©IDOSR PUBLICATIONS
International Digital Organization for Scientific Research ISSN: 2550-7931
IDOSR JOURNAL OF APPLIED SCIENCES 2(3) 68-82, 2017.

Comparative Antiviral Activities of *Diospyros Mespiliformis* and *Quisqualis indica* Leaf Extracts on Newcastle Disease.

Onwuatuegwu Joseph Taiwo Chukwuma

Department of Microbiology, Tansian University, Umunya, Anambra State, Nigeria.
Phone number: 08037463879. Email: joeonwuatuegwu@gmail.com

ABSTRACT

A comparative analysis was done on the antiviral activities of aqueous, ethanolic and methanolic extracts of two Nigerian plants which were used traditionally to treat illnesses. The plant extracts of *Diospyros mespiliformis* Hochst, ex A.DC., and *Quisqualis indica* A.L., 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 haemagglitination 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 were 91.0%, 86.0% and 85.0%, respectively, whereas, *Quisquali indica* (QI) yielded 50.0%, 50.0% and 43.0% respectively. Similarly, the percentage inhibition of the ethanolic extracts were DM: 95.0%, 90.5% and 89.0%; QI: 86.0%, 50.0% 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% respectively. The extracts of *Diospyros mespiliformis* exhibited a better antiviral result in aqueous, ethanolic and methanolic conditions when compared to *Quisqualis indica*. 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 Disease Virus affecting the poultry industry. However, the active components responsible for the antiviral activity need to be evaluated.

Keywords: Comparative, anti-viral, Newcastle disease, *Diospyros Mespiliformis* and *Quisqualis indica.*

INTRODUCTION

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 [1, 2, 3, 4]. The plant is used as dressing for burns, antibacterial agent and astrigent in diarrhea. Over dose of concentrated decoction can cause abortion [4]. 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 [5].

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. 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 [6]. Barks and roots are used for serious infections such as malaria, pneumonia, syphilis, leprosy and dermatomycosis, as an anthelmintic and to facilitate delivery [7]. Different parts of the tree are used against diarrhea, skin infections, headache, toothache and similar pains and as a psycho-pharmacological drug [8].

Quisqualis indica A. L., 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 [9].

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 in apparent to highly virulent forms, depending on the virus strain and the host species. The continuous spectrum of virulence displayed by NDV strains enabled the grouping of them into three different pathotypes; Lentogenic. mesogenic and velogenic [10]. 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 [11]. Hence there is need to continue the search for antiviral agents with more satisfactory results.

Medical practices (Traditional and Veterinary) 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 [12]. 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 [13, 14, 15]. Within the past decade therapeutic options 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 [16, 17, 18, 19]. 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 [20, 21, 22]. 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 [23, 24, 25]. 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 [26, 27, 28].

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 [29]. 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 pharmacentical industry, resistance to these drugs have also increased [30]. 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 [30]. 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 [31]. 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. 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.

Materials and methods

The Collection of *Diospyros mespiliforms* and *Quisqualis indica* 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 Prof. C.U. Okeke of the Department of Botany, Nnamdi Azikiwe University, Awka.

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 [32] 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 (KH_2 Po_4) 0.20g and Disodium hydrogen phosphate (Na_2 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 121^0c and 151bs per square inch (Pis) pressure. The PH was taken as 7.2 [33].The lid of the bottle was tightened, labelled and stored in the refrigerator at 4^0C 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 labelled.

Preparation of washed chicken red blood cells (RBCs) for haemagglitination Assay (HA): The washed RBCs from a 10weeks – old ND unvaccinated chicken were used to carry out haemagglitination 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 descriptions of Krishnaiah et al. (2009) and Mikail (2010) [16, 17].

Virus: Newcastle disease virus (NDV) thermo stable strain, its 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^{0}C deep freezer.

The procedures applied in the egg inoculation were as described by [34]. Using asceptic technique, the harvested allantoic fluids were pooled, centrifuged at 5000rpm for 5mins and aliquots of the supernatant prepared and there after the Haemagglunation (HA) titre of the viral suspension was established using the standard method described by Office International des Epiziooties (OIE), 1996. 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) 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 (10log2). 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 autibiotics (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 haemagglitination 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 4^0C in an Eppendorf centrifuge. It was evaporated to dryness by using stream of cold air (16^0C 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^oc. 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 – 75um, 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 (16^0C, 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 4^0C 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 forcepts, 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 haemagglitination 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 haemagglitination test.
Haemagglutination Assay was used to determine the presence of a haemagglutinating agent in one minute.

RESULTS

Table1. Phytochemical Analysis of *Diospyros mespiliformis* Extracts from Various Solvents

Phytochemical	Aqueous	Ethanol	Methanol
Alkaloids	---	(++)	(++)
Saponins	(+)	(++)	(++)
Triterpenes	---	(+)	(+++)
Flavonoids	(+)	(+)	(++)
Tanins	(+)	(++)	(++)
Cyanogenic glucosides	---	(+)	(+)

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

Table 2. Phytochemical Analysis of *Qusqualis Indica* Extracts from Various Solvents

Phytochemical	Aqueous	Ethanol	Methanol
Alkaloids	---	(+)	(+)
Saponins	(+)	(+)	(+)
Triterpenes	---	---	---
Flavonoids	---	(+)	(+)
Tanins	(+)	(++)	(++)
Cyanogenic glucosides	---	---	---

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

Table 3. 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 4: Toxicity of *Diospyros mespiliformis* extracts under study in embryonated chicken eggs, estimated as percentage egg mortality

Extraction	Extract concentration mg/ml	Egg mortality			Percentage Egg mortality (%)
		Inoculated	Dead	Alive	
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

Table 5: Toxicity of *Qusquali Indica* extracts under study in embryonated chicken eggs, estimated as percentage egg mortality

Extraction	Extract concentration mg/ml	Egg mortality			Percentage Egg mortality (%)
		Inoculated	Dead	Alive	
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

Table 6. Reduction of Newcastle Disease Viral Load by Extracts of *Diospyros mespiliformis (DM)* leaves in Embryonated chicken eggs.

Extraction	Test Extract concentration	Inoculums	No of Eggs inoculated	No of Eggs survived	Percentage egg mortality	Geometric mean virus titre	Percentage (%) inhibition
Aqueous	400mg/ml	0.2ml(Virus+Extract)	5	4	20%	36.0	91.0
Extract	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	==
Ethanol	400mg/ml	0.2ml(Virus+Extract)	5	5	0%	18.4	95.0
Extract	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	==
Methanol	400mg/ml	0.2ml(Virus+Extract)	5	5	0%	==	100.0
Extract	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 7. Reduction of Newcastle Disease Viral Load by Extracts of *Qusquali Indica* leaves in Embryonated chicken eggs

Extraction	Test Extract concentration	Inoculums	No of Eggs inoculated	No of Eggs survived	Percentage egg mortality	Geometric mean virus titre	Percentage (%) inhibition
Aqueous	400mg/ml	0.2ml(Virus+Extract)	5	0	100%	194.0	50.0
Extract	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	==
Ethanol	400mg/ml	0.2ml(Virus+Extract)	5	2	60%	55.7	86.0
Extract	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	==
Methanol	400mg/ml	0.2ml(Virus+Extract)	5	5	0%	21.1	94.6
Extract	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 8. Virucidal Effect of 4mg/ml concentration of Various Fractions of Methonolic 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 results of the antiviral activities of plant extracts studied with their various degree of inhibitory properties invitro have been reported and some of the tested Nigeria plant extracts have some inhibitory activity against Newcastle disease virus. A hundred percent (100%) inactivation has been used to define an extract with antiviral activity [34]. However, a higher antiviral activity was observed in extracts of *Diospyros mespiliformis* leave than *Quisquali indica* leave. Preparations which exert antiviral effects invivo may not be detected with invitro 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 at various concentrations.

In this study, extracts of *Diospyros mespiliformis* (African Ebony) exhibited the best antiviral results in both aqueous, ethanolic and methanolic conditions. At 400mg/ml concentration the aqueous, ethanolic and methnolic 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 [35]. *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 [35]. At the concentration of 400mg/ml, using aqueous, ethanolic and methanolic extract, the plant achieved viral inhibitions of 50%, 86% and 94.6% respectively.

The Phytochemical analysis of the plant materials unravelled a number of chemical substances with antiviral activity. 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 Eloff (2008) [9] who examined a variety of extractants 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 Agatemor (2009) [2] 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.

REFERENCES

1. Adzu, B., Amos, S., Dzarma, 1., Mauzzam, and Gamaniel, K. S. (2002). Pharmacological Evidence favouring the folkloric use of Diospyros mespiliformis Hochst in the relief of pain and fever. *Journal of Ethnopharma.* **82**: 191-195.
2. Agatemor, C. (2009). Antimicrobial activity of aqueous and ethanol extracts of nine Nigeria species against four food borne bacteria. *Eletronic Journal of Environmental, Agricultural and Food Chemistry.*8(**3**):195- 200.
3. Alexander, D. J. (1997). Newcastle disease and other avian paramyxoviridae infection. In: Disease of poultry. B. W. Calnek H. J. Barnes., C. W. Beard., L. R. Mcdougald, and Y. M. Saif, ed. *Iowa State University Press, Ames*, I.A. Pp541 – 569.
4. Bizimana, N. (1994). Traditional Veterinary Practice in Africa. Schrifteib der GTZ.No.243, Eschborn, Germany.
5. Coates Palgrave, M. (2002). Keith Coates Palgrave of Southern African, ed.3.Struik Cape Town.
6. Dalziel, J. M., (1995). The useful plants of West Tropical Africa. *Crown Agents,* London. Pp. 364.
7. Eisikowitch, D.; Rotem, R. (1987). "Flower Orientation and Color Change in *Quisqualis indica* and Their Possible Role in Pollinator Partitioning". *Botanical Gazette. **148** (2): 175.* doi:10.1086/337645. JSTOR 2995472.
8. Ekpa, O. D. (1996). Nutrient composition of three Nigeria medicinal plants.
9. Eloff, J.N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? *Journal of Ethnopharmacology* **60** : 1-8. *Food Chemistry*. 57(2): 229 – 232
10. Esimone, C.O., Ofokansi, K.C., Adikwu, M.U., Ibezim, E.C., Abonyi, D.O., Odaibo, G.N. and Oladeye, D.O (2007). In vitro evaluation of the antiviral activity of extracts from the lichen Parmelia parlata (L.) Ach.against three RNA viruses. *Journal of infections of Developing Countries.***1** (13): 315-320.
11. Gbile, G.O.and Adesina, S.K. (1986). Nigeria flora as pharmaceutical potentials. *Journal of Ethnopharmacology.* **19**: 1 – 16.

12. Gill, L.S. (1992). Ethnomedical uses of plants in Nigeria. Uniben press, University of Benin, Benin – city, Edo State, Nigeria. ISBN 978 – 2027 – 20 – 0 Pp. 102 – 103.
13. ITDG and IIRR, (1996). Ethnoveterinary medicine in Kenya; A field manual of traditional animal healthcare practices. Intermediate Technology
14. Kambewa, B.M.D., Mfilitilodze, M. W., Huttner, K, Wolling, C.B.A.and Phoya, R.K.D., (1997). The use of indigenous veterinary remdies in Malawi. In: The proceedings of an International, conferece held in Pune. India. 4 – 6 November 1997. Http: //www.vetwork. org. uk. pune/India.
15. Kerharo, J. and Adam, J.C.(1974). La pharmacopee senegalaise traditionnelle, vigot, Paris.
16. Krishnaiah, D., Devi, T., Bonno, A. and Sarbartly, R. (2009) Studies on phytochemical constituents of six Malaysian plants. *Journal of medicinal Plant Research*. **3**: 67 – 72.
17. Mikail, H.G. (2010). Phytochemical screening, elemental analysis and acute toxicity of aqueous extract of *Allium sativum* L. *bulbs* in experimental rabbits. *Journal of Medicinal Plant Research* **4**: 322 – 326.
18. Mohamed, I.E., EI Nur, E.E., and Abdelrahman, M.E., (2010). The antibacterial, antiviral activities and phytochemical screening of some Sudanese medicinal plants. *Eur Asia journal of Biosciences* 4, 2, 8, - 16.
19. Mtambo, M.M.A., Mushi, E.J., Kinabo, L.D.B., Maeda-Machangu, A., Mwamegele, G.L.M., Yongolo, M.G.S. and Temu. R.P.C.(1999). Evaluation of the efficiency of the crude extracts of *Capsicum frustescens, citrus limon* and *Opuntia vulgaris* against Newcastle disease in domestic fowl in Tanzania. *Journal of Ethnopharmacology*. **68**:55-61.
20. Nascimento Gislen, G. F., Lukatelli, J. Frentas, P. C., and Silva, G. L. (2000). Antimicrobial activity of plant extracts and phytochemical on antibiotic resistant bacteria, *Brazillian Journal of Microbiology*, **31**: 247 – 256.
21. National Research Council (2008). "Ebony". Lost Crops of Africa: Volume III: Fruits. Lost Crops of Africa. **3**. National Academies Press. ISBN 978-0-309-10596-5. Retrieved 2008-07-25.
22. Nwude, N., and Ibrahim, M. A., (1980). Plants used in traditional veterinary practice in Nigeria. *Journal of Veterinary Pharmacology.* Pp 261 – 273.
23. O.I.E., (1996). The Newcastle disease In: Manual of standards for diagnostic Test and vaccines. 161 – 169.
24. Ojo, O.O., Oluyege, J.O. and Famurewa, O. (2009). Antiviral Properties of two Nigerian Plants. *African Journal of Plant Sciences.* **3** (7): 157-159.

25. Pillay, D., Emery, V. C., and Mutimer, D. (2002). Guidelines for laboratory monitoring of treatment of persistent virus infections. *Journal of Clinical Virology* 25: 73 – 92.
26. Reed, L.J., and Muench, L.H. (1938). A simple method of estimating fifty percent end points. *American Journal of Hygiene* **27:** 493 – 497.
27. Ros, J. L., Recio, M. C., (2005). Medicinal plants and Antimicrobial Activity, *Journal of Ethnopharmacology*, **100**: 80 – 84.
28. Senne, D.A. (1998). Virus propagation in embryonating eggs. In: A laboratory manual for the Isolation and Identification of Avian Pathogens. Forth Edition. David E. Swayne, John, R., Clisson, Mark, W. Jackwood, James, E., Pearson and Willie M. Read. 235 – 240.
29. Sherrif, Z. U., (2001). Modern herbal therapy for common ailments, Nature Pharmacy series Spectrum Books Ltd, Ibadan, Nigeria in Association with Safari Books (Export) Ltd. United Kingdom., **1**: 9 – 84.
30. Sofowora, E. A., (1982). Medicine plants and Traditinal Medicine in Afircan. Wiley, New York.
31. Venter, F. and Venter , J.A. (1996) .Making the most of indigenous trees. Briza publications, Pretoria. 1(**1**): 24 – 30.
32. Vijayan, P., Raghu,C., Ashok, G., Dhanaraj, S.A.and Suresh, B.(2004). Antiviral activity of medicinal plants of Nilgiris. *India Journal of Medical Research.* **120**: 24 – 29.
33. Waihenya, R. K., Mtambo, M. M. A., and Nkwengulila, G.(2002). Evaluation of efficacy of the crude extract of *Aloe secundiflora* in chicken experimentally infected with Newcastle disease virus. *Journal of Ethnopharmacology*: 299 – 304.
34. Williams, J.E.(2001). Review of antiviral and immunodulating properties of plants of the Penyian rainforest with particular emphasis on Una de gata and Sangre de Grado. Alternative *Medicine Review*. **6**(6): 567 – 567.
35. Zaher, K.S. (2007). In vitro studies on the antiviral effect of olive leaf against infectious laryngotracheitis virus. *Global veterinaria.*