, 2002; Chadare et al., 2009) and some commercial enterprises produce standardized preparations derived from its parts. The medicinal applications include treatment of intestinal and skin disorders and various uses as anti-inflammatory, anti-pyretic and analgesic agents (Ramadan et al., 1994; Palombo, 2006;

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Abbreviation: DMSO, Dimethylsulfoxide; **EID_{50},** embryo infective dose 50; **HA,** haemagglutination; **ND,** Newcastle disease; **NVRI,** Nigerian Veterinary Research Institute; **SAN,** specific antibody negative.

Ajose, 2007; Karumi et al., 2008). In addition, anti-bacterial, antiviral and anti-trypanosomal activities of the plant extracts have been reported (Anani et al., 2000; Hudson et al., 2000; Atawodi et al., 2003; Vimalanathan and Hudson, 2009). Most of the reports were on the efficacy of the leaves, fruit pulp, seeds and bark fibers of this plant with very little information on the antiviral properties associated with the root-bark extracts. However, the root bark is commonly used in traditional African Medicine in the treatment of fever (SCUC, 2006; Wickens, 2008). This study was therefore carried out to evaluate the antiviral property of the methanolic root bark extracts of the African Baobab tree (*A. digitata Lin*) against Newcastle disease (ND) virus.

MATERIALS AND METHODS

Extract preparation

Root-bark of *A. digitata* was harvested, cut into small pieces and air-dried on a clean surface for 10 days. Two kilograms of the root-bark was soaked (completely immersed) in 5 L of methanol in a covered container for 3 days (Wickens, 2008; Egunyomi et al., 2010). The methanol was decanted and fresh methanol was added to the root-bark. The procedure was repeated twice and the extract was poured into a small glass container and refrigerated.

Antiviral activity test

Preparation of virus: Extract inoculum

The virus used was a field strain of ND virus from the Virology Research Laboratory of the National Veterinary Research Institute (NVRI), Vom, Nigeria. The virus was propagated and titrated in 9 day-old specific antibody negative (SAN) embryonated chicken eggs and the $100EID_{50}$ was calculated. The extract was diluted to the desired concentrations of 250, 200, 100, 50, 25, 10, 5 and 2 mg/ml. These extract concentrations were substituted for the virus buffer diluent at the end point dilution of the virus to achieve the $100EID_{50}$. The virus/extract suspensions were kept at 4°C for 2 h to allow reaction to occur.

Egg inoculation

The embryonated chicken eggs were labeled according to the extract concentrations used. A set of plastic egg trays were thoroughly cleaned with Virkon ®, the eggs were swabbed with 70% alcohol in cotton wool and transferred into the cleaned trays. The swabbed eggs were placed in the micro-safety cabinet where they were punched and immediately inoculated with the extract/virus suspension via the allantoic route. Each egg was inoculated with 0.1 ml of the inoculum and 5 eggs were inoculated with each con-centration of the extract/virus suspension, while inoculating the 250 mg/ml extract only (without virus) and the $100EID_{50}$ concentration of the virus (without extract- virus control) as negative and positive controls, respectively. The eggs were sealed with molten wax and incubated at 37°C. The inoculated eggs were monitored for embryonic death 12-hourly for up to 96 h post inoculation. Dead embryos were removed and chilled in the refrigerator for 3 h after which they were opened to check for haemagglutinating (HA) activity by spot testing.

Spot haemagglutination test

Dead embryos that had been chilled were brought out of the refrigerator and kept at room temperature for about 30 min. The eggs were swabbed and placed in the biosafety cabinet. The shell of each egg was opened to reveal the air space and a pipette was used to dispense a drop of 1% washed chicken red blood cells on a white tile. A wire loop was thoroughly flamed and used to pick a drop of the allantoic fluid which was mixed with the drop of blood. The tile was gently rocked and observed for visible agglutination, indicating viral activity (Thayer and Beard, 1998; Murakawa et al., 2003). This was done for every egg and the observations were recorded.

RESULTS AND DISCUSSION

All eggs inoculated with the virus control, 5 and 2 mg/ml of the extract/virus suspension died between 48 and 72 h of inoculation and showed positive haemagglutination by spot-testing. However, no mortality was observed amongst the embryonated eggs inoculated with the extract only, 250 and 200 mg/ml of extract/virus suspension up till 96 h post inoculation. There were varying percentages of embryo mortality amongst the 100, 50, 25 and 10 mg/ml extract/virus suspension groups (Table 1). The haemagglutination test was carried out to associate embryo mortality with viral activity. The results of mortalities and HA spot-testing are shown in Table 1. Only the group of eggs inoculated with 5 mg/ml extract/virus suspension had a dead embryo at 24 h post inoculation.

This study has evaluated the potentials of the methanolic root-bark extract of *A. digitata Lin* as an antiviral agent against ND virus using multiplication of the ND virus in embryonated egg as an indicator for antiviral property. The results of the virus propagation showed that 250 and 200 mg/ml concentrations of the extract completely inhibited the growth of ND virus in embryonated chicken eggs, indicating that the methanolic root bark extract of *A. digitata* at these doses are effective against the virus when exposed *in-ovo.*

The results of this study corroborrates the findings of Vimalanathan and Hudson (2009) that the methanolic, water and dimethylsulfoxide (DMSO) extract of the leaf, stem and pulp of *A. digitata* has both antiviral effect, especially against influenza virus and anti-inflammatory effects. The antiviral property observed might be ascribed to the presence of various potentially bioactive ingredients (Chadare et al., 2009), including triterpenoids, flavonoids and phenolic compounds, but at present, the antiviral activity observed in this study could not be ascribed to specific compounds.

At extract concentrations of 100, 50 and 25 mg/ml, there were 20, 40 and 40% mortalities due to viral activity when compared with 0% at concentrations of 250 and 200 mg/ml. This indicates inhibitory rather than virucidal effect of the extract on the ND virus at these doses. Whereas, at concentrations of 2 to 10 mg/ml, as well as at 0 mg/ml (virus control), the study recorded total or near

Table 1. Embryo mortality and haemagglutination (HA) test result of embryonated chicken eggs inoculated with graded doses of *A. digitata* extract/ND virus suspension.

Virus (mg/ml)	Mortality				HA Test		Mortality due to viral activity (%)
	24 h pi	48 h pi	72 h pi	96 h pi	+ve	-ve	
250	0/5	0/5	0/5	0/5	0	5	0
200	0/5	0/5	0/5	0/5	0	5	0
100	0/5	0/5	1/5	0/4	1	4	20
50	0/5	1/5	3/4	1/1	2	3	40
25	0/5	0/5	2/5	0/3	2	3	40
10	0/5	0/5	3/5	2/2	5	0	100
5	1/5	0/4	4/4	-	4	1	80
2	0/5	0/5	5/5	-	5	0	100
Vc	0/5	0/5	5/5	-	5	0	100
Uic	0/5	0/5	0/5	0/5	0	5	0
Ec	0/5	0/5	0/5	0/5	0	5	0

Vc = Virus control; Uic = uninoculated control; Ec = egg control; pi = post inoculation.

total mortalities due to viral activity, indicating the ineffectiveness of the extract at these doses. The dead embryos in the 50 and 5 mg/ml extract concentrations that were negative for haemagglutinating activity must be a result of nonspecific deaths from manipulations during inoculation (Villegas, 1998).

In conclusion, this work has shown that the methanolic root-bark extract of *A. digitata* Lin has direct antiviral activity against ND virus and could therefore be useful in the control of the disease in poultry birds.

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