Journal of Advanced Pharmacy Education & Research
2 (3) 82-92 (2012) ISSN 2249-3379

Antiviral Potential of Aqueous Extracts of Some Parts of *Momordica balsamina* Plant against Newcastle Disease Virus

Chollom S.C.[1*], Olawuyi A.K.[1], Danjuma L.D.[2], Nanbol L.D.[3], Makinde I.O.[3], Hashimu G.A.[4], Alesa M.U.[5], Esilonu J.T.[6], Ogundeji E.B.[4], Kwatjel J.S.[6]

1. Viral Research Department, National Veterinary Research Institute, Vom, Nigeria
2. Dermatophilosis Unit, National Veterinary Research Institute, Vom, Nigeria
3. Viral Vaccine Production Department, National Veterinary Research Institute, Vom, Nigeria
4. Bacterial Vaccine Production Department, National Veterinary Research Institute, Vom, Nigeria
5. Central Diagnostic Laboratory, National Veterinary Research Institute, Vom, Nigeria
6. Parasitology Laboratory, Federal College of Animal Health and Production Technology, Vom, Nigeria

*Corresponding author: cholloms_12@yahoo.com

ABSTRACT

Investigation was made on the fruit pulp and leaf extracts of *Momordica balsamina* plant to determine their antiviral potential against Newcastle Disease Virus (NDV) using chicken embryo fibroblast (CEF) cell lines. Three experimental sets were assembled: virus and extract were made to react before infection of cell line; virus and cell were made to react before addition of extract and cell and extract made to react before infection with virus. Cytotoxicity test showed that both extracts are safe at concentrations lower than 30mg/ml. Phytochemistry of the two extracts revealed the presence of novel antiviral agents just as elemental studies showed the presence of important elements. Antiviral assay showed inhibition of the virus from 10mg/ml concentration of fruit pulp extract and 20mg/ml concentration of leaf extract upwards. With both extracts, the experimental sets that pre-treated the virus with the extract and cell with extract prior to infection of cells showed greater sensitivity relative to the one that allowed the virus to infect the cell before

introduction of the plant extract. It suggests that the plant has the ability to prevent attachment of the virus to host cells hence affirming its antiviral potential against the virus.

Keyword*: Momordica balsamina,* Newcastle Disease Virus, antiviral, chicken embryo fibroblast, cytotoxicity, phytochemistry

INTRODUCTION

Newcastle disease virus (NDV) is a single stranded RNA-containing virus with helical capsid symmetry (26), which belongs to the family Paramyxoviridae and the genus Aulavirus (21). The disease still remains a serious economic challenge to all segments of the poultry industry (11) because of its contagious and mortality records (2). The only control method so far still remains vaccination, which does not confer 100% immunity in all vaccinated birds (33). Thus there is a need to search for alternative measure.

Momordica balsamina Linn, belongs to the family Cucurbitaceae, it is a climber that grows over native huts. It is annual to perennial herb found wild throughout most states in Northern Nigeria (7). Different parts of the plant have been used for different medical purposes. The Wollofs in Senegal and Gambia have used it as sponge in treating skin disease; elsewhere it is also used as tranquillizer in the treatment of mental illness (3). Tender shoots are usually consumed with okra soup by the Kanuris of Borno and Yobe States (Nigeria) where the plant is locally known as Dagdawu (14). Phytochemical screening of *M. balsamina* Linn has revealed the presence of tannins, saponins and lectins (3). The seed of *M. balsamina* is known to contain glycosides, saponins and steroids. The leaves and fruits were observed to have hypoglycaemic effects in rats (15, 16). Furthermore, the fruits were observed to be toxic to various organs and tissues of rats in very high dose (29).

MATERIAL AND METHODS

Collection and Identification of the Plant Material

Fresh fruits and leaves of *M. balsamina* were randomly collected from different areas in Heipang, Plateau State, Nigeria. The plant was first identified at the field using standard keys and descriptions (10). It's botanical identity was further confirmed and authenticated at the Federal College of Forestry in Jos.

Preparation of Plant Extract

The fruit pulps and leaves of M balsamina were air dried and pulverized before the commencement of the extraction. The extraction was carried out as described by Njar et al. (13) and Raji (27). The pulverized fruit pulps and leaves weighing 450g and 400g respectively were exhaustively extracted with distilled water by means of cold extraction and extracts evaporated in vacuo. The extracts were concentrated in vacuo using a rotary evaporator at 40^{o}C. The solvent remaining in the extract was finally removed by placing the extracts in porcelain dishes in temperature-controlled oven to give a residue weighing 10.55g and 8.75g for fruits and leaves respectively. The resulting extracts were reconstituted in 10.55ml and 8.75ml for fruit and leaf extracts respectively of sterile distilled water to give a final concentrations of 1000mg/ml each.

Phytochemical Screening

Phytochemical screening was carried out using the methods of Trease and Evans (32) as reported by Chollom et al. (9).

Elemental analysis

0.2 g each of the *M. balsamina* fruit pulp and leaf extract was weighed into a porcelain crucible and ashed in a muffle furnace pre-heated to 600^{o}C for 4 h. The crucible was then transferred directly to desiccators and allowed to cool. The weight of the ash was noted. The ash was treated with a few milliliters of 5N HCl and a few drops of concentrated HNO_3 and boiled. This was cooled, filtered and the filtrate made up to 50ml in a volumetric flask with de-ionized water. This solution was used for the determination of cations using atomic absorption spectrophotometer (AAS) as described by AOAC (4).

Preparation of cell culture suspension

The continuous cell line of Chicken Embryo Fibroblast (CEF) was used according to the method of Levy et al. (19). A confluent monolayer was washed twice with prewarmed phosphate buffered saline (PBS) and then layered with 1-5 ml of prewarmed 0.25% trypsin for 5 min to dislodge the cells. As soon as there was evidence of cell rounding, the trypsin was tipped off and 1-2 ml of growth medium (Eagles Minimum Essential Medium was added) to neutralize the action of trypsin. With a sterile 5 ml pipette, the cells were

dislodged by pipetting the medium against the wall of the bottle. Viable cells were estimated using the 1:10 suspension of cells in neutral red in a haemocytometer. The cells were seeded into tubes using 100,000 cells/ml. They were allowed to form a monolayer before being used for viral assay.

Viral stocks were prepared as aliquots of culture medium from CEF cells infected at multiplicity of infection (m.o.i.) of one virion per 10 cells and cultured for 3 days according to the method of Levy et al. (19). They were stored at -80°C. Working stocks were prepared by serial dilution of viral stock in culture medium and assayed in triplicate on CEF cells monolayer in the wells of microtitre tray. The working stock suspensions were then stored at 4°C until used.

Cytotoxicity Assay

Each extract was separately dissolved in 1ml of distilled dimethyl sulphoxide (DMSO) and volume was made up to 10 ml with maintenance medium to obtain a stock solution of 50 mg/ml concentration, sterilized by filtration and further dilutions were made from the stock. The cytotoxicity assays were carried out using 0.1ml of cell suspension, containing 10,000 cells seeded in each well of a 96-well microtitre plate. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Control cells were incubated without test sample and with DMSO present in the wells (maximum 0.2%) was found not to affect the experiment. The microtitre plates were incubated at 37^{o}C in a humidified incubator with 5% CO_2 for a period of 72 h. Sixteen wells were used for each concentration of the test sample. The morphology of the cells was inspected daily and observed for microscopically detectable alterations, that is, loss of monolayer, granulation and vacuolization in the cytoplasm. The cytopathogenic effect (CPE) was scored. The 50% cytotoxic concentration (CTC_{50}), was determined by the standard MTT assay (13, 17), trypan blue dye exclusion method (22), cell metabolic function by protein estimation (20), and total cellular DNA content by ^{3}H thymidine labeling (12).

Antiviral assay of plant extracts

For the antiviral investigation, the experimental design of Obi et al. (24) was adopted. Briefly, each plant extract in graded doses of (5, 10, 15 and 20 mg/ml) was mixed with equal volume of 100 $TCID_{50}$/0.1 ml of velogenic strain of NDV stock. The mixture was incubated for 1 h and agitated at intervals of 15 min. One hundred microlitres of extract-virus mixture

were inoculated into CEF cells in a 96-well tissue culture plate. For the control, in the same plate, the last row of wells was reserved for controls that were not treated with extract or not treated with virus. The culture was incubated with the un-inoculated controls at 37°C. The entire setup was examined daily for seven days.

The reactions in the three different experimental sets: virus and extract before cell line; virus and cell before extract and cell and extract before virus were stopped by adding 70% methanol into the wells using multichannel micropipette. The mixtures were allowed to stand on the bench for 10 min for complete inactivation of cells to take place after which they were discarded and crystal violet solution added. After about 3 min, they were rinsed with water, and examined visually. Antiviral activity was identified as confluent, relatively unaltered monolayer of stained CEF cells that were previously treated with the virus. Cytotoxicity was identified as the loss of the cell monolayer as a result of the plaques formed by the measles virus (19).

RESULT AND DISCUSSION

The Phytochemical contents of the fruit and leaf extracts of *M. balsamina* are as displayed in Table 1. The leaf appears less endowed pharmacologically relative to the fruit with conspicuous absence of tannins and flavonoids. Also, elemental analysis of the two extracts showed the presence of important minerals (Table 2). Again, the fruit has higher deposits of all the minerals except manganese and copper. Cytotoxicity test showed that the extracts were safe at concentrations lower than 30mg/ml. Antiviral exploits of the two extracts as shown in tables 3 and 4 showed varied responses against the virus. Fruit extract inhibited virus growth in as low as 10mg/ml concentration while leaf extracts inhibited it at 20mg/ml concentration. With both extracts, the experimental set that pre-treated the virus with the extract prior to infection of cell lines was more sensitive against the virus followed by the set that pre-treated the cell lines with the extract prior to virus inoculation. The least sensitive set was that which allowed for virus infection of cells prior to introduction of plant extract.

The degree of virus inhibition was however greater with fruit pulp extract than with the leaf extract of the plant under investigation.

Table 1: Result of Phytochemical Screening of *Momordica balsamina* Fruit Pulp and Leaf Extracts

Extracts	Tannin	Resins	Alkaloids	Glucosides	Flavonoids	Saponins
Fruit	++	+	+	+	+	++
Leaf	-	+	-	+++	+	+

KEY

+ = Trace

++ = Present in appreciable quantity

_ = Absent

Table 2: Result of Elemental Studies of *Momordica balsamina* Fruit Pulp and Leaf Extracts

Elements	Concentration (ppm)	
	Fruit	Leaf
Calcium	1.1276	0.9001
Lead	ND	ND
Iron	1.1210	1.0032
Manganese	0.0340	1.2500
Sodium	0.4552	0.4500
Potassium	45.0012	40.3322
Copper	0.1032	1.1031
Selenium	ND	ND
Zinc	0.4111	0.1091

Key:

ND= Not Detected

Table 3: Result of Antiviral Activity of *Momordica balsamina* Fruit Pulp Extract against NDV

	Concentration (mg/ml) of Extract			
Assay	**5mg**	**10mg**	**15mg**	**20mg**
Virus + Extract+ Cell	0	-/+	+	++
Virus+ Cell + Extract	0	0	0	+
Cell + Extract + Virus	0	0	+	++

Key:

0=No inhibition

-/+=Faint inhibition (Between 5-10%)

+= Inhibition (Between 20-30%)

++=Inhibition (40% and above)

Table 4: Result of Antiviral Activity of *Momordica balsamina* Leaf Extract against NDV

	Concentration (mg/ml) of Extract			
Assay	**5mg**	**10mg**	**5mg**	**20mg**
Virus + Extract+ Cell	0	0	0	+
Virus+ Cell + Extract	0	0	0	0
Cell + Extract + Virus	0	0	0	-/+

Key:

0=No inhibition

-/+=Faint inhibition (Between 5-10%)

+=Inhibition (Between 20-30%)

++= Inhibition (40% and above)

The result of this study showed that the plant investigated has potential in the management of Newcastle disease. The aqueous extracts of parts of *M. balsamina* showed promise in inhibiting NDV. This is essentially due to the pharmacologically active substances as revealed by the phytochemical analysis (Table 1). Alkaloids, flavonoids, saponins and tannins found in the plant are regarded as novel antiviral agents (18). This is the reason

behind the ethno-veterinary application of the plant. *M. balsamina* had hitherto been screened against some viruses including NDV and was found to hold good promise as candidate for antiviral drug development (8). It has also been screened against parasites such as trypanosomes and Plasmodium species with significant efficacies (1, 28).

It is also rich in elements as revealed by elemental studies (Table 2). The presence of these elements is no doubt responsible for the leading roles the plant plays in boosting immune responses against microbial infections including NDV and human immunodeficiency virus (25, 8). These elements have been reported previously to potentiate haemopoeisis in experimental rats without altering homeostasis (14).

The three experimental sets are possible ways of administering the extract in field situation. In the experimental set where the virus was pre-treated with the extract before administration, inhibition of virus growth was observed with lower extract concentration (10mg/ml). Previous studies have earlier revealed the possibility of virus inactivation when a potent antiviral candidate is incubated with the virus at controlled conditions (30, 6). The possibility that the extract interferes with the neuraminidase-haemagglutinin sites necessary for attachment and penetration of the virion into the living cell is very likely.

The second successful experimental set was when the cell line was pre-treated with the plant extract prior to infection with the virus. Here, the degree of virus inhibition was also good. Scientists have earlier explained that if the receptor sites of susceptible hosts are bound or altered prior to virus infection, the ability of the virus to attach and penetrate the living cell would be greatly reduced (24).

The last experimental set was the least productive. Here, the virus was made to infect the cell before introduction of the plant extract. Although at 20mg/ml concentration of fruit extract, it inhibited virus growth, it generally did not show the degree of potency and sensitivity with smaller concentrations; as it was the case with the other two. This presupposes that the extracts may exhibit minimal antiviral activity once the virus has attached, penetrated and uncoated in the cell. It may be right to conclude that the ability of the extracts to alter virus replication in stages subsequent to attachment and penetration of host cell is not as effective as prior to the aforementioned stages. Although a greater degree of success may be achieved with higher extract concentrations. Antiviral agents are known to interfere with virus replication at different stages of virus replication (24).

Although both extracts have potentials for antiviral activity against NDV, the fruit extract seem to have more of the antiviral components than the leaf extract. This is based on the

fact that it inhibited virus growth at lower concentrations (10mg/ml) relative to the leaf extract (20mg/ml). The reason for this is not far-fetched as phytochemistry revealed more antiviral components in the fruit extract than in the leaf (Table 1).

REFERENCES

1. Abubakar A , Iliyasu B, Yusuf AB, Igweh AC, Onyekwelu NA, Shamaki BU, Afolayan DO, Ogbadoyi EO. Antitrypanosomal and haematological effects of selected Nigerian medicinal plants in Wistar rats. Biochem, 2005; 17(2):95-99.
2. Adeboyega MA. Newcastle Disease. In: serological Evidence of Newcastle Disease and Infectious Bursal Agents in Ducks and Guinea fowls. African Press, Ibadan; 1999; 5:11.
3. Akiniyi JA, Sultanbawa MUA and Mnawaku D. Ethnobotany and ethnopharmacology of Nigerian Medicinal Plants. In: The State of Medicinal Plant Research in Nigeria. University Press, Ibadan, Nigeria, 1986; pp: 154-165.
4. AOAC. Official methods of Analysis. Association of analytical Chemists. 1975.
5. Washington, D.C, USA, pp 450.
6. Bakari GG, Max RA, Mdegela RH, Phiri EC, Mtambo MM. Antiviral activity of crude extracts from Commiphora swynnertonii against Newcastle disease virus in ovo. Trop. Anim. Health. Prod. 2012.
7. Bokhari MH and Ahmed MSCH. Food plants in Borno State, Nigeria, Gulani Publishers Lahore, India, 1980; pp: 31-32.
8. Bot YS, Mgbojikwe LO, Nwosu C, Abimiku A, Dadik J, and Damshak D. Screening of the fruit pulp extract of *Momordica balsamina* for anti HIV property. Afr J Biotechnol; 2007; 6(1), 047-052
9. Chollom SC, Agada GOA, Gotep JG, Mwankon SE, Dus PC, Bot YS, Nyango DY,Singnap CL, Fyaktu EJ and Okwori AEJ. Investigation of aqueus extracts of Moringa oleifera lam seed for antiviral activity against Newcastle Disease Virus in ovo. J. Med. Plants Res. 2012; 6(22) pp. 3870-3875.
10. Dalziel JM. Useful plants of west tropical Africa. Crown Agents for Oversea Government and Administration, London, 1956; 23pp.
11. David-West KB. Newcastle Disease in Nigeria, retrospection and Anticipation Bull. Epiz. Afr. 1972; 20:291-295.

12. De Clereq E, Holy A, Rosenberg I. Efficacy of phosphonyl methoxyalkyl derivatives of adenine in experimental herpes simplex virus and vaccinia virus infections in vivo. Antimicrob Agents Chemother. 1989; 33: 185-91.
13. Francis D, Rita L. Rapid colorimetric assay for cell growth and survival: modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J. Immunol. Methods. 1986; 89: 271-7.
14. Geidam MA, Pakmam I, Laminu H. Effects of aqueous stem bark extract of *M. balsamina* Linn on serum electrolytes and some Hematological parameters in normal and alcoholic fed rats. Pakistan Journ of Biol Sci; 2004; 7(8): 1430-1432.
15. Karumi Y and Bobboi A. Hypoglycemic effects of Balsam apple (*M. balsamina* L) in alloxan diabetic male rabbits. Biochemistry; 1999; 9:0795-8080.
16. Karumi Y, Geidam MA and Shettima Y. Hypoglycemic properties of M.balsamina L fruits could be due to inhibition of glucose uptake in gastro-intestinal tract. West African J Biol Sci. 1999; 10:84-91.
17. Ke H, Hisayoshi K, Aijun D, Yonngkui J, Shigeo I, Xinsheng Y. Antineoplastic agents-III: steroidal glycosides from Solanum nigrum. Planta Med. 1999; 65: 35-8.
18. Jassim SSA and Naji MA. Novel antiviral agents: a medicinal plant perspective. J. Appl. Microbiol. 2003; 95:412-427.
19. Levy F, Hay DA, McStephen M, Wood C, Waldman I. ADHD: A category or a continuum? Genetic analysis of a large-scale twin study. J. Am. Acad. Child Adolesc. Psychiatry. 1997; 36:737–744
20. Lowry ON, Roseborough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem. 1951; 193: 265-75.
21. Mayo MA. A summary of taxonomic changes recently approved by International Committee in taxonomy of Viruses (ICTV). Arch. Virol. 2002; 147: 1655-1656.
22. Moldeus P, Hogberg J, Orrhenius S, Fleischer S, Packer L. Methods in enzymology. 1978; vol.52, New York: Academic Press 60-71.
23. Njar VCO, Alao TO, Okogun JI, Holland HL. 2 methoxy cathin-6-one: A new alkaloid from the stem wood of Quassia amara. Planta Med. 1993; 59: 259-261.
24. Obi RK, Iroagba II and Ojiako OA. Virucidal potential of some edible Nigerian vegetables. Afr. J. Biotechnol; 2005; 5 (19). 1785-1788.

25. Olabode HOK, Eghafona NO and Mgbojikwe LO. The role of *Momordica balsamina* fruit pulp extract in development of immunity to avian Newcastle Disease Virus. Nigerian Veterinary Journal. 2007; 28 (1) 41-47.
26. Pringle CR. Paramyxoviridae. In: Heath, RB, Collier, LH, Timbury, MC, Topley and Wilson (eds). Principles of Bacteriology, Virology and Immunity Vol. 4 Edward Arnold, Hodder and Sloghton: Kent, England; 1990; 286-287.
27. Raji Y. Hormone and Drug stimulation of testosterone secretion in rat leydig cell. Ph.D. Thesis. Dept of Physiology University of Ibadan. 1995; XXVIII +227.
28. Ramalhete C, Lopes D, Mulhovo S, Rosario VE, Ferreira MJU. Antimalarial Activity of Some Plants Traditionally Used in Mozambique. Workshop on medicinal plants and phytotherapeutics in the tropics. 2008.
29. Shettima Y, Karumi Y and Addy EO. Effect of *M.Balsamina* L fruit on liver enzymes and histopathology in rats. Nigerian J. Exp Appl. Biol. 2001; pp 195-198.
30. Sulaiman LK, Oladele OA, Shittu IA, Emikpe, BO, Oladokun AT and Meseko CA (2011).
31. In-ovo evaluation of the antiviral activity of methanolic root-bark extract of the African Baobab (Adansonia digitata Lin). Afr. J. of Biotech. Vol. 10(20), pp. 4256-4258.
32. Trease GE and Evans WC. Trease and Evans Pharmacognosy. A physician guide to Herbal medicine. 1989; 13th Edition: Ballere Tindal London.
33. USDA/APHIS (2003). Exotic Newcastle Disease (US Department of Agriculture/ Animal Plant Health Inspection Service). Fact Sheet.