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Antiviral property of crude ethanolic extract of *Mangifera indica* leaves on embryonated henss' eggs infected with Newcastle disease virus

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ABSTRACT

Mangifera indica is a plant with varied medicinal properties including the treatment of infectious diseases. It also has antimicrobial and antiviral activities against *Bacillus subillis, Staphylococcus albus, Vibro cholerae* and herpes simplex virus type 2. Newcastle disease virus causes Newcastle disease and this disease is a highly contagious viral disease of poultry which causes huge economic losses to the local and commercial poultry industry. There is no known cure for this disease except prophylactic measures by means of vaccination. There is therefore the need to research into alternative means of treating the disease. This study is aimed at determining the toxicity and antiviral properties of crude ethanolic extract of *Mangifera indica* leaves on embryonated eggs infected with Newcastle disease virus. Soxhlet extraction was carried out using about 100g powdered plant and ethanol as solvent. At the end of the extraction process, the solvent was evaporated to recover the extract. Fifty embryonated hen eggs were arranged in ten groups of five. Toxological assay was carried out on two groups, B_1 and B_1 repeat with an extract concentration of 250mg/ml and 200mg/ml with their controls. Antiviral assay was carried out on four groups using concentrations of 250mg/ml, 200mg/ml, 100mg/ml and 50mg/ml with an uninoculated control. This was observed for four days. The result showed that crude ethanolic extract of *Mangifera indica* has mild toxicity on embryonated eggs ($P<0.05$) but the antiviral property of the extract depend on the concentration. At a higher concentration of 250mg/ml and 200mg/ml, the extract showed antiviral properties but at lower concentration of 100mg/ml and 50mg/ml, it showed no antiviral property ($P<0.05$). The ethanolic extract is mildly toxic against the embryonated eggs and has antiviral property only at high concentration.

Keywords:Antiviral property, ethanolic extract, *Mangifera indica* leaves, embryonated eggs, Newcastle disease virus

INTRODUCTION

Newcastle disease is a viral disease of birds with a wide range of clinical signs from mild to severe. The disease is one of the most important poultry diseases worldwide. It is caused by Newcastle disease virus [1]. Newcastle Disease Virus (NDV) is an Avian Paramyxovirus (APMV) belonging to the genus *Avulavirus* in the family *Paramyxoviridae* [2]. Newcastle castle disease virus strains are classified into three pathotypes based on their virulence in chickens as lentogenic, mesogenic and velogenic strains [3]. It is further grouped into five pathotypes depending on tissue tropism and hence, clinical signs seen in infected chicken [4]. Newcastle disease is the major disease of both local and commercial chickens and is characterized by high mortality and morbidity which hinders the development of poultry industries in developing countries. Outbreaks are seasonal and in Nigeria, it occurs in the dry harmattan period which is further worsened by the cold harmattan wind [5].Occurrence in man manifests as mild conjunctivitis, influenza-like disease with fever and headache [6]. Clinical signs exhibited by infected chickens and other poultry birds includes, diarrhoea, dropped wings, paralysis of

legs, coughing and sneezing, nasal discharge, ruffled feathers, torticollis (moving backwards), incordination, muscular spasm, ocular discharges, congested eyes, cloudy eyes, sitting on the hock, facial swelling, star gazing etc [6]. Virulent Newcastle disease virus strains are endemic in poultry in most of Africa, Asia, countries of North, Central and South America, Mexico, Meddle east US and Canada [7]. Current diagnosis of Newcastle disease is by conventional virus isolation and serological tests such as Haemagglutination Inhibition (HI), Elisa Technique, and Serum Neutralization [8]. Reverse Transcription Polymerase Chain Reaction (RT – PCR) coupled with red blood cell adsorption is a much more sensitive method [9].

Manigifera indica commonly known as mango is a tropical fruit belonging to the genus *Mangifera* and family *Anacadiaceae* [10]. Mango is a large evergreen tree that grows to a height of 10-45m, dome-shaped with dense foliage. The leaves are greenish to pale yellow and are spirally arranged on branches. Fruits are large, fleshy drupes with varying sizes [11]. Mango is native to the Indian subcontinent from where it spread all over the world [12]. It is now widely distributed in most tropical countries and accounts for half of all tropical fruit produced worldwide [13]. Varied medicinal properties are attributed to different parts of mango tree e.g. Mangiferin is a pharmacologically active flavanoid. A natural Xanthone C-glycoside, Homomangiferin is Mangiferin monomethylether. Both are extracts from *Mangifera indica* and are widely used to relieve many symptoms e.g. cough and asthma [14]. Newcastle disease has no curative treatment but can be prevented. (Controlled by vaccination and sanitary measures). These vaccines have been reported not to give adequate protection to birds as outbreaks are reported in vaccinated birds every year [15]. The disease is also endemic in local chicken especially in Nigeria, where it serves as a source of new outbreaks, resulting in the death of virtually all young birds especially during the harmattan season. Vaccination coverage is poor and does not cover local chicken. There is also vaccines failure due to poor keeping or holding during transportation and storage, resulting in loss of potency [16]. There is therefore, the need to research into cheaper, affordable and available means of control of the diseases. There is therefore the need to look for an alternative means of treatment, thus, this research work determined the toxic potentiality and antiviral effects of crude ethanolic extracts of *Mangifera indica* leaves on Newcastle disease virus of embryonated eggs.

MATERIALS AND METHODS

COLLECTION AND IDENTIFICATION OF PLANT

The leaves of *Mangifera indica* were collected from Inikpi hostel in Federal Polytechnic Idah, Kogi State by stem cutting and identified by Prof. P.C Okeke, a Taxonomist in the Department of Botany, Nnamdi Azikiwe University Awka (NAU). The leaves were kept in a voucher in the herbarium of the Nnamdi Azikiwe University, Awka.

EXPERIMENTAL SITES

The extraction process was carried out in the Department of Applied Microbiology Laboratory, National Veterinary Research Institute (NVRI) Vom, Jos, Plateau State; while the antiviral assay of ethanolic extract of *Mangifera indica* leaves on Newcastle disease virus (Hert isolate) was carried out in the Avian Viral Research unit of the National Veterinary Research Institute (NVRI) Vom, Jos, Plateau State.

PREPARATION OF LEAVES AND EXTRACTION

Preparation of Plant Materials

The leaves of *Mangifera indica* were air-dried for about 30 days at room temperature in the Biology Laboratory of the Department of Science Laboratory Technology, Federal Polytechnic, Idah, Kogi State. The leaves were then pulverized by pounding in a clean mortar with pestle to increase surface area.

PREPARATION OF EXTRACT (EXTRACTION).

About 100g of the pulverized leaves of the *Manigifera indica* was weighed, wrapped in filter paper (Whatman filter paper) and placed in the thimble of the soxhlet extractor which was fitted to a round bottom flask containing the extraction solvent (ethanol). A lie-big condenser was fitted on the thimble and tap water was

allowed to flow in-end- out of the condenser in order to cool the system. The flask was subjected to heating on a heating mantle and the solvent evaporated into the condenser where it was converted into liquid that trickle into the extraction chamber containing the sample. The chamber is designed such that when the solvent surrounding the sample exceeds a certain level, it over flows and trickles back into the boiling flask (containing the solvent). A clear solution in the extraction chamber indicates complete extraction. At the end of the extraction process, the flask containing the solvent and sample extract was evaporated using a rotary evaporator leaving the extract to concentrate. The powder was collected and weighed.

QUALITY CONTROL TEST OF THE EXTRACT

The extract was tested for possible contamination by plating the extract on blood agar and incubated at 37% for 24hrs and PSGA (Penicillin, Streptomycin Gentamycin and Amphotericin β) was used to clear the contaminants.

PREPARATIONS OF EGGS.

The 11-day-old embryonated or fertile hen eggs were candled by holding them against the aperture of the candling lamp in a darkened room. And the position and movement of the embryo was noted. The air sac was marked by drawing a line round the region while the position of the embryo was marked with X and the site of inoculation was marked approximately 2mm above the line of the air sac region on the side opposite the side marked X with the aid of a pencil.

COLLECTION OF VIRUS STRAIN / ISOLATE

The Newcastle disease Virus Hert isolate (Hertforthshire isolate) was obtained from the Avian Viral Research unit of the National Veterinary Research Institution (NVRI) Vom, Jos, Plateau State

NDV- VIRUS TITRATION

Newcastle disease virus (NDV) titration is done by the Supplemental Assay method (SAM). The NDV Vaccines are reconstituted and inoculated into the embryonated chicken eggs in ten fold dilutions such that the 50% egg infective doses (EID_{50}) can be calculated directly on a per field dose basis Reed- Muench methods [16]. A stock of the Virus isolate/stain was serially diluted from 10^{-1} to 10^{-9}. 0.1ml of each dilution from 10^{-3} to 10^{-9} was inoculated into the allantoic cavity of each of the disinfected 5 eggs (30 eggs were used in all). 0.1ml of Phosphate Buffer Saline (PBS) was inoculated into each of 5 eggs as control, incubated in an egg incubator at 37°C and observe for viral activity for every 24hrs for 48hrs (the $100LD_{50}$ of the Virus suspension was used).

ANTIVIRAL DETERMINATION OF THE EXTRACT.

Two concentrations (250mg/ml and 500mg/ml) of the extract were tested for antiviral activities against Newcastle disease virus (the Hert isolate) in 5eggs for each concentration. 5ml of 250mg/ml as well as 5ml of 500mg/ml of the extracts were prepared. 2.5ml of each extracts concentration was diluted with 2.5ml of 100 LD_{50} of Virus suspensions and incubated at 37% for 1hour. 5ml of the $100LD_{50}$ of the Virus was prepared as control and incubated at 37% for 1hour. 0.1ml of the Virus and extract combination was inoculated into the allantoic cavity of each of 5 eggs and 0.1ml of virus control was inoculated into the allantoic cavity of each of 5 eggs. 0.1ml of extract alone was inoculated into each of 5 eggs and 0.1ml of PBS was also inoculated into the allantoic cavity as uninoculated control of each of 5 eggs (all the 20 eggs were properly disinfected with alcohol). The same procedure was repeated for the 500mg/ml of the extract and the eggs were then incubated in an egg incubator at 37°C and observed for viral activity, extract activity and antiviral activity for every 24 hours for 72 hours

SPOT HAEMAGLUTINATION (HA) TEST.

Spot agglutination test was carried out for each egg to determine virus activity or presence of the virus in each egg. 5ml of heparinized chicken Red Blood Cell (RBC) was collected into a sterile heparinized test tube by bleeding the chicken via wing vein. Sterile beads were added and shaken vigorously for 5mins to defribrinate. The RBC was decanted into a centrifuge tube and washed with sterile PBS 3 times in a centrifuge at 3000rpm for 5mins. The supernatant was poured off until the

supernatant become cleared and 10% RBC (1ml of the washed RBC was added to 9ml of PBS) was obtained. A drop of the 10% RBC was placed on a sterile white tile using a pipette, the egg was opened at the air sac and a loopful of the allantoic fluid was picked using a sterile wire loop and mixed with the RBC on the tile. The same was repeated for all the eggs and the tile rocked side ways and observed for agglutination.

STATISTICAL ANALYSIS

Data were analyzed using the chi-square test at a confidence interval of 95% and a probability value (P value < 0.05).

RESEARCH HYPOTHESIS

Null hypothesis (H_o) Ethanolic extract of *Mangifera indica* L is not toxic to embroyonated eggs. Alternatives hypothesis (H_A) – Ethanolic extract of *Mangifera indica* L. is toxic to embryonated eggs.

Null hypothesis (H_o): Ethanolic extract of *Mangifera indica* L. has antiviral activity on Newcastle disease virus.
Alternative hypothesis (H_A) – Ethanolic extract of *Mangifera indica* L. does not have antiviral activity on Newcastle disease virus.

RESULTS

TOXICOLOGICAL ASSAY OF CRUDE ETHANOLIC EXTRACTS OF *Mangifera indica* ON THE EMBRYONATED EGGS.

Five embryonated were inoculated with 0.2ml of 250mg/ml of the extract and was observed for 96hours. 5 embryonated eggs were also used as control. None of the eggs died during the period of observation for both the test and the control (Table 1).

TABLE 1:TOXICOLOGICAL ASSAY OF CRUDE ETHANOLIC EXTRACTS OF *Mangifera indica* ON THE EMBRYONATED EGGS.

Sample Concentration	24hours	48hours	72hours	96 hours
B1		0/5	0/5	0/5
CONTROL		0/5	0/5	0/5
TOTAL		0/5	0/5	0/5

REPEAT OF TOXICOLOGICAL ASSAY OF CRUDE ETHANOLIC EXTRACTS OF *Mangifera indica* ON THE EMBRYONATED EGGS.

The procedure was repeated and after 24hours one out of the five eggs died, 2 died after 48hours, and none die after 72 and 96 hours for the test. For the control, none died after 24hours, one died after 48hours and none died after 72 and 96 hours(Table2).

TABLE 2: REPEAT OF TOXICOLOGICAL ASSAY OF CRUDE ETHANOLIC EXTRACTS OF *Mangifera indica* ON THE EMBRYONATED EGGS.

NO. OF HOURS	B1		Total
B1	1/5	0/5	1/5
CONTROL	2/4	1/5	3/5
72hours	0/2	0/4	0/5
96hours	0/2	0/4	0/5
TOTAL	3/5	1/5	4/5

AN ANTIVIRAL ASSAY ON EMBROYNATED EGGS

An antiviral assay was carried out on four groups of the embroynated eggs using concentrations of 250mg/ml, 200mg/ml, 100mg/ml and 50mg/ml with another group for the uninoculated control. This was observed for 96 hours. At a concentration of 250mg/ml and 200mg/ml, all the eggs died after 24 hours. At a concentration of 100mg/ml, 2 eggs died after 24 hours and 3 died after 46 hours. At a concentration of 50mg/ml, no death was observed after 96 hours. The heamagglutination inhibition test was negative at concentrations of 250mg/ml and 200mg/ml but, was positive at 100mg/ml and 50mg/ml (Table 3).

TABLE 3: ANTIVIRAL ASSAY ON EMBRYONATED EGGS

Sample Concentration	24hours	48hours	72hours	96hours
250mg/ml	$^{5}/_{5}$	-	-	-
200mg/ml	$^{5}/_{5}$	-	-	-
100mg/ml	$^{2}/_{5}$	$^{3}/_{3}$	-	-
50mg/ml	$^{0}/_{5}$	$^{5}/_{5}$	-	-
Uninoculated control	$^{1}/_{5}$	$^{0}/_{4}$	$^{0}/_{4}$	$^{0}/_{4}$

SPOT TEST – (HA)

NO. OF EGGS	POSITIVE	NEGATIVE
5	-	5
5	-	5
5	5	-
5	5	-

Positive (Spot test) = Virus recovered; Negative (spot test)= No virus recovered

DISCUSSION

From table 1, the result shows that the ethanolic extract has no toxic effect on the embroynated eggs (P<o.05). This may be as a result of lack of toxicity of extract. From table 2, after 24 hours, one of the eggs died and after 48 hours, 2 out of the remaining four eggs died (P<o.05). This can also be compared to the result obtained by [16] that the toxicity of *Magnifera indica* has mild toxicity on embryonated eggs. From table 3, it can deduce that the antiviral property is dependent on concentration (P<o.05). At high concentration, 250mg/ml and 200mg/ml, it shows antiviral property but at lower concentrations, 100mg/ml and 50mg/ml. it shows no antiviral property. The result can compared with the studies done by [8] that the antiviral property of ethanolic extract is dependent on concentration. This may be due to the extract or other immunity the eggs have against Newcastle disease virus.

CONCLUSION

The crude ethanolic extract of *Magnifera indica* leaves has mild toxicity on embryonated eggs and it has antiviral property at higher concentration but shows no antiviral property at lower concentration. Further research work should be carried out to improve on the ethanolic extract of *Mangifera indica* as this can serve as a better means of controlling the disease. The federal government should provide fund to encourage more research on this work.

REFERENCES

1. Munir, M., Abbas, M., Khan, M, T., Zohani, S., Berg, M. (2012): Genomic and Biological Characterization of a velogenic Newcastle Disease Virus Isolated from a Healthy Backyard Poultry flock in 2010. *Virology Journal.* 9: 46, 1180-1186.
2. Jindal,N.,Chander,Y., Chockalingam, A, K., Abin, M., Readig, P, T., Goyal, S, M. (2009): Phylogenetic Analysis of Newcastle Disease Virus isolated from water fowl in the upper Midwest Region of the United state: *Virology Journal*, 6: (9) do; 1156/1743-422x-6-191.
3. Aldous,E.W.,Alexander,D.J.,(2008);Newcastle Disease in Pheasants: A Review. *Vet J.*; 175:181- 185.
4. Alexandra, D, J., Jose, A., Norma, C. (2011): Detecting Newcastle Disease Virus in Combination of RT-PCR with red blood cell absorption. *Virology Journal.* 8: 202 do; 10.1186/1743-422x-8-202
5. Andreas, S., Wieland, U., Reinhold, C. (2000): Characterization of Polyphenols in mango pure concentrate by HPLC with Diode Array and mass spectrometer detection. *Int. J. Food Sci. Nutr.* 1; 161-166.
6. Barreto, J, C., Trevisan, M, T, S., Hull, W, E., Erben, G., De Brito, E, S., Pfundstein, B., Wurtele, G., Spiegelhalder, B., Owen, R, W. (2008): "Characterization and Quantization of Polyphenolic Compounds in Bark, Kernel, Leaves and Peels of Mango (*Mangifera indica*)".*Journal of Agriculture and Food Chemistry*. 54(14): 5599-5610.

7. Berardine, N., Fezer, R., Conrad, J., Beifuss, U., Carle, R., Schieber, A. (2005): "Screening of Mango (*Mangifera indica*) Cultivars for their content of flavonol O- and XanthoneC-glycosides,Anthocyanins and Pectin". *J. Agric Food Chem*. 53(5): 1563 1570.
8. Chakrabarti, S., King, D.J., Afonso. C., Swagne, D., Cardona, C.J., Kuney, D.R., Gerry, A, D. (2007): Detection and isolation of exotic Newcastle disease Virus from field-collected flies. *J. Med entomol*. 44: 840- 844.
9. Chen, J.P., Tai, C.Y., Chen, B.H. (2004):ImprovedLiquidChromatographic Method for Determination of Carotenoids in Taiwanese Mango (*Mandifera indica* L.). *J Chromatogr A*. 10(54); 261-268.
10. Creed, Richard (2010): "Relative obscurity; Variations of Antigodlin grow".*Winston- Salem Journal*. Retrieved 2010-09-06.
11. Daud,N.H.,Aung,C.S.,Hewavitharana, A.K., Wilkinson, A.S., Pierson, J.T., Roberts-Thomson, S.J., Shaw, P.N., Monteith, G.R., Gidley, M.J., Parat, M.O. (2010): "Mango Extracts and the Mango components Mangiferin promoteEndothelial cell migration". *J Agric Food Chem*. 58(8): 5181-5186.
12. Delgodo- Hernandez, R., Rodeiro, I., Garcia, O., Pardo-Andreu, G.L., Garrido, B., Morales, C.I., Reynaldo, G., Nunez- Selles, A.J. (2009): "From the Ethnomedcine to the clinical controlled investigation in Cuba. *Mangifera indica*. Stem bark extract (Vimang), like an instructive Example." *Basic and Clinical Pharmacology and Toxicology Suppl*. 1(144): 10-11.
13. Farkas, T., Antal, M., Sami, L., Germain, P., Kecskemeti, S., Kardos, G., Belak, S., Kiss, I. (2007): Rapid and simultaneous detection of avian influenza and Newcastle disease Viruses by duplex polymerase chain reaction assay. *Zoonoses Public Health*, 54(1): 38-43.
14. Flint, S.J., Enquist, W., Racaniello, V.R., and Skalk, A.M. (2009): "Virological Methods" *Principles of Virology*. Asm Press. (ISBN-1555814433).
15. Fratnik Steyer A., Rojs O.Z., Krapez, U., Slavec, B., Barlic Maganja, D. (2010): A diagnostic method based on MGB probes for rapid detection and simultaneous differentiation between virulent and vaccine strains of avian paramyxovirus type 1. *J. Virol Methods*, 166(1-2): 28-36.
16. Garcia- Rivera, D., Delgado, R., Bougarne, N., Haegeman, G., Berghe, W.V. (2011): "Gallic acid Indanone and Mangiferine xanthone are strong determinants of immumo suppressive anti-tumor effects of *Mangifera indica* bark in MDA- MB 231 breast cancer cells". *Cancer Lett*;305(1):21-31.