

## Comparative analysis of the antiviral activities of three selected indigenous plant extracts against Newcastle disease virus

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### ABSTRACT

A comparative analysis on the antiviral activities of aqueous, ethanolic and methanolic extracts of three Nigerian plants which were used traditionally to treat illnesses were evaluated against an avian virus. The plant extracts of *Diospyros mespiliformis* Hochst, ex A.DC., *Quisqualis indica* A.L., and *Aframomum melegueta* K, Schum. were screened for their activities against Newcastle disease virus (NDV). The assay was performed in ten day old embryonated chicken eggs by chorioallantoic membrane (CAM) and the allantoic sac inoculation. The viral replication in the tests and controls were estimated by haemagglutination assay of harvested allantoic fluid. The percentage inhibition of aqueous extracts concentrations of 400mg/ml, 200mg/ml and 100mg/ml of *Diospyros mespiliformis* (DM) on the virus (NDV) were 91.0%, 86.0% and 85.0%, respectively. *Quisqualis indica* (QI) yielded 50.0%, 50.0% and 43.0% respectively, whereas *Aframomum melegueta* (AM) achieved 86.0%, 50.0% and 50.0% respectively. Similarly, the percentage inhibitions of the ethanolic extracts were DM: 95.0%, 90.5% and 89.0%; QI: 86.0%, 50.0% and 50.0%; AM: 89.0%, 86.0% and 50.0% respectively. The percentage inhibition of methanolic extracts were DM: 100.0%, 92.8% and 90.5%; QI: 94.6%, 90.5% and 42.6%; AM: 95.05%, 87.5% and 50.0% respectively. The extracts of *Diospyros mespiliformis* exhibited a better antiviral result in aqueous, ethanolic and methanolic conditions when compared to *Quisqualis indica* and *Aframomum melegueta*. The phytochemical analysis of crude extract of *Diospyros mespiliformis* and fractions exhibited the presence of Alkaloids, Tannins, Saponins, Triterpenes, Flavonoids and Cyanogenic glycosides. These results obtained in this study suggest that *Diospyros mespiliformis* could be a potential candidate in the management of Newcastle diseases affecting the poultry industry. However, the active components responsible for the antiviral activity need to be evaluated.

Keywords: Antiviral activities, avian virus, Newcastle diseases and plants.

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### INTRODUCTION

Newcastle Disease Virus (NDV) causes a highly contagious and fatal disease affecting all species of birds in poultry industry. The disease can vary from clinically inapparent to highly virulent forms, depending on the virus strain and the host species [1,2]. The continuous spectrum of virulence displayed

by NDV strains enabled the grouping of them into three different pathotypes; Lentogenic, mesogenic and velogenic [3,4]. The disease has a worldwide distribution and remains a constant major threat to commercial poultry production. As with other viral disease, there is no known specific treatment for ND

[5,6,7]. Hence there is need to continue the search for antiviral agents with more satisfactory results.

Traditional medical practices still play an important role in many areas of Nigeria; as a result, large number of plants is used on the continent of Africa for the treatment of various kinds of diseases and ailments [8,9,10]. Many natural plants have been sourced and used as valuable medicinal agent for many years with proven potential of treating infectious disease and with lesser side effects compared to synthetic agents [11,12,13,14]. Within the past decades therapeutic option for viral infections have improved significantly and has witnessed intensive studies on extracts and biologically active compounds isolated from plants species used for natural therapies or herbal medicine [15]. However, simultaneous antiviral treatment is associated with the emergence of resistant viruses, which is one of the various problems associated with synthetic drugs. These problems include high cost, increasing adulteration and side effects, coupled with their inadequacies in disease treatment as found most especially in developing countries of the world [16]. Many screening efforts have been made to find antiviral agents from natural sources. Plants have long been used as remedies and many are now being collected and examined in an attempt to identify possible sources of antiviral [17]. Studies conducted in laboratories around the world revealed that traditional medicinal plants can provide a rich source of antiviral activities. There are reports on the use of ethno- veterinary herbal practices in the management of diseases of chickens caused by infectious viral pathogens [18,19]. The plant extracts of *Diospyros mespiliformis*, *Quisqualis indica*, and *Aframomum melegueta* were screened for their activities against Newcastle disease

virus (NDV), *Diospyros mespiliformis* (African ebony) is a large deciduous tree found mostly in the savannas of Africa. It is confined to tropical and sub-tropical regions notably in Africa [20]. The plant is used as dressing for burns, antibacterial agent and astrigent in diarrhea. Over dose of concentrated decoction can cause abortion [21]. The leaves are eaten by elephant, giraffe, black rhino, baboons etc; a definite asset to any farm. Extracts from leaves and barks of *Diospyros mespiliformis* have long been credited for their medicinal properties that include being antiviral, anti-bacterial, anti-inflammatory among others [22]. Ripe fruits of *Diospyros mespiliformis* are relished by indigenous people. The edible fruits is used fresh in fermented drink or dried and stored for later use [23]. Several ethno-pharmacological applications have been reported for *Diospyros mespiliformis* which include the use of leaf decoction as extraordinary remedy for fever, whooping cough and for wounds [24]. Barks and roots are used for serious infections such as malaria, pneumonia, syphilis, leprosy and dermatomycosis, as an anthelmintic and to facilitate delivery [25]. Different parts of the tree are used against diarrhea, skin infections, headache, toothache and similar pains and as a psycho-pharmacological drug [26]. *Quisqualis indica* also known as the Rangoon creeper is a ligneous vine that can reach from 2.5 meters to 8 meters, it belongs to the family *Combretaceae*. Its leaves are elliptical with an acuminate tip and a rounded base. The plant is mainly used for traditional medicine. Decoction of the root, seed or fruit can be used as antihelminthic to expel parasitic worms or for alleviating diarrhea. The fruits and leaves can be used to combat nephritis and to relieve pain caused by fever [27]. *Aframomum melegueta* is specie in the ginger family,

*Zingiberaceae*. The specie, commonly known as grains of paradise, melegueta pepper, alligator pepper, Guinea grains, or Guinea pepper, is obtained from the ground seed; it gives a pungent, peppery flavour. The alligator pepper is extensively used as a common ingredient of many traditional medicines. The decoction of the leaves is used for small pox and chicken pox. As a purgative, galactagogue (to increase the production of breast milk), antihelminthic and haemostatic agent (purifies the blood) in medicinal applications. Also used as a vermifuge and stimulant. Further it is used against intestinal infections, infestations, to calm indigestion and heartburn. It also posses potent anti-inflammatory activity with favourable gastric tolerability profile [28]. Many natural plants have also been sourced and used as valuable medicinal agent for many years with proven potential of treating infectious disease and with lesser side effects compared to synthetic agents [29].

Studying medicinal plants with ethnobotanical importance and folklore reputation has become the more important need in recent times in order to promote the use of herbal medicines and to determine their potential as source of new drugs [30]. In recent years, use of antimicrobial drugs in treatment of infectious disease has developed multiple drug resistance and with increase in production of news antimicrobials, by pharmaceutical industry,

#### MATERIALS AND METHODS

The Collection of *Diospyros mespiliformis*, *Quisqualis indica* and *Aframomum melegueta* leaves was done from their natural habitats in Dekina, Dekina Local Government Area of Kogi State. Collection for extraction was done during rainy season (May 2010 and July 2010). The indigenous plant was identified by

resistance to these drugs have also increased [31]. Hence, scientists are shifting their attention to folk medicine in order to find new leads for better drugs against microbial infections. Plant materials are known as source of new antimicrobial agents as a result search has been to discover new antiviral drugs of plant origin. A number of compounds like quinine, Garlic acid, morphine, codeine, vitexin and lupeol, etc, have been derived from plants which are having enormous therapeutic potentials [29]. There are fewer substances available for the treatment of viral infection when compared with the large amount of available antibiotics for the treatment of bacteria infections [17]. With the enormous amount of antimicrobial drugs present at the moment, the lack of success in developing antiviral drugs is due to the nature of the infectious viral agents, which totally depend upon the cell they infect for their multiplication and survival. Since many of the disinfectants and antiseptics fail to kill all pathogenic viruses, the demand for new antiviral agents that are affordable, available safe and dependable is great and needs all possible approaches towards the development of new antiviral drugs [22]. The main objective of this study was to investigate and compare the antiviral effects of the crude extracts of the plants against Newcastle Disease Virus using 9 - 11 day - old embryonated chicken eggs and live chickens.

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#### Preparation and Extraction

The plant materials were rinsed with clean water; air dried at room temperature in locally designed and constructed wire trays for two weeks. The dried leaves were then blended to

fine powdered forms using a household electric blender. Phytochemical analysis were carried out on some Quantity of each of the ground crude samples while the remaining dried leaves homogenized powder were stored in sealed air-tight plastic containers at room temperature, pending commencement of extraction.

Distilled water was used for aqueous extraction, while Soxhlet extraction method was used to obtain the ethanol and methanol extracts of the plants.

**Preparation of Phosphate Buffered Saline**

**(PBS):** The following salts were carefully weighed out: Sodium chloride (NaCl) 8g, Potassium chloride (KCl) 0.20g, Potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) 0.20g and Disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) 0.92g. These were added to a 2500ml capacity bottle, and 1000ml (1 liter) of distilled water was added to it. The solution was allowed to dissolve and was autoclaved for 15mins at 121°C and 15lbs per square inch (Pis) pressure. The PH was taken as 7.2(Grimes, 2002).The lid of the bottle was tightened, labelled and stored in the refrigerator at 4°C until ready for use.

**Preparation of Antibiotic Solution (PSGA):**

The following reagents were carefully weighed out: Benzyl penicillin 6g, Streptomycin 500mg, Gentamicin 250mg, and Amphotericin B 4mg. These were dissolved in 200ml capacity bottle and 1000ml (1liter) of sterile phosphate buffered saline (PBS) was added to it and allowed to dissolve. The solution was sterilized by passing through a 0.2micron filter. The solution was dispensed into 100ml sterile glass bottles, lid and labeled.

**Preparation of washed chicken red blood cells (RBCs) for haemagglutination Assay (HA):**

The washed RBCs from a 10weeks - old ND unvaccinated chicken were used to carry out haemagglutination test to quantify the virus in

allantoic fluid and ascertain the level of inhibition of virus replication by the different plant extracts used for the study.

**Concentration and Reconstitution of Extracts:**

Phosphate buffered saline (PBS) was used to reconstitute the aqueous, methanol and ethanol extracts. Three (3) different concentrations in dilutions were made namely: 400mg/ml, 200mg/ml and 100mg/ml. The reconstituted extracts were sterilized by filtration using Millipore micro-filter (0.45um pore size).

**Phytochemical Analysis of Plant Materials:**

The ground samples were examined for the presence of chemical constituents such as tannins, alkaloids, flavonoids, terpenoids, cyanogenic glycosides, saponins, following the methods of Mikail (2010) [14].

**Virus:** Newcastle disease virus (NDV) thermo stable strain, its  $\text{EID}_{50}$  was  $10^{8.6}$  /ml, was prepared from infected tissues - were supplied by the Avian Viruses Research Disease Laboratory Section, Viral Research Division, National Veterinary Research Institute Vom, Plateau State, Nigeria. The virus was transported in an ice pack in its lyophilized form and stored at -20°C deep freezer.

The procedure applied in the egg inoculation was as described by [20]. Using aseptic technique, the harvested allantoic fluids were pooled, centrifuged at 5000rpm for 5mins and aliquots of the supernatant prepared and there after the Haemagglutination (HA) titre of the viral suspension was established using the standard method described by Office International des Epizooties (OIE), 1996 [20]. The concentration of Newcastle disease virus in a suspension was expressed as an infectivity titre. The infectivity titre was established by carrying out a titration. The end point of the titration was used to calculate the infectivity titre of the original suspension of virus. The Reed and Muench (1938) [23] mathematical technique was used to

calculate this end point from the result of the HA test on each of the inoculated eggs.

(% infected at dilution immediately above 50%)  
- (% infected at dilution immediately below 50%),

The virus was quantified by HA using the harvested allantoic fluid from the inoculated fertilized eggs. The aim was to check if the virus actually grew in the eggs and if so, determine the percentage inhibition by the extract using the HA titres of positive control and test. The HA titres of the tests (virus inoculated with extracts) were compared with those of positive control (virus alone) and the difference used to compute the percentage inhibition of virus replication by the extracts.

Newcastle disease virus (wild type), obtained from the National Veterinary Research Institute, Vom, was transported in an ice pack in its lyophilized form. The NDV strain supplied was further expanded in ten-day old embryonic chicken eggs and diluted with phosphate buffered saline (PBS) to obtain a titre of 1024 Haemagglutination unit HA (10log<sub>2</sub>). The eggs used for cultivation must be sterile and the shell should be intact and healthy. Candling is the process of holding a strong light above or below the egg to observe the embryo. The crude extracts of the test sample at 400mg/ml 200mg/ml 100mg/ml were prepared in sterile PBS containing antibiotics (PSGA). Blank controls and positive controls were included in the experiment.

Antiviral testing of the plant extracts were carried out invitro using allantoic sac routes of developing chick embryos. NDV: Inhibition of the haemagglutination by each extract was calculated as follows:

$$\text{HA inhibition \%} = \frac{C - T}{C}$$

Where C = the HA titre (GM) of the virus control, and T - the HA titre (GM) treated with the extracts.

**Partial Chromatographic Separation of Extract:** The crude methanolic extract (8ml) of *Diospyros mespiliformis* was centrifuged at 5000rpm for 30min at 4°C in an Eppendorf centrifuge. It was evaporated to dryness by using stream of cold air (16°C for 36hrs). This was achieved by exposing the extract in small amounts (2ml) in petridishes to cool air emanating from an air conditioner at 16°C. The stream of cold air was complimented with air from a standing fan. About 165mg of the dry extract was dissolved in 3ml of 20mM phosphate buffered saline, PH 7.2. About 2.5ml of the re-dissolved extract was applied to a high purity silica gel (220 - 440 mesh, 35 - 75µm, Sigma - Aldrich) column (1.2 x 45cm) previously equilibrated with dimethyl sulphoxide (DMSO). The extract was eluted with the above solvent at a flow rate of 2ml/hour. A total of 17 (seventeen) fractions (each containing 10ml) were collected. The active fractions were evaporated to dryness by exposing them to a stream of cold air (16°C, 36hours). The dry extracts were weighed to 3 - decimal places and re-suspended in 2ml of phosphate buffered saline (PH 7.2). Fraction 9 which exhibited the highest antiviral activity was selected for further studies. The partially purified extract of *Diospyros mespiliformis* was also examined for the presence of chemical constituents as illustrated in crude extract method.

**Harvesting of Allantoic Fluid:** To harvest the infected tissues, the eggs were first chilled overnight at 4°C to induce contraction of the blood vessels and to kill embryo. The chilled eggs were placed in the egg crates with the blunt ends up. Starting from the point of inoculation, the shell over the airspace was carefully cut open using a small pair of sterile

scissor. The exposed membrane was then held up with a pair of sterile forceps, and then cut open further with the scissors. Sterile 5ml syringes and needles or Pasteur pipette (one for each egg) were used to aspirate the allantoic fluid (to be used for haemagglutination assay) into sterile carefully labelled vials. The

aspirated allantoic fluid was clarified by centrifuging at 5000rpm for 5minutes and then the supernatant taken and used for haemagglutination test.

**Haemagglutination Assay** was used to determine the presence of a haemagglutinating agent in one minute.

## RESULTS

**Table 1: Phytochemical Analysis of *Diospyros mespiliformis*, *Qusquali Indica* and *Aframomum melegueta* Extracts from Various Solvents.**

| Plant sample                   | Extraction | Phytochemical |          |             |            |        |                       |
|--------------------------------|------------|---------------|----------|-------------|------------|--------|-----------------------|
|                                |            | Alkaloids     | Saponins | Triterpenes | Flavonoids | Tanins | Cyanogenic glucosides |
| <i>Diospyros mespiliformis</i> | Aqueous    | -             | +        | -           | +          | +      | -                     |
|                                | Ethanol    | ++            | ++       | +           | +          | ++     | +                     |
|                                | Methanol   | ++            | ++       | +++         | ++         | ++     | +                     |
| <i>Qusquali Indica</i>         | Aqueous    | -             | +        | -           | -          | +      | -                     |
|                                | Ethanol    | +             | +        | -           | +          | ++     | -                     |
|                                | Methanol   | +             | +        | -           | +          | ++     | -                     |
| <i>Aframomum melegueta</i>     | Aqueous    | -             | +        | -           | -          | +      | -                     |
|                                | Ethanol    | +             | ++       | -           | ++         | ++     | -                     |
|                                | Methanol   | +             | ++       | -           | ++         | ++     | -                     |

Key: +++ = high concentration; ++ medium concentration; + = low concentration; -- = not detected.

**Table 2: Result of some phytochemical compounds in *Diospyros mespiliformis***

| Phytochemical        | Crude extract | Partial chromatographic separation of extract |
|----------------------|---------------|---|
| Alkaloid             | ++            | +   |
| Saponin              | ++            | +   |
| Triterpenes          | +++           | ++  |
| Flavonoids           | ++            | +   |
| Tannins              | ++            | +   |
| Cyanogenic glucoside | +             | -   |

Key: +++ = high concentration present; ++ = moderate concentration present; + = mild concentration present; - = absence

Table 3: Toxicity of Plant extracts under study in embryonated chicken eggs, estimated as percentage egg mortality

| Plant extract                  | Extraction          | Extract concentration mg/ml | Egg mortality |      |       | Percentage Egg mortality (%) |
|--------------------------------|---------------------|-----------------------------|---------------|------|-------|------------------------------|
|                                |                     |                             | Inoculated    | Dead | Alive |                              |
| <i>Diospyros mespiliformis</i> | Aqueous Extraction  | 500                         | 5             | 2    | 3     | 40.0                         |
|                                |                     | 400                         | 5             | 0    | 5     | 0.0                          |
|                                |                     | 200                         | 5             | 0    | 5     | 0.0                          |
|                                |                     | 100                         | 5             | 0    | 5     | 0.0                          |
|                                | Ethanol Extraction  | 500                         | 5             | 3    | 2     | 60.0                         |
|                                |                     | 400                         | 5             | 0    | 5     | 0.0                          |
|                                |                     | 200                         | 5             | 0    | 5     | 0.0                          |
|                                |                     | 100                         | 5             | 0    | 5     | 0.0                          |
|                                | Methanol Extraction | 500                         | 5             | 3    | 2     | 60.0                         |
|                                |                     | 400                         | 5             | 0    | 5     | 0.0                          |
|                                |                     | 200                         | 5             | 0    | 5     | 0.0                          |
|                                |                     | 100                         | 5             | 0    | 5     | 0.0                          |
| <i>Qusquali Indica</i>         | Aqueous Extraction  | 500                         | 5             | 1    | 4     | 20.0                         |
|                                |                     | 400                         | 5             | 0    | 5     | 0.0                          |
|                                |                     | 200                         | 5             | 0    | 5     | 0.0                          |
|                                |                     | 100                         | 5             | 0    | 5     | 0.0                          |
|                                | Ethanol Extraction  | 500                         | 5             | 2    | 3     | 40.0                         |
|                                |                     | 400                         | 5             | 0    | 5     | 0.0                          |
|                                |                     | 200                         | 5             | 0    | 5     | 0.0                          |
|                                |                     | 100                         | 5             | 0    | 5     | 0.0                          |
|                                | Methanol Extraction | 500                         | 5             | 2    | 3     | 40.0                         |
|                                |                     | 400                         | 5             | 0    | 5     | 0.0                          |
|                                |                     | 200                         | 5             | 0    | 5     | 0.0                          |
|                                |                     | 100                         | 5             | 0    | 5     | 0.0                          |
| <i>Aframomum melegueta</i>     | Aqueous Extraction  | 500                         | 5             | 1    | 4     | 20.0                         |
|                                |                     | 400                         | 5             | 0    | 5     | 0.0                          |
|                                |                     | 200                         | 5             | 0    | 5     | 0.0                          |
|                                |                     | 100                         | 5             | 0    | 5     | 0.0                          |
|                                | Ethanol Extraction  | 500                         | 5             | 2    | 3     | 40.0                         |
|                                |                     | 400                         | 5             | 0    | 5     | 0.0                          |
|                                |                     | 200                         | 5             | 0    | 5     | 0.0                          |
|                                |                     | 100                         | 5             | 0    | 5     | 0.0                          |
|                                | Methanol Extraction | 500                         | 5             | 3    | 2     | 60.0                         |
|                                |                     | 400                         | 5             | 0    | 5     | 0.0                          |
|                                |                     | 200                         | 5             | 0    | 5     | 0.0                          |
|                                |                     | 100                         | 5             | 0    | 5     | 0.0                          |

Table 4. Reduction of Newcastle Disease Viral Load by Extracts of *Diospyros mespiliformis* (DM) leaves in Embryonated chicken eggs.

| Extraction              | Test | Extract concentration | Inoculum             | No of Eggs inoculated | No of Eggs survived | Percentage egg mortality | Geometric mean virus titre | Percentage inhibition (%) |
|-------------------------|------|-----------------------|----------------------|-----------------------|---------------------|--------------------------|----------------------------|---------------------------|
| <b>Aqueous Extract</b>  |      | 400mg/ml              | 0.2ml(Virus+Extract) | 5                     | 4                   | 20%                      | 36.0                       | 91.0                      |
|                         |      | 200mg/ml              | 0.2ml(Virus+Extract) | 5                     | 3                   | 40%                      | 55.7                       | 86.0                      |
|                         |      | 100mg/ml              | 0.2ml(Virus+Extract) | 5                     | 3                   | 40%                      | 56.7                       | 85.0                      |
|                         |      | Positive control      | 0.2ml(Virus+PBS)     | 5                     | 0                   | 100%                     | 388.0                      | 0.0                       |
|                         |      | Negative control      | 0.2ml PBS only       | 5                     | 5                   | 0.0%                     | 1.0                        | ==                        |
| <b>Ethanol Extract</b>  |      | 400mg/ml              | 0.2ml(Virus+Extract) | 5                     | 5                   | 0%                       | 18.4                       | 95.0                      |
|                         |      | 200mg/ml              | 0.2ml(Virus+Extract) | 5                     | 5                   | 0%                       | 36.8                       | 90.5                      |
|                         |      | 100mg/ml              | 0.2ml(Virus+Extract) | 5                     | 4                   | 20%                      | 42.0                       | 89.0                      |
|                         |      | Positive control      | 0.2ml(Virus+PBS)     | 5                     | 0                   | 100%                     | 388.0                      | 0.0                       |
|                         |      | Negative control      | 0.2ml PBS only       | 5                     | 5                   | 0%                       | 1.0                        | ==                        |
| <b>Methanol Extract</b> |      | 400mg/ml              | 0.2ml(Virus+Extract) | 5                     | 5                   | 0%                       | ==                         | 100.0                     |
|                         |      | 200mg/ml              | 0.2ml(Virus+Extract) | 5                     | 5                   | 0%                       | 27.8                       | 92.8                      |
|                         |      | 100mg/ml              | 0.2ml(Virus+Extract) | 5                     | 5                   | 0%                       | 36.8                       | 90.5                      |
|                         |      | Positive control      | 0.2ml(Virus+PBS)     | 5                     | 0                   | 100%                     | 388.0                      | 0.0                       |
|                         |      | Negative control      | 0.2ml PBS only       | 5                     | 5                   | 0.0%                     | 1.0                        | 1.0                       |

**Table 5. Reduction of Newcastle Disease Viral Load by Extracts of *Qusquali Indica* leaves in Embryonated chicken eggs.**

| Extraction              | Test concentration | Extract | Inoculum             | No of Eggs inoculated | No of Eggs survived | Percentage egg mortality | Geometric mean virus titre | Percentage (%) inhibition |
|-------------------------|--------------------|---------|----------------------|-----------------------|---------------------|--------------------------|----------------------------|---------------------------|
| <b>Aqueous Extract</b>  | 400mg/ml           |         | 0.2ml(Virus+Extract) | 5                     | 0                   | 100%                     | 194.0                      | 50.0                      |
|                         | 200mg/ml           |         | 0.2ml(Virus+Extract) | 5                     | 0                   | 100%                     | 194.0                      | 50.0                      |
|                         | 100mg/ml           |         | 0.2ml(Virus+Extract) | 5                     | 0                   | 100%                     | 222.0                      | 43.0                      |
|                         | Positive control   |         | 0.2ml(Virus+PBS)     | 5                     | 0                   | 100%                     | 388.0                      | 0.0                       |
|                         | Negative control   |         | 0.2ml PBS only       | 5                     | 5                   | 0.0%                     | 1.0                        | ==                        |
| <b>Ethanol Extract</b>  | 400mg/ml           |         | 0.2ml(Virus+Extract) | 5                     | 2                   | 60%                      | 55.7                       | 86.0                      |
|                         | 200mg/ml           |         | 0.2ml(Virus+Extract) | 5                     | 1                   | 80%                      | 194.0                      | 50.0                      |
|                         | 100mg/ml           |         | 0.2ml(Virus+Extract) | 5                     | 1                   | 80%                      | 194.0                      | 50.0                      |
|                         | Positive control   |         | 0.2ml(Virus+PBS)     | 5                     | 0                   | 100%                     | 388.0                      | 0.0                       |
|                         | Negative control   |         | 0.2ml PBS only       | 5                     | 5                   | 0%                       | 1.0                        | ==                        |
| <b>Methanol Extract</b> | 400mg/ml           |         | 0.2ml(Virus+Extract) | 5                     | 5                   | 0%                       | 21.1                       | 94.6                      |
|                         | 200mg/ml           |         | 0.2ml(Virus+Extract) | 5                     | 1                   | 20%                      | 36.8                       | 90.5                      |
|                         | 100mg/ml           |         | 0.2ml(Virus+Extract) | 5                     | 4                   | 80%                      | 222.9                      | 42.6                      |
|                         | Positive control   |         | 0.2ml(Virus+PBS)     | 5                     | 0                   | 100%                     | 388.0                      | 0.0                       |
|                         | Negative control   |         | 0.2ml PBS only       | 5                     | 5                   | 0.0%                     | 1.0                        | ==                        |

**Table 6. Reduction of Newcastle Disease Viral Load by Extracts of *Aframomum melegueta* (AM) leaves in Embryonated chicken eggs.**

| Extraction              | Test concentration | Extract | Inoculum             | No of Eggs inoculated | No of Eggs survived | Percentage egg mortality | Geometric mean virus titre | Percentage inhibition (%) |
|-------------------------|--------------------|---------|----------------------|-----------------------|---------------------|--------------------------|----------------------------|---------------------------|
| <b>Aqueous Extract</b>  | 400mg/ml           |         | 0.2ml(Virus+Extract) | 5                     | 3                   | 40%                      | 55.7                       | 86.0                      |
|                         | 200mg/ml           |         | 0.2ml(Virus+Extract) | 5                     | 4                   | 20%                      | 194.0                      | 50.0                      |
|                         | 100mg/ml           |         | 0.2ml(Virus+Extract) | 5                     | 0                   | 100%                     | 194.0                      | 50.0                      |
|                         | Positive control   |         | 0.2ml(Virus+PBS)     | 5                     | 0                   | 100%                     | 388.0                      | 0.0                       |
|                         | Negative control   |         | 0.2ml PBS only       | 5                     | 5                   | 0.0%                     | 1.0                        | ==                        |
| <b>Ethanol Extract</b>  | 400mg/ml           |         | 0.2ml(Virus+Extract) | 5                     | 4                   | 20%                      | 42.0                       | 89.0                      |
|                         | 200mg/ml           |         | 0.2ml(Virus+Extract) | 5                     | 3                   | 40%                      | 55.7                       | 86.0                      |
|                         | 100mg/ml           |         | 0.2ml(Virus+Extract) | 5                     | 1                   | 80%                      | 194.0                      | 50.0                      |
|                         | Positive control   |         | 0.2ml(Virus+PBS)     | 5                     | 0                   | 100%                     | 388.0                      | 0.0                       |
|                         | Negative control   |         | 0.2ml PBS only       | 5                     | 5                   | 0%                       | 1.0                        | ==                        |
| <b>Methanol Extract</b> | 400mg/ml           |         | 0.2ml(Virus+Extract) | 5                     | 5                   | 0%                       | 18.4                       | 95.05                     |
|                         | 200mg/ml           |         | 0.2ml(Virus+Extract) | 5                     | 1                   | 20%                      | 48.5                       | 87.5                      |
|                         | 100mg/ml           |         | 0.2ml(Virus+Extract) | 5                     | 3                   | 60%                      | 194.0                      | 50.0                      |
|                         | Positive control   |         | 0.2ml(Virus+PBS)     | 5                     | 0                   | 100%                     | 388.0                      | 0.0                       |
|                         | Negative control   |         | 0.2ml PBS only       | 5                     | 5                   | 0.0%                     | 1.0                        | ==                        |

**Table 7. Virucidal Effect of 4mg/ml concentration of Various Fractions of Methanolic Extracts of *Diospyros mespiliformis* on Newcastle Disease virus**

| Fraction no                  | No of Eggs inoculation | No of Eggs survived | Percentage Egg mortality | Geometric mean virus | Percentage inhibition | Virucidal effect |
|------------------------------|------------------------|---------------------|--------------------------|----------------------|-----------------------|------------------|
| 7                            | 5                      | 0                   | 100%                     | 230                  | 33.3%                 | 1.5 times        |
| 8                            | 5                      | 3                   | 40%                      | 46.6                 | 86.4%                 | 7.4 times        |
| 9                            | 5                      | 5                   | 0%                       | 37.1                 | 89.2%                 | 9.3 times        |
| 10                           | 5                      | 3                   | 40%                      | 50.7                 | 85.3%                 | 6.8 times        |
| 11                           | 5                      | 2                   | 60%                      | 86.3                 | 75.0%                 | 4.0 times        |
| 12                           | 5                      | 0                   | 100%                     | 265.5                | 23.1%                 | 1.3 times        |
| Positive control(virus+DMSO) | 5                      | 0                   | 100%                     | 345                  | 0%                    |                  |
| Negative control (DMSO only) | 5                      | 5                   | 0%                       | 1.0                  | ==                    |                  |

## DISCUSSION

The comparative analysis on the antiviral activities of plant extracts studied with their various degree of inhibitory properties *in vitro* have been reported and some of the tested Nigeria plant extracts have some inhibitory activity against Newcastle disease virus, which reveals a higher antiviral activity for *Diospyros mespiliformis* leave extract than *Quisquali indica* and *Aframomum melegueta* leave. A hundred percent (100%) inactivation has been used to define an extract with antiviral activity [28]. Preparations which exert antiviral effects *in vivo* may not be detected with *in vitro* assays because of the extremely low concentrations of extracts tolerated by cells in the artificial system. Even with this limitation, the plant extracts studied exhibited various degrees of antiviral activity against the virus at various concentrations.

In this study, extracts of *Diospyros mespiliformis* exhibited the best antiviral results in both aqueous, ethanolic and methanolic conditions. At 400mg/ml concentration the aqueous, ethanolic and methanolic extract (NDV) it achieved antiviral inhibitions of 91%, 95%, and 100% respectively against Newcastle disease virus. Studies have shown that *D. mespiliformis* has antimicrobial activities, especially antibacterial, antifungal and antiviral [16]. *Quisquali indica* leaves are reputed for use as an antihelminths to expel parasitic worms and for alleviating diarrhea, combating nephritis and to relieve pains caused by fever [12]. At the concentration of 400mg/ml, using aqueous, ethanolic and methanolic extract, the plant achieved viral inhibitions of 50%, 86% and 94.6% respectively. Higher virus inhibition was achieved by *Aframomum melegueta* extracts with 86%, 89% and 95.05% respectively aqueous,

ethanolic and methanolic extracts at the concentration of 400mg/ml. *Aframomum melegueta* (alligator pepper) is extremely used as a common ingredient of many traditional medicines. It is extensively used as a purgative, antihelminthic, haemostatic agent and has a potent anti-inflammatory activity with favourable gastric tolerability profile [5 and 6].

The Phytochemical analysis of the plant materials unravelled a number of chemical substances with antiviral activity. Flavones, for instance which are known to be synthesized by plants in response to microbial infection, inhibit the initiation, promotion and progression of tumours [10,11 and 13]. Flavonones exhibit inhibitory effects against viruses [23] including HIV and respiratory syncytial virus [3]. Terpenoids were reported to be active against bacteria, fungi, viruses and protozoa [16,17,18]. It is believed to be active against viruses by envelope disruption by the lipophilic compounds. Alkaloids have been commonly found to have antimicrobial properties [19,20,21]. It is also useful against HIV infection as well as intestinal infections associated with AIDS [24,27,29]. Tannins are found in almost every plant part; wood, leaves, bark, roots and fruits [14] and tannin containing beverages can cure or prevent a variety of viral infections [12]. At least two studies [15,16] have shown tannins to be inhibitory to viral reverse transcriptases. Tannins (hydrolysable) also show anti-carcinogenic and anti-mutagenic effects [30]. These phytochemical were significantly demonstrated in methanolic extracts of *Diospyros mespiliformis* and *Aframomum melegueta*.

The ethanolic extracts and methanolic extracts appeared to have had more antiviral phytochemical than the aqueous extracts which accounted for better reduction in the Newcastle

disease viral load for the most part of the experiment. Thus the efficacy of plant extract evaluated as antimicrobial agents was dependent on the solvent of extraction. This finding is in agreement with the finding of [8] who examined a variety of extracts for their ability to solubilize antimicrobials from plants and ranked them in the order; methanol, ethanol, and water and posited also that most active inhibitors extracted are not water soluble. It is also in agreement with results of [10] who found that ethanolic extracts of some Nigerian spices were more potent than the aqueous extracts against common food borne microorganisms including; *Staphylococcus aureus*, *Klesbsiella pneumonia*, *Proteus vulgaris* and *Streptococcus faecalis*.. In this study, a good number of the ethanolic and aqueous extracts of the plants employed portrayed some level of antiviral activity. Nevertheless, less than 100% activity was obtained in all cases, and might be taken to be that the antiviral compounds present were in amounts insufficient to inactivate all infectious virus particles. The extracts and the fractions delayed embryo mortality. The low embryo mortality exhibited from some extracts concentrations could be explained by the corresponding arrested virus multiplication which was reflected by the low virus titres harvested from the eggs by the end of day- 5 PI. The results of embryo mortality also clearly showed that both the crude

*Diospyros mespiliformis* extract and some phytochemical fractions and controls were not lethal to the chicken embryonated eggs as all the embryos were alive by day-5 when the experiment was terminated. The results also showed that within the experimental group, methanolic crude extract of *D. mespiliformis* and phytochemical fractions delayed embryo mortality.

The various traditional uses of the plants tested in this study; especially *D.mespiliformis* correlate well with the present findings. The results gave evidence that a good number of the aqueous ethanolic and methanolic extracts of the plants employed in this study portrayed some level of antiviral activity. Preliminary results of this investigation appear to indicate that leaves of *D.mespiliformis* have high potential antiviral activity. Further investigations are necessary in order to draw solid conclusion. The bioactive compounds from the leaves need to be isolated and screened for their pharmaceutical and biotechnological applications in order to cure chronic and infectious diseases. The development of more potent antiviral agents for human race may be enhanced, perhaps, by harvesting these plant constituents and harnessing their potencies.

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