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In ovo antiviral potency of the leaf constituents of Tanzanian *Toussaintia* species against Infectious Bursal Disease Virus and Newcastle Disease Virus

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ABSTRACT

The chemical constituents of *Toussantia orientalis* and *T. patriciae* (Annonaceae) leaf extracts were evaluated for their antiviral activities *in ovo* against Infectious Bursal Disease Virus (IBDV) and Newcastle Disease Virus (NDV). The nine-day-old embryonated eggs in a set of five were used for testing through the allantoic route inoculation assay for each tested compound and controls. For NDV assay, the allantoic fluids from the specimens were further harvested to determine viral contagion. The tested compounds exhibited potency with varying levels of significance at a screening concentration of 360 µg/ml against the two viral strains. Embryos infected with IBDV survived, grew to normal size with complete organ formation and had mean weights comparable to those of the uninfected ones when treated with the aminocinnamoyl tetraketides 1, **2**, and **4**, glucosylflavonoid **7** from *T. orientalis* and ursolic acid derivatives **9** and **10** from *T. patriciae* demonstrating high efficacy against IBDV. The compounds also exhibited antiviral activity against NDV, showing viral titre reduction ranging from 1:16 - 1:256 in the haemagglutination test, with compound **11** having the lowest titre value (1:16) followed by compound **7** (1:32). The compounds that exhibited significant antiviral efficacy could be considered potential leads for the development of antiviral agents.

Keywords: Aminocinnamoyl tetraketides, ursolic acids, *Toussaintia orientalis*, *Toussaintia patriciae*, Annonaceae.

INTRODUCTION

Poultry production in Tanzania and other low income countries is dominated by the free-range local chickens (FRLC) forming one of the major economic activities for majority of the people living in the rural areas. It has been established that rural poultry industry contributes significantly to improving income at household levels (Kisungwe et al., 2012; Moreki, 2013). The industry is characterized by low productivity arising from such constraints as diseases, predation, poor management and marketing strategies (Kisungwe et al., 2012). The major diseases affecting FRLC include the devastating Newcastle Disease (ND), Infectious Bursa

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Disease (IBD, Gumboro) among the viral diseases and fowl typhoid, callibacillosis among bacterial diseases (Moreki, 2013). Interventions towards control of these diseases have mostly involved the use of vaccines, which are practically inaccessible and expensive to the poor rural villagers. Hence, many people in the villages resort to the use of the cheap and easily available ethnoveterinary plants (Waihenya et al., 2002; Adejeji et al., 2013; Moreki, 2012; Moreki, 2013). The evidence and importance of plant-based natural products in the treatment and control of poultry viral diseases has been reported (Waihenya et al., 2002; Bakari et al., 2012; Bakari et al., 2013; Mabiki et al., 2013), calling for more research endeavors to the untapped biomedical potential of bio-resources endowment of tropical and subtropical floral diversity.

In our recent papers, we reported the isolation of a series of novel bioactive aminocinnamoyl tetraketides (Samwel et al., 2011), a new glucosylglycero-*N*-octenyl adipate and a known glycoflavonoid afzelin from the leaf extracts (Nyandoro el al., 2012) of the red-listed endangered medicinal plant *Toussiantia orientalis* Verdc. (EAM-CF-CEPF-PAP, 2009), several aristolactams, a *pseudo*-nucleoside 1-(2-C-methyl-β-D-ribofuranosyl)-uracil and other bioactive metabolites from the stem and root bark extracts of the same plant species (Odalo et al., 2010). These results inspired us to include the recently described and red-listed endangered *Toussaintia* species, namely *Toussaintia patriciae* Q. Luke & Deroin (Deroin and Luke 2005) in the phytochemical investigations to supplement the existing chemical information on the constituents of the genus *Toussaintia* and to further assess effectiveness of the metabolites isolated there from on poultry viruses. In the analysis, the leaf extracts of *T. patriciae* did not yield any of the metabolites that were previously obtained from *T. orientalis*, instead ursolic acids and ethyl gallate were obtained. The metabolites from *T. patriciae* together with the recently reported aminocynnamoyl tetraketides from *T. orientalis* were evaluated for their *in ovo* antiviral activity against the two poultry viruses; the Infectious Bursal Disease Virus (IBDV) and Newcastle Disease Virus (NDV) that are economically important to the poultry industry in many countries, Tanzania included.

MATERIALS AND METHODS

Plant materials collection

The leaves of *Toussaintia patriciae* Q. Luke & Deroin were collected on 4th June 2008 from Ndundulu Forest Reserve, 12 km from Udekwa Village, Kilolo District in Iringa Region, Tanzania. The plant species was authenticated at the Herbarium of the Department of Botany, University of Dar es Salaam where a voucher specimen is preserved (# FMM 3521).

Extraction, isolation and identification compounds

The air-dried and powdered leaves (1.4 kg) of *T. patriciae* were subjected to sequential extraction using petroleum ether, dichloromethane (CH_2Cl_2) and ethanol (CH_3CH_2OH, EtOH) at room temperature, each soaking lasting for 48 h so as to obtain less, medium and polar constituents of the plant materials, respectively. Removal of residual solvent under reduced pressure in a rotavap furnished the crude extracts. The pet ether and CH_2Cl_2 extracts were obtained in small quantities which did not show any interesting spots on thin layer chromatography (TLC) [Precoated plates (Merck, Kieselgel 60 F254, 0.20 mm)]. Hence, the extracts were not analysed further. The EtOH extract was obtained as a dark green gelatinous material (18.2 g), whose TLC analysis showed one conspicuous UV positive spot and other three UV negative spots [UV/VIS (254 and 365 nm) and anisaldehyde spray reagent/heat at *ca* 110 ºC]. EtOH extract was subjected to a repeated chromatographic separation (Silica gel 60 (Merck 230–400 mesh, 10–100% Ethylacetate (EtOAc)/pet ether gradient) followed by gel

filtration (Sephadex LH-20, 1:1 $MeOH/CH_2Cl_2$) to isolate the compounds.

Chemical structures of the isolated compounds were identified upon analysis of their spectroscopic [^{1}H- (300 MHz) and ^{13}C-NMR (75 MHz): Bruker Topspin Spectrometer and CD_3OD or DMSO-d_6 as deuterated solvent, TMS as internal standard for ^{1}H-NMR and solvent signal for ^{13}C-NMR] and spectrometric data [high-resolution electron ionisation mass spectra (HREIMS): GCT Premier TOF mass spectrometer, 70 eV and 180 ºC source temperature].

In ovo antiviral assays

Allantoic cavity inoculation assay

The embryonated eggs collected from healthy flocks were used for the determination of antiviral activity of the isolated natural products against IBDV (KMRG-48 strain) and velogenic NDV strain (13C/SUA). Both viral strains used in the experiment were supplied by the Department of Microbiology and Parasitology, Sokoine University of Agriculture and they originated from field outbreaks. The exterior surface of fertilized eggs were sterilized with 70% ethanol and incubated for 9 days at 37 ºC and 65-70% humidity in an egg incubator (Memmert 854, Schwabach, Germany) prior to inoculation. The nine-day-old embryonated eggs were used for the antiviral testing through the allantoic cavity route of inoculation (Hsiung 1973). The eggs were candled to confirm their fertility and a small puncture was made through the shell over the air sac as previously described by Grimes (2002). The stock solutions containing the tested compounds were prepared by dissolving each sample (2 mg) in dimethylsulphoxide (DMSO, 5 ml) to make a solution of 0.4 mg/ml concentration. The tested compounds were mixed with the viral strains and allowed to equilibrate for 30 min. before inoculation. The eggs were inoculated with the specimen [0.1 mL, having been prepared by mixing tested compound (0.9 ml) with the viral strain (0.1 ml) in allantoic fluid treated with the antibiotic Gentamycin to make up a concentration of 360 µg/ml of the tested sample], using a sterile insulin needle and syringe. The inoculated site of the shell was sealed with paraffin wax and each egg was dated and labeled. For each set of the tested compound, five eggs were used per specimen (compound + virus), and three sets of controls were employed [egg control (eggs without any inoculums) acting as a negative control, solvent control (DMSO + virus) and virus control (virus only) acting as a positive control. The eggs were then incubated at 37 ºC and 65-70% humidity for 72 and 96 h for IBDV and NDV, respectively.

The eggs were candled after every 24 h to monitor embryonic development and survival. The egg shells were then opened after 72 or 96 h of incubation to record mortality, survival, embryo size, weight, and organ formation. The specimen treated embryos were then compared and contrasted with the control sets in relation to survival, embryo size, weight, and completeness in tissue/organ formation. Furthermore, InStat 3 statistical package was used to compare the logarithm of the mean weight of the egg control (negative control) with those of the untreated virus-infected-embryos (positive control) and the treated embryos (compound + virus).

Viral haemagglutination inhibition test

In case of NDV assay, the allantoic fluid from the sample treated specimens and control eggs were further harvested to determine viral infectivity. The haemagglutination inhibition test was carried out as previously described by Allan and Gough (1974). A phosphate buffer solution (PBS, 25 µL) was placed in each well of the microtitre plate. Then allantoic fluids (25 µL each) from negative and positive controls and treated specimens (virus + tested compound) were added to the 1st well of column 1, and each thoroughly mixed. A two fold serial dilution was done by transferring 25 µl of the suspension to the next well and the last one discarded. The 25 µl of 1% chicken RBCs in 99% PBS was added to each

well of the microtitre plate. The microtitre plate was shaken, covered and incubated at room temperature for about 30 min. The results were observed to see whether there was haemagglutination or not, and the lowest titre values were recorded at the end point beyond which there was no further haemagglutination.

IBDV being inactive in haemaglutination inhibition test and having no other test kits available at our exposal to quantitate IBDV antibodies during the investigation reported hereby, no attempt was made to establish the presence of IBDV following infection of the embryos.

RESULTS

Isolation and identification of compounds

Isolation and structural elucidation for compounds **1-7** (Figure 1) from *T. orientalis* is reported in our recent papers (Samwel et al., 2011; Nyandoro et al., 2012). Repeated chromatography of the leaf ethanol extract of *Toussaintia patriciae* yielded ursolic acid (**8**) (Budzikiewicz et al., 1963; Seebacher et al., 2003), its hydroxyl derivatives 30-hydroxyursolic acid (**9**) (Budzikiewicz et al., 1963; Talapatra et al., 1981; Woo et al., 1984) and 19α,23-dihydroxyursolic acid (**10**) (Budzikiewicz et al., 1963; Soares et al., 1998), together with ethyl gallate (ethyl 3,4,5-trihydroxybenzoate, **11**) (Ooshiro et al., 2009) (Figure 2). Structures of the isolated compounds were established based on analysis of both 1 & 2D spectroscopic as well as spectrometric data, which were consistent with those reported in the literature.

In ovo antivirial activity

When evaluated for the *in ovo* antiviral activity at a concentration of 360 µg/ml, the compounds demonstrated effective control of viral virulence at different levels of significance, with the nitrogenous compounds and and the glycosylflavonoid **7** isolated from *T. orientalis* exhibiting significant antiviral activity. These compounds effectively controlled the IBDV activity and enabled the embryo to grow to normal size with complete organ formation (Table 1). The mean weights of chicken embryos treated with each of these compounds showed no significance difference when compared with the mean weights of uninfected embryos (Table 2). However, compounds **3**, **5** and **6**, also isolated from *T. orientalis* showed insignificant activity against IBDV, with compound **6** being the least active. Furthermore, compounds **1-7** demonstrated antiviral activity against NDV by enabling the treated chicken embryos to grow to normal size and to complete organ formation (Table 3). However, the mean weights of embryos treated with each of these compounds against NDV were either very or highly significant compared to uninfected ones, indicating that the treatment was less effective (Table 4).

The ursane-type triterpenoids **8-10** as well as ethyl gallate (**11**) isolated from *T. patriciae* also showed high antiviral potency against IBDV. Thus, while the untreated chicken embryos died within 24 h of inoculation, the embryos treated with compounds 8-11 survived, and grew to normal size with complete organ formation similar to the uninfected embryos (Table 5). Compounds 9 and 10 were particularly effective against IBDV, with the mean weights being comparable to those of uninfected embryos, indicating effectiveness of the treatment (Table 6). Although compounds **8-11** also exhibited antiviral activity against NDV by enabling the treated chicken embryos to grow to normal size and to complete organ formation (Table 7), the difference in the mean weights of embryos treated with these compounds compared to the uninfected ones ranged from very to highly significant (Table 8), which meant that the treatment was less effective against this virus.

The haemagglutination inhibition test for NDV was used as an alternative method to evaluate the antiviral effect of the isolated compounds by generating viral titre values, which showed the extent to which the virus

was cleared after treatment. The results from the haemagglutination test as were observed in the microtitre plate assays are represented in Table 9. Thus, on the basis of viral titre reduction, ethyl gallate (**11)** had the lowest titre value (1:16) followed by glycosylflavonoid **7** (1:32), while the other compounds had viral titre values of 1:128 and 1:256. These values were by far lower (128, 64, 16, or 8 times, respectively) than for the positive control (1:2048), indicating effectiveness of the compounds against NDV.

Table 1: Antiviral activity (at 360 µg/ml) of compounds from *Toussaintia orientalis* against IBDV.

	Egg 1	**Egg 2**	**Egg 3**	**Egg 4**	**Egg 5**	**Embryo size**	**Embryo average weight (g)**	**Embryo organ formation**
Negative control (Eggs without any inoculums)	Alive	Alive	Alive	Alive	Alive	Normal	7.626	Complete
Solvent control (Virus +DMSO)	Dead	Dead	Dead	Dead	Dead	Abnormal	1.670	Incomplete
Positive control (Virus alone)	Dead	Dead	Dead	Dead	Dead	Abnormal	1.647	Incomplete
Virus + **1**	Alive	Alive	Alive	Alive	Alive	Normal	6.939	Complete
Virus + **2**	Alive	Alive	Alive	Alive	Dead	Normal	6.921	Complete
Virus + **3**	Alive	Alive	Alive	Alive	Alive	Normal	7.309	Complete
Virus + **4**	Alive	Alive	Alive	Dead	Alive	Normal	6.908	Complete
Virus + **5**	Alive	Alive	Alive	Alive	Alive	Normal	6.564	Complete
Virus + **6**	Dead	Dead	Dead	Dead	Dead	Abnormal	2.464	Incomplete
Virus + **7**	Alive	Alive	Alive	Alive	Alive	Normal	7.720	Complete

Table 2: Comparison of the logarithm of the mean weight of untreated (infected with IBDV), treated (with compounds from *Toussaintia orientalis*) against uninfected embryos (Mean ± SEM = 0.882 ± 0.004).

Treatment	**Mean ± SEM**	**T-value**	**P-value**	**95 % CL**	**Comment**
IBDV only	0.215 ± 0.020	32.512	< 0.0001	0.159-0.270	Highly significant
Virus + **1**	0.837 ± 0.033	1.386	0.1015	0.745-0.927	Not significant
Virus + **2**	0.837 ± 0.026	1.679	0.0659	0.076-0.911	Not significant
Virus + **3**	0.845 ± 0.011	3.079	0.0076	0.814-0.876	Very significant
Virus + **4**	0.862 ± 0.022	0.8993	0.1974	0.799-0.924	Not significant
Virus + **5**	0.813 ± 0.003	12.565	< 0.0001	0.805-0.823	Highly significant
Virus + **6**	0.349 ± 0.098	5.436	0.0003	0.077-0.620	Highly significant
Virus + **7**	0.887 ± 0.008	0.5415	0.3014	0.864-0.911	Not significant

Note: Level of significance, $\alpha = 0.05$ [Not significant ($p \geq 0.05$), significant ($p < 0.05$), very significant ($p < 0.01$), highly significant ($p < 0.001$)].

Table 3: Antiviral activity (at 360 µg/ml) of compounds from *Toussaintia orientalis* against NDV.

	Egg 1	Egg 2	Egg 3	Egg 4	Egg 5	Embryo size	Embryo average weight (g)	Embryo organ formation
Negative control (Eggs without any inoculums)	Alive	Alive	Alive	Alive	Alive	Normal	9.438	Complete
Positive control (Virus +DMSO)	Dead	Dead	Dead	Dead	Dead	Abnormal	3.837	Incomplete
Positive control (Virus alone)	Dead	Dead	Dead	Dead	Dead	Abnormal	3.204	Incomplete
Virus + **1**	Alive	Alive	Alive	Alive	Alive	Normal	6.206	Complete
Virus + **2**	Alive	Alive	Alive	Alive	Alive	Normal	6.734	Complete
Virus + **3**	Alive	Alive	Alive	Alive	Alive	Normal	6.656	Complete
Virus + **4**	Alive	Alive	Alive	Alive	Alive	Normal	6.372	Complete
Virus +**5**	Alive	Alive	Alive	Alive	Alive	Normal	6.227	Complete
Virus + **6**	Alive	Alive	Alive	Alive	Alive	medium	5.000	Complete
Virus + **7**	Alive	Alive	Alive	Alive	Alive	Normal	7.374	Complete

Table 4: Comparison of the logarithm of the mean weight of untreated (infected with NDV), treated (with compounds from *Toussaintia orientalis*) against uninfected embryos (Mean ± SEM = 0.972 ± 0.026).

Treatment	Mean ± SEM	T-value	P-value	95 % CL	Comment
NDV only	0.505 ± 0.015	16.171	< 0.0001	0.463-0.546	Highly significant
Virus + **1**	0.792± 0.015	6.003	0.0002	0.754-0.833	Highly significant
Virus + **2**	0.827 ± 0.013	4.965	0.0006	0.792-0.863	Highly significant
Virus + **3**	0.804 ± 0.011	5.919	0.0002	0.772-0.836	Highly significant
Virus + **4**	0.822 ± 0.013	5.069	0.0005	0.784-0.861	Highly significant
Virus + **5**	0.794 ± 0.007	6.611	< 0.0001	0.805-0.823	Highly significant
Virus + **6**	0.699 ± 0.005	10.250	< 0.0001	0.684-0.714	Highly significant
Virus + **7**	0.868 ± 0.005	3.934	0.0022	0.855-0.881	Very significant

Table 5: Antiviral activity (at 360 µg/ml) of compounds from *Toussaintia Patriciae* against IBDV.

	Egg 1	Egg 2	Egg 3	Egg 4	Egg 5	Embryo size	Embryo average weight (g)	Embryo organ formation
Virus + **8**	Alive	Alive	Dead	Alive	Alive	Normal	8.418	Complete
Virus + **9**	Alive	Alive	Alive	Alive	Alive	Normal	7.971	Complete
Virus + **10**	Dead	Alive	Alive	Alive	Alive	Normal	7.159	Complete
Virus +**11**	Alive	Alive	Alive	Alive	Alive	Normal	8.218	Complete

Table 6: Comparison of the logarithm of the mean weight of untreated (infected with IBDV), treated (with compounds from *Toussaintia patriciae*) against uninfected embryos (Mean ± SEM = 0.882 ± 0.004).

Treatment	Mean ± SEM	T-value	P-value	95 % CL	Comment
IBDV only	0.215 ± 0.020	30.618	< 0.0001	0.159-0.270	Highly significant
Virus + **8**	0.924 ± 0.015	2.772	0.0121	0.884-0.965	Significant
Virus + **9**	0.900 ± 0.014	1.229	0.1270	0.861-0.960	Not significant
Virus + **10**	0.924 ± 0.018	1.562	0.0784	0.803-0.903	Not significant
Virus + **11**	0.914 ± 0.013	2.273	0.0263	0.877-0.951	Significant

Table 7: Antiviral activity (at 360 µg/ml) of compounds from *Toussaintia patriciae* against NDV.

	Egg 1	Egg 2	Egg 3	Egg 4	Egg 5	Embryo size	Embryo average weight (g)	Embryo organ formation
Virus + **8**	Alive	Alive	Dead	Alive	Alive	Normal	7.004	Complete
Virus + **9**	Alive	Alive	Alive	Alive	Alive	Normal	5.926	Complete
Virus + **10**	Alive	Alive	Alive	Alive	Alive	Normal	7.075	Complete
Virus + **11**	Alive	Alive	Alive	Alive	Alive	Normal	7.518	Complete

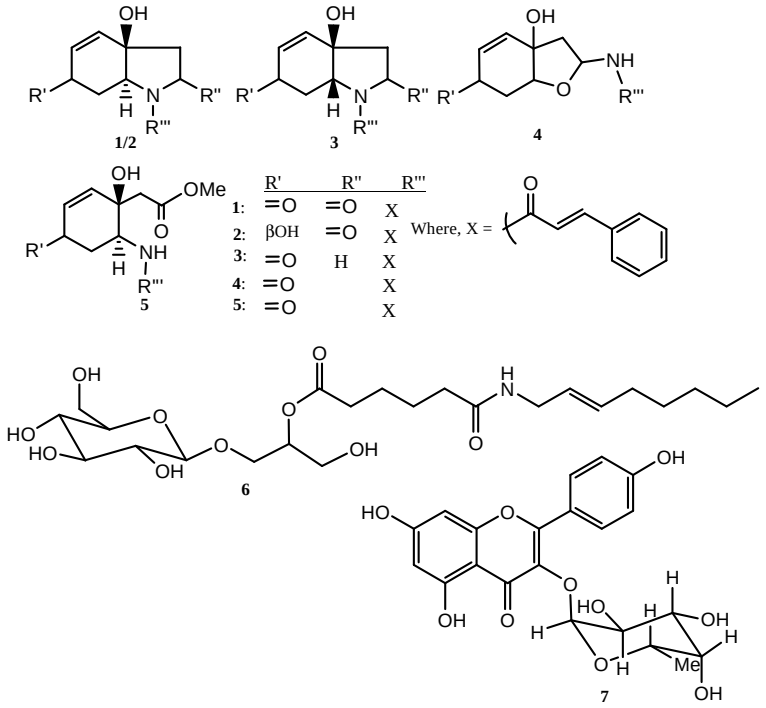
Note: Control results the same as for **Table 3**

Table 8: Comparison of the logarithm of the mean weight of untreated (infected with NDV), treated (with compounds from *Toussaintia patriciae*) against uninfected embryos (Mean ± SEM = 0.826 ± 0.013).

Treatment	Mean ± SEM	T-value	P-value	95 % CL	Comment
NDV only	0.505 ± 0.015	15.573	< 0.0001	0.463-0.546	Highly significant
Virus + **8**	0.846± 0.003	4.831	0.0007	0.855-0.852	Highly significant
Virus + **9**	0.827 ± 0.013	6.299	0.0001	0.720-0.822	Highly significant
Virus + **10**	0.849 ± 0.003	4.662	0.0008	0.843-0.857	Highly significant
Virus + **11**	0.876 ± 0.004	3.622	0.0034	0.864-0.888	Very significant

Table 9: NDV Haemagglutination assay results.

Specimen	+ve/-ve	Well #	Titre	Specimen	+ve/-ve	Well #	Titre
Negative control (Eggs without any inoculums)	-ve	-ve	-ve	Virus + **5**	+ve	8	1:256
Positive control (NDV alone)	+ve	11	1:2048	Virus + **6**	+ve	8	1:256
Solvent control (NDV +DMSO)	+ve	10	1:1024	Virus + **7**	+ve	5	1:32
Virus + **1**	+ve	8	1:256	Virus + **8**	+ve	7	1:128
Virus + **2**	+ve	8	1:256	Virus + **9**	+ve	8	1:256
Virus + **3**	+ve	8	1:256	Virus+ **10**	+ve	7	1:128
Virus + **4**	+ve	8	1:256	Virus + **11**	+ve	4	1:16



	R'	R''	R'''
1:	=O	=O	X
2:	βOH	=O	X
3:	=O	H	X
4:	=O		X
5:	=O		X

Figure 1: Compounds isolated from *Toussaintia orientalis* (**1–7**).

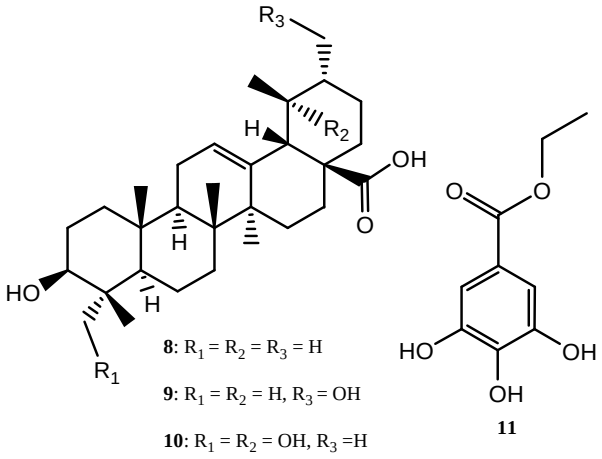


Figure 2: Compounds isolated from *Toussaintiapatriciae* (**8–11**).

DISCUSSION

The results of the antiviral assays as presented in these investigations have demonstrated the compounds isolated from *Toussantia orientalis* and *T. patriciae* to have activity against the two tested avian viruses, albeit with unequal efficacy. Whereas the tetraketide nitrogenous compounds **1**, **2** and **4**

and the glycosylflavonoid **7** isolated from *T. orientalis*, and ursolic acid derivatives **9** and **10** obtained from *T. patriciae* exhibited significant activity against IBDV, the other compounds showed moderate or weak activity. Generally, the tested compounds were more potent to IBDV than NDV. The observed differences in activity against the two assayed viruses and between the compounds tested could probably be attributed to structural differences hence the corresponding different modes of action of the compounds against the tested viruses. Thus, the compounds could have virucidal, inhibitory or combinations of both effects.

The nitrogenous compounds with nitrogen sandwiched between two carbonyl groups as in **1** and **2**, or with electronegative oxygen and a carbonyl group as in **3** and **4** showed better activity than **5** that lacks a heterocyclic atom-containing fused ring. Such a structural-activity trend could be attributed to the variation in the degree of electrophilicity of the *N*-atom adjacent to electron withdrawing species (carbonyl group and oxygen), making the corresponding compounds to react at different rates with nucleophilic targets in the viral protein coat or nucleoside.

The triterpenoids **8** - **10** have some similarities in their structural features; that is, each has a hydroxyl group at C-3, a C_{12}-C_{13} double bond and a carboxylic acid group at C-17. The compounds differ only in the extra hydroxylation at C-30 for 9, C-19 and C-23 for 10. Thus, the higher antiviral efficacy of compounds **9** and **10** as compared to **8** could have been due to the extra hydroxylation in the first two compounds. In fact, ursolic acid (**8**) and its derivatives similar to **9** and **10** are well known for their antiviral properties, which in most cases has been associated with the presence of an *oxo*- and hydroxyl-functionalities (Sun et al., 2006), as further corroborated in the present studies for the tested avian viral strains. On the other hand, alkyl gallates such as **11** are also known for their virucidal and inhibitory effects against viruses, particularly Herpes Simplex Virus (HSV). The previous structure activity relationship (SAR) studies of *n*-alkyl gallates indicated the three hydroxyl units and the alkyl group to play a role in the antiviral potency of such compounds (Kratz et al., 2008). Therefore, the same structural features could have also attributed to the observed antiviral activity of compound **11** reported in these investigations.

Although the viral load reduction and virulence control was evident as indicated by titre values (for NDV) and embryo survival, normal size, complete organ formation, and mean weights of chicken embryos treated with some compounds showing no significance difference as compared with uninfected embryos, the mode of action, elucidation of the exact target sites of the active compounds and their toxicity remains a subject of future research. Nevertheless, the *in ovo* antiviral results hereby reported indicate that the two *Toussaintia* species accumulate metabolites that are potential leads for the development of antiviral agents against IBDV and NDV. The results further underscore the need for the concerted efforts to conserve these bio-resources vulnerable to extinction.

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