©IDOSR PUBLICATIONS
International Digital Organization for Scientific Research ISSN: 2579-0811
IDOSR JOURNAL OF BIOCHEMISTRY, BIOTECHNOLOGY AND ALLIED FIELDS 3(1): 1-10, 2018.

The Antiviral Activities of leaf extract of *Quisqualis indica* and *Aframomum melegueta* on Fowl Pox Virus (FPV) and Infectious Bursal Disease Virus (IBDV)

Onwuatuegwu Joseph Taiwo Chukwuma

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

ABSTRACT

The aqueous, ethanolic and methanolic leave extract of *Quisqualis indica* and *Aframomum melegueta* were analyzed for its antiviral activities on Fowl pox virus (FPV) and Infectious Bursal Disease Virus (IBDV) respectively. The assay was performed in ten day old embryonated chicken eggs by chorioallantoic membrane (CAM). The viral replication in the tests and controls were estimated by reduction in pocks formation when compared with controls as an indication of viral inhibition in FDV and IBDV. The percentage inhibition of aqueous extracts concentrations of 400mg/ml, 200mg/ml and 100mg/ml of *Quisquali indica* (QI) on the viruses were 50.0%, 50.0% and 43.0% respectively, whereas *Aframomum melegueta* (AM) yielded 86.0%, 50.0% and 50.0% respectively. Similarly, the percentage inhibitions of the ethanolic extracts were QI: 86.0%, 50.0% and 50.0%; AM: 89.0%, 86.0% and 50.0% respectively. The percentage inhibitions of methanolic extracts were QI: 94.6%, 90.5% and 42.6%; AM: 95.05%, 87.5% and 50.0% respectively. At 400mg/ml crude extract concentration, the challenged virus FPV recorded a moderate activity with *Aframomum melegueta* and no activity with *Quisquali indica*. One hundred percentage (100%) egg mortality was observed in all plant extracts investigated at the end of the experiment with infectious bursal disease virus (IBDV). The phytochemical analysis of crude extract of Diospyros mespiliformis and fractions exhibited the presence of Alkaloids, Tannins, Saponins, Triterpenes, Flavonoids and Cyanogenic glycosides.

Keywords: Antiviral activities, IBDV, FPV, CAM and phytochemicals.

INTRODUCTION

Traditional and veterinary 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 [1,2,3]. Plants have long been used as remedies and many are now being collected and examined in an attempt to identify possible sources of antiviral [4,5,6]. 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 [6,7].

Quisqualis indica A. L., also known as the Rangoon creeper belongs to the family

Combretaceae. 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 [8]. 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 [9]. 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 [10]. 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 [11]. *Aframomum melegueta (*K. Schum.*)* 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, galactogogue (to increase the production of breast milk), antihelmintic 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 [12]. 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 [13,14,15].

This study investigated the invitro antiviral activities and the phytochemical constituents of *Quisqualis indica* and *Aframomum melegueta* crude extracts. The plant extracts were screened for their antiviral activities against Fowl pox virus (FPV) and Infectious Bursal Disease Virus (IBDV) using 9 - 11 day – old embryonated chicken eggs and live chickens. These diseases have a worldwide distribution and remain a constant major threat to commercial poultry production. Hence there is need to continue the search for antiviral agents with more satisfactory results. Infectious Bursal Disease (IBD) is a highly contagious disease of young chickens caused by infectious bursal disease virus (IBDV) [16,17] also known as Gumboro disease, characterized by immunosuppression and mortality generally at 3 to 6 weeks of age. Newcastle disease virus 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. Fowl pox virus (FPV) is an infectious disease of worldwide distribution that affects commercial poultry e.g. chickens, turkeys, domestic pets and free living birds of many species [18,19,20,21].

MATERIALS AND METHODS

The Collection of *Quisqualis indica* and *Aframomum melegueta* leaves was done from its natural habitats in Dekina, Dekina Local Government Area of Kogi State and Eziani village, Ichi in Ekwusigo Local Government Area of Anambra State. Collection for extraction was

done during rainy season. The indigenous plant was identified by Prof. C.U. Okeke of the Department of Botany, Nnamdi Azikiwe University, Awka.

Preparation and Extraction

The sample was 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 (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^{0}C and 151bs 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^{0}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 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 Mikail (2010) [22].

Viruses

Two (2) animal viruses, Fowl pox virus (FPV) and Infections Bursal Disease Virus (IBDV) – which were 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.

Fowl Pox Virus (FPV): It cultivation was done by inoculating about 0.1ml virus suspension on the chorioallontoic membranes (CAMs) of 10days old developing chicken embryo. These were incubated at 38^{0}C for 7days and then

examined for focal pock lesions or generalized thickening of the CAMs.

Infectious Bursal Disease Virus (IBDV): The virus was cultivated by inoculation of a viral suspension containing 10^5 EID_{50} onto the chorioallontoic membranes of 10day old chicken embryos .The eggs were incubated and observed daily for viability. Dead embryos during the first 24hours post inoculation (Pl) were discarded. Mortality was recorded between 2 - 4days Pl. At the end of 4thday Pl effects were compared with controls. The reduction or no pock/lesion formation was observed as an indicator for antiviral activity. The results were compared to the sample without treatment as a positive and PBA solution as a negative control.

RESULTS

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

Plant sample	Extraction	Phytochemical					
		Alkaloids	Saponins	Triterpenes	Flavonoids	Tanins	Cyanogenic glucosides
Qusquali Indica	Aqueous	_	+	_	_	+	_
	Ethanol	+	+	_	+	++	_
	Methanol	+	+	_	+	++	_
Aframomum melegueta	Aqueous	_	+	_	_	+	_
	Ethanol	+	++	_	++	++	_
	Methanol	+	++	_	++	++	_

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

Table 2: 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 3: Toxicity of *Aframomum melegueta* (AM) 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	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 Fowl Pox Virus (FPV) pocks extracts of *Quisquali indica* leaves in Embryonated chicken Eggs.

Extract	Test Extract concentration	FPV pock lesion formation	FPV virus inhibition
Aqueous Extract	400mg/ml	Present	0
	200mg/ml	present	0
	100mg/ml	Present	0
Ethanol Extract	400mg/ml	Present	0
	200mg/ml	Present	0
	100mg/ml	Present	0
Methanol Extract	400mg/ml	Present	0
	200mg/ml	Present	0
	100mg/ml	Present	0
Positive control	Virus+PBS	Present	0
Negative control	PBS only	Absent	== ==

Key:++++=complete inhibition,++=moderate inhibition, += mild inhibition, 0 = no inhibition.

Table 5: Reduction of Fowl Pox Virus (FPV) pocks extracts of *Aframomum melegueta* (AM) leaves in Embryonated chicken Eggs.

Extract	Test Extract concentration	FPV pock lesion formation	FPV virus inhibition
Aqueous Extract	400mg/ml	Present	+
	200mg/ml	present	+
	100mg/ml	Present	0
Ethanol Extract	400mg/ml	Present	++
	200mg/ml	Present	+
	100mg/ml	Present	0
Methanol Extract	400mg/ml	Present	++
	200mg/ml	Present	+
	100mg/ml	Present	0
Positive control	Virus+PBS	Present	0
Negative control	PBS only	Absent	== ==

Key:++++=complete inhibition,++=moderate inhibition, += mild inhibition, 0 = no inhibition

Table 6: Reduction of infectious Bursal Disease virus (IBDV) viral load by Extracts of *Quisquali indica* leaves in Embryonated Chicken Eggs.

Extracts	Test Extract concentration	IBDV pock lesion formation	IBDV virus inhibition
Aqueous Extract	400mg/ml	Present	0
	200mg/ml	present	0
	100mg/ml	present	0
Ethanol Extract	400mg/ml	Present	0
	200mg/ml	present	0
	100mg/ml	present	0
Methanol Extract	400mg/ml	Present	0
	200mg/ml	present	0
	100mg/ml	present	0
Positive control	Virus+PBS	Present	0
Negative control	PBS only	Absent	== ==

Key: ++++ = complete inhibition, 0 = no inhibition.

Table 7: Reduction of infectious Bursal Disease virus (IBDV) viral load by Extracts of *Aframomum melegueta* (AM) leaves in Embryonated Chicken Eggs.

Extracts	Test Extract concentration	IBDV pock lesion formation	IBDV virus inhibition
Aqueous Extract	400mg/ml	Present	0
	200mg/ml	present	0
	100mg/ml	present	0
Ethanol Extract	400mg/ml	Present	0
	200mg/ml	present	0
	100mg/ml	present	0
Methanol Extract	400mg/ml	Present	0
	200mg/ml	present	0
	100mg/ml	present	0
Positive control	Virus+PBS	Present	0
Negative control	PBS only	Absent	== ==

Key: ++++ = complete inhibition, 0 = no inhibition.

DISCUSSION

The results of 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. A hundred percent (100%) inactivation has been used to define an extract with antiviral activity [23]. However, partial antiviral activity was observed in extracts used in this study when tested with Fowl pox virus whereas when tested with Infectious bursal disease virus, no activity was recorded. 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 FPV at various concentrations.

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 [5]. 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 Afromomum 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 [7].

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 [9]. Flavonones exhibit inhibitory effects against viruses [7] including HIV and respiratory syncytial virus [6,7]. Terpenoids were reported to be active against bacteria, fungi, viruses and protozoa [8,9]. It is believed to be active against viruses by envelope disruption by the lipophilic

compounds. Alkaloids have been commonly found to have antimicrobial properties [10,11]. It is also useful against HIV infection as well as intestinal infections associated with AIDS [12,13]. Tannins are found in almost every plant part; wood, leaves, bark, roots and fruits [8] and tannin containing beverages can cure or prevent a variety of viral infections [9]. At least two studies [6,9] have shown tannins to be inhibitory to viral reverse transcriptases. Tannins (hydrolysable) also show anti-carcinogenic and anti-mutagenic effects [11]. These phytochemical were significantly demonstrated in methanolic extracts.

The ethanolic extracts and methanolic extracts appeared to have had more antiviral phytochemical than the aqueous extracts which accounted for better reduction in the 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 [24] 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 [25] 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 various extracts studied against FPV and IBDV were considered active, if there was reduction in formation of pocks as compared to the controls. In the study, there were moderate inhibitions by aqueous and, high inhibitions by both ethanolic and methanolic extracts at the concentration of 400mg/ml. The various extracts exhibited no activity when tested with IBDV. The extract of *Quisquali indica* exhibited no activity at various concentrations (400mg/ml, 200mg/ml and 100mg/ml) when tested with FPV and IBDV. 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.

REFERENCES

1. 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.
2. Ayatoa, J.F., Tchendem, M.H.K. and Nyasse, B. (1994). Novel bioactive diterpenoids from *Aframomum aulacocarpos. Journal of Natural Products* **57**: 917-923.
3. Bernard, D.L., Huffman, H.J., Meyerson, L.R. and Sidwell, R.W. (1993). Mode

of Inhibition of respiratory syncytial virus by plant flavonoid. Chemotherapy **39**: 21-217.

4. Bizimana, N. (1994). Traditional Veterinary Practice in Africa. Schrifteib der GTZ. No. 243, Eschborn, Germany.
5. Caston, J.A. (2008), "Infections Bursal Disease virus (IBDV)." (HTTP: //WWW. horizon press. Com/Ma). Segmented Double Stranded RNA viruses: structure and Molecular Biology. Caster *Academic Press*. ISBN 978 – 1– 90 4455 – 21 - 9
6. Ekpa, O. D. (1996). Nutrient composition of three Nigeria medicinal plants.
7. 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
8. 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.
9. Gbile, G.O.and Adesina, S.K. (1986). Nigeria flora as pharmaceutical potentials. *Journal of Ethnopharmacology*. **19**: 1 – 16.
10. Ghoshal,S.,Krishna,B.N. and Lakshmi, V. (1996). Anti amoebic activity of Piperlongum fruits against Entamoeba histolytica *in vitro* and *in vivo. Journal of Ethnopharmacology.* **50**: 167-170.
11. 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.
12. Hasegawa, H. Matsumiya, S., Uchiyama, M., Kurokawa, T., Inovye, Y., Kasai, R., Ishibashi, S. and Yamasaki, K. (1994). Inhibitory effect of some triterpenoid saponins on glucose transport in tumor cells and its application *to in vitro* cytotoxic and antiviral activities. *Planta Medica*. **6**: 240 - 243
13. ITDG and IIRR, (1996). Ethnoveterinary medicine in Kenya; A field manual of traditional animal healthcare practices. Intermediate Technology Development Group (ITDG) and International Institute of Rural reconstruction (IIRR), Nairobi, kennya ISBN 9966 – 9606 – 2 – 7.
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. Kaul , T.N., Middle town, E.Jr. and Ogra, P.L. (1985). Antiviral effect of flavonoids on human viruses. Journal of Medical Virology. **15**: 71-79
16. Kerharo, J. and Adam, J.C. (1974). La pharmacopee senegalaise traditionnelle, vigot, Paris
17. Kirmse, P:, (1969): Host specificity and pathogenicity of pox viruses from wild birds. *Bull wildlife Disease Association*. **5**: 376 – 389.
18. 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.

19. McMahon, J.B., Currens, M.J., Gulakowaki. R.J., Buckheit. R.W.J., Lackman- Smith, C., Hallock, Y.F. and Boyd, M.r. (1995). Mochellamine B, A Novel plant alkaloid, inhibits human immunodeficient virus – induced cell killing by at least two distinct mechanisms. *Antimicrobial Agents Chemotherapy*. **39**:484-488.
20. McMillan, J.K., Cochran, M.D. Junker, D.E., Redd, D.N., and Valencia, D.M., (1994). The safe and effective use of fowl pox virus as a vector for poultry vaccines. *Development Biological Standard.* **82**: 137 – 165.
21. Mendooza, L., Wikkens, M. and Urzua, A. (1997). Antimicrobial study of the resinous exudates and of diterpenoids and flavonoids isolated from some ChileanPseudognaphalium (Asteraceae). *Journal of Ethnopharmacology.* **58** : 85-88.
22. 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.
23. 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.
24. Nonaka, G.I., Nishioka, I., Nishizawa, M., Yamagishi, T., Kashiwada, Y., Dutschman, G.E., Bodner, A.j., Kilkushie, RE., Chang, Y.C. and Lee, K.H.(1990). Anti-AIDS agents, 2. Inhibitory effects of tannins on HIV reverse transcriptase and HIV replication in H9 Lymphocyte cells. *Journal National Products*. **53**:587-595.
25. Nwude, N., and Ibrahim, M. A., (1980). Plants used in traditional veterinary practice in Nigeria. *Journal of Veterinary Pharmacology.* Pp 261 – 273.