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RESEARCH ARTICLE

Anti-Newcastle Disease Virus activity of 3β and 3α Friedelanol Triterpenoids from the leaves of Synadenium glaucescens Pax

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ABSTRACT

Newcastle Disease (ND) is a highly pathogenic disease of avian species which is caused by Newcastle Disease Virus (NDV). It is one of the major causes of mortality and morbidity to poultry industry in the third world countries. Currently, there is no treatment measures against ND; the only existing measure is vaccination, though it is incapable to offer 100% immunity. In Tanzania, the leaves of *Synadenium glaucescens* Pax. are traditionally used for treatment of various ailments including ND. Previously, its leaves extract has been scientifically confirmed to exhibit anti-NDV activity though bioactive compound(s) responsible for this activity is/are unknown. Therefore, this study was aimed to evaluate anti-NDV activity of 3β-Friedelanol (1) and 3α-friedelanol (2) isolated from its leaves extract. Isolation of these compounds was achieved by column chromatography method whereas, their chemical structures were determined by Nuclear Magnetic Resonance (NMR) data and by comparing with the available literature NMR data. Anti-NDV activity study was done in embryonated chicken eggs (ECEs). Treatment of NDV inoculated ECEs with 3β-Friedelanol (1) reduced the viral load to zero and maintained the survival of embryos, this was revealed by continuous organs formation and increase in embryo weights with no significant different ($p > 0.05$) from un-inoculated ECE. These effects suggest that, 3β-Friedelanol (1) possesses anti-NDV activity. Therefore, existence of 3β-Friedelanol (1) in the leaves of *S. glaucescens* may justify its earlier described anti-NDV activity and traditional use in the treatment of ND. Hence, its leaves extract may be considered for development of anti-NDV herbal formulation while 3β-Friedelanol could either serve as a drug or lead compound for synthesis of anti-NDV drugs.

Keywords: Antiviral activity; bioactive compounds; friedelane triterpenoids; *in ovo* assay; poultry viral diseases.

INTRODUCTION

Newcastle Disease (ND) is a pathogenic disease in avian species which is caused by Newcastle Disease Virus (NDV). Newcastle Disease Virus is a pleomorphic single stranded and negative strain ribonucleic acid (-ssRNA) genomic enveloped virus under the family Paramyxoviridae (Ganar *et al*., 2014). Newcastle Disease is among of the serious challenges which hinders the development of poultry industry in developing countries including Tanzania (Mabiki *et al*., 2013c). It is a serious danger due to its high morbidity and mortality rates which are said to be up to 100% in naive or poorly vaccinated chickens (Absalón *et al*., 2019). Currently, there is no treatment measures against this disease; the only control measure currently available against it, is vaccination. Apart from its limited accessibility in the third world countries, vaccine is said to be incapable to offer 100% immunity (Ganar *et al*., 2014). Therefore, there is an urgent need to search for more effective and affordable alternative measures. Some medicinal plants have been used as the alternative for treatment of ND in various third world countries (Lagu & Kayanja, 2010; Al-Hadid, 2016). Medicinal plants produce bioactive compounds which are responsible for their therapeutical potentials (Barbieri *et al*., 2017; Credo *et al*., 2018). Several medicinal plants have been scientifically confirmed to have anti-NDV activity (Lagu & Kayanja, 2010; Sulaiman *et al*., 2011; Bakari *et al*., 2012; Raza *et al*., 2015; Al-Hadid, 2016; Ashraf *et al*., 2018; Doostmohammadian *et al*., 2020; Shahzad *et al*., 2020) though their bioactive compounds responsible for it, are not mentioned.

Synadenium glaucescens Pax (Family Euphorbiaceae) is a useful medicinal plant which is used traditionally for treatment of numerous diseases including ND in Njombe region - Tanzania (Mabiki *et al*., 2013a, 2013b, 2013c). The crude extracts from its leaves, roots and stem barks have been scientifically confirmed to have anti-NDV

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activity (Mabiki *et al*., 2013c). However, the bioactive compound(s) responsible for its anti-NDV activity is/are unknown. However, previous phytochemical screening of its crude extracts have shown the presence of terpenoids, steroids, tannins, coumarins, glycosides and phenolic compounds (Mabiki *et al*., 2013a). The only existing information about this plant is the isolation of euphol (3) shown in Figure 3 and β-sitosterol (4) shown in Figure 4 from its root barks and leaves respectively (Nyigo *et al*., 2016). So far, euphol and β-sitosterol have been not mentioned to have anti-NDV activity.

Nevertheless, previous studies of euphol isolated from other plant species of the genus Synadenium and other genera of the same family (Euphorbiaceae) have shown anti-inflammatory, anti-tumor, anti-nociceptive and anticancer effects (Nyigo *et al*., 2016). Furthermore, β-sitosterol previously isolated from other plant species have exhibited hypercholesterolemia, immunomodulation, anticancer, anti-rheumatoid arthritis, anti-tuberculosis, anti-hair loss and anti-benign prostatic hyperplasia (Saeidnia *et al*., 2014).

Therefore, in order to reveal whether the current isolated bioactive compounds exhibit activity against NDV or not, the purpose of this study was to investigate anti-NDV activity of 3β-Friedelanol (1) and 3α-friedelanol (2) isolated from the leaves of *S. glaucescens*.

MATERIALS AND METHODS

Plant authentication, collection and preparation

Plant authentication was done by Mr. Haji O. Seleman (a botanist) from the Department of Botany, University of Dar es Salaam (UDSM). Plant leaves were collected from Njombe district (08°34′ to 08°49′ S and 034°55′ to 035°10′ E), Njombe region -Tanzania in December 2018. The voucher specimen (voucher no. 3672) was stored in the herbarium of the Department of Botany- UDSM. The leaves were air dried in an open space under the shade at room temperature. The dried leaves were then pulverized into fine powder by using a milling machine type Y (Hangyu®, China).

Chemicals

All chemicals used in this study were of analytical grade. They were obtained from either Loba Chemie, Mumbai-India i.e. Dichloromethane (DCM) and Petroleum ether (PE) or Finar Chemical, Gujarat-India i.e. Methanol (MeOH). Moreover, silica gel 60 (70-230 mesh, 60 angstrom pore size) and Thin Layer Chromatography Aluminium sheets (TLC, silica gel 60 F_{254}) were obtained from Merck KGaA group, Darmstadt, Germany.

Extraction of plant leaves

About 1 kg of leaves powder was extracted exhaustively with 100% dichloromethane (DCM) by Soxhlet method (Azwanida, 2015). The filtrate obtained was evaporated using a rotary evaporator (Büchi Labortechnik, Flawil, Switzerland) at 40°C.

Isolation and purification of compounds

The leaves extract was initially analyzed by using TLC to establish the solvent system for use in the isolation process. Then, 40 g of extract was subjected to isolation process through column chromatography (CC) method over silica gel. Then, gradient elution by using several solvent systems of varying polarities was conducted. One hundred seventy fractions (F1-170), each of 50 ml were collected. Basing on the TLC profiles, the fractions collected were combined together to give twelve (12) combinations: F1-12, F13-21, F22-30, F31-38, F39-65, F66-74, F75-80, F81-101, F102-143, F144-151, F152-165 and F166-170 as shown in Figure 6. The eighth (F81-101) and ninth (F102-143) combinations were observed to form white precipitates. The precipitates were filtered, washed with 100% MeOH and dried to give white powdered pure compound 1 and 2 which shown single spot on TLC. The other combinations were stored for later further purification.

Identification of compounds

The structures of pure compounds were determined by using ^{1}H NMR and ^{13}C NMR spectroscopic data and by comparing with the available literature NMR data. The NMR data were recorded on a 600 Megahertz Bruker Avance III HD 600 (UltraShield™) NMR spectrometer. The chemical shifts of the data (δ) were expressed relative to the standard, tetramethylsilane (TMS) in parts per million (ppm). Acquired data were processed by using Topspin software version 3.6.3.

Anti-Newcastle Disease Virus evaluation

Embryonated Chicken Eggs (ECEs)

The 9 days old, ECEs from the chickens of the same breed were obtained from the local supplier in Morogoro- Tanzania.

Maximum non-toxic concentration of isolated compounds in ECEs

The maximum non-toxic concentration of isolated compounds in ECEs was determined. The compounds were diluted in 80% dimethylsulphoxide (DMSO) at the desired concentrations of 0.1, 0.2, 0.3 and 0.4 mg/ml. Then 0.1 ml of each compound dilution was inoculated into allantoic cavity of six ECEs. The eggs were incubated for 4 days with daily monitoring. Six ECEs injected only with 0.1 ml of solvent were used as negative control. The highest concentration (0.2 mg/ml) which caused no embryo death after incubation was considered as the maximum non-toxic concentration and was selected for later experiments.

Newcastle Disease Virus

The virulent NDV was obtained in the Bacteriology and Mycology Laboratory, Department of Veterinary Microbiology and Parasitology, Sokoine University of Agriculture. The virus was propagated in ECEs by inoculating via the allantoic route.

The suitable dilution of NDV for inoculation to ECEs in the experiments was initially determined, 10-fold dilutions (10^{-1} to 10^{-10}) were made from the virus stock and 0.1 ml of each dilution was injected to allantoic cavity of six, 9 days old ECEs according to the previous described procedure (Young *et al*., 2012) with some minor modifications. The lowest dilution of the virus with haemagglutination (HA) positive activity in 50% of infected ECEs was 10^{-5}.

Then, calculation of the starting titre of NDV used in the inoculation as the embryo infectious dose (EID_{50}) of the virus was done according to the mathematical technique invented by Reed and Muench (1938) as previous described (Young *et al*., 2012). Hence, the calculated starting titre of NDV used in the inoculation of ECEs in this study was $10^{5.3}$ EID_{50} per 0.1 ml.

Experimental design

With some minor modifications*, in ovo* assay was done to evaluate anti-NDV activity of 3β-Friedelanol (1) and 3α-Friedelanol (2) as per previous procedure (Bakari *et al*., 2012; Mabiki *et al*., 2013c).

In brief, 9 days old ECEs were initially observed for viability by candling. Then compound 1 and 2 were prepared in 80% DMSO at a concentration of 0.2 mg/ml. The ECEs were randomized into 5 groups (n = 6) in each received the following treatment:

Group 1: Negative control (un-inoculated eggs)
Group 2: Positive control (inoculated with NDV suspension only)
Group 3: Positive control (inoculated with NDV + 80% DMSO)
Group 4: Inoculated with NDV + 80% DMSO + 3β -Friedelanol (1)
Group 5: Inoculated with NDV + 80% DMSO + 3α -Friedelanol (2)

The inocula were made by mixing 0.1 ml virus suspension and 0.9 ml of either 3β-Friedelanol (1) or 3α-Friedelanol (2). Then, a hole was made through the eggshell above the air sac to allow vertical inoculation of 0.1 ml of the inoculum. After inoculation, inoculated location was sealed with paraffin wax. Then, the eggs were

incubated at 37°C for 4 days with the air sac uppermost position. Embryo survivals were checked daily for 4 days through candling of eggs. The observations were made based on embryo movements, blood vessels, embryo weight and time of embryo death. The dead embryos were removed followed by harvesting allantoic fluid for assessment of viral titres. At day 5, allantoic fluid from the surviving embryos was harvested to analyze virus titres. Evaluation of antiviral activity was based on embryo survival, mean embryo weight and viral load in the allantoic fluid during harvesting.

The mean embryo weight (MEW) at each group was determined by using the equation:

MEW = total weights of all embryos harvested from treated ECEs/ number of treated ECEs

Haemagglutination test of the allantoic fluid

Haemagglutination (HA) test of the allantoic fluid to quantify the amount of NDV was done using by two fold dilutions in a V-shaped 96 well microtitre plates as follows:

Sterile, 96-well polystyrene microtitre plates were first preloaded with 25 µL phosphate buffered saline (PBS) in each well followed by addition of 25 µL of allantoic fluid into the first well of each row to make a total volume of 50 µL. To the first well, the PBS and allantoic fluid were mixed and 25 µL was drawn from each well and transferred to the subsequent wells to the last wells. Then, 25 µL of the mixture from the last well was discarded. Thereafter, 25 µL of the 1% chicken red blood cells were added to each well followed by allowing the plates to stand for 45 min at room temperature (28°C). Then, the endpoint was recorded for interpretation of the HA titre as per previous procedure (Mabiki *et al*., 2013c).

In brief, wells with a sharp button of red blood cells at the bottom were considered HA negative, whereas the wells with a hazy film of red blood cells or without button at the bottom of the V-bottom well were considered to be HA positive. The endpoints for HA were considered to be virus inhibition titres.

Statistical analysis

The results of mean embryo weights were expressed as mean ± Standard Error of the Mean (SEM). Statistical analysis of results was carried out using Microsoft Excel statistical package (2016). The different in mean embryo weights among the groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons at probability value, $p < 0.05$. The endpoints for HA tests were considered to be virus titres.

RESULTS

Isolation of compounds

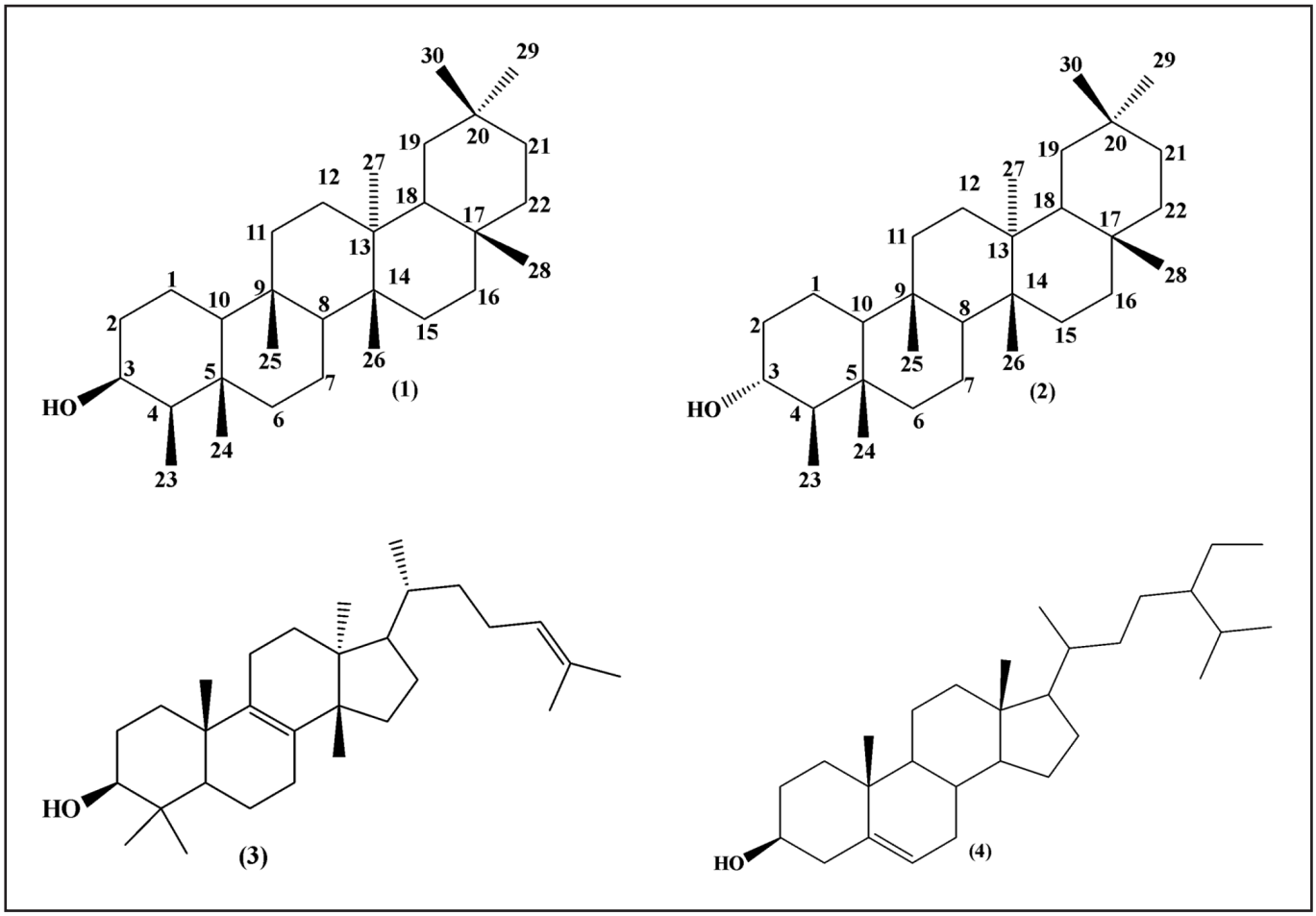
The compound 1 and 2 were isolated as white powder from the leaves extract of *S. glaucescens* with weights of 57 and 194 mg respectively. All compounds were tested negative under Ultraviolet lamp (UV-lamp).

Structure determination of compounds

Based on experimental NMR data and comparison with other published spectroscopic data, chemical structures of compounds **1** and **2** were determined as follows:

Compound 1

Based on the obtained ^{13}C NMR and ^{1}H NMR data and by comparing with the literature data (Chang *et al*., 2012; Salazar *et al*., 2000), the structure of compound 1 was revealed to be in consistent with the skeleton of friedelane triterpenoid and it was identified as 3β-Friedelanol as shown in Figure 1.



Figures 1-4. Structures of 3β -Friedelanol (1), 3α -Friedelanol (2), Euphol (3) and β-sitosterol (4).

The ^{13}C NMR spectra of compound 1 indicated 30 carbon signals at chemical shifts, δ_C (ppm) as follows: 16.4 (C-1), 35.8 (C-2), 73.1 (C-3), 49.8 (C-4), 37.7 (C-5), 42.3 (C-6), 18.1 (C-7), 53.7 (C-8), 38.9 (C-9), 61.9 (C-10), 35.9 (C-11), 30.5 (C-12), 38.4 (C-13), 40.2 (C-14), 32.9 (C-15), 36.7 (C-16), 30.3 (C-17), 43.4 (C-18), 36.1 (C-19), 28.6 (C-20), 33.4 (C-21), 39.8 (C-22), 11.9 (C-23), 16.8 (C-24), 18.6 (C-25), 19.0 (C-26), 20.5 (C-27), 32.1 (C-28), 35.3 (C-29) and 32.4 (C-30).

The ^{1}H NMR spectra showed the presence of seven methyl singlets at δ 0.95 (3H, *s*, C-24), 0.86 (3H, *s*, C-25), 1.02 (3H, *s*, C-26), 0.99 (3H, *s*, C-27), 0.99 (3H, *s*, C-28), 0.94 (3H, *s*, C-29) and 1.18 (3H, *s*, C-30); one methyl doublets that appeared at δ 0.92 (3H, *d*, *J* = 7.0 Hz, C-23) and the proton attached to carbon bearing hydroxyl group attachment (C-3) appeared as a broad (br) peak at δ 3.70 (1H, *br*, C-3).

The comparisons between experimental and literature ^{13}C NMR and ^{1}H NMR chemical shift values for carbons and protons of compound 1 are as shown in Table 1 and 2 respectively.

Compound 2

With the aid of experimental ^{13}C NMR and ^{1}H NMR data obtained and by comparing with the literature data (Chang *et al*., 2012; Salazar *et al*., 2000), the structure of compound **2** was also shown to be in consistent with the skeleton of friedelane triterpenoid and was identified as 3α-Friedelanol as shown in Figure 2.

The ^{13}C NMR spectra of compound 2 indicated 30 carbon signals at chemical shifts, δ_C (ppm) as follows: 19.8 (C-1), 36.9 (C-2), 72.4 (C-3), 53.4 (C-4), 37.3 (C-5), 41.6 (C-6), 18.1 (C-7), 53.2 (C-8), 38.5 (C-9), 60.3 (C-10), 35.6 (C-11), 30.2 (C-12), 39.9 (C-13), 38.3 (C-14), 32.6 (C-15), 35.8 (C-16), 29.9 (C-17), 43.0 (C-18), 36.3 (C-19), 28.4 (C-20), 32.3 (C-21), 39.5 (C-22), 10.1 (C-23), 14.8 (C-24), 18.4 (C-25), 20.4 (C-26), 18.9 (C-27), 30.8 (C-28), 32.0 (C-29) and 35.2 (C-30).

The ^{1}H NMR spectra indicated the presence of seven methyl singlets at δ 0.75 (3H, *s*, C-24), 0.79 (3H, *s*, C-25), 0.96 (3H, *s*, C-26), 0.99 (3H, *s*, C-27), 1.15 (3H, *s*, C-28), 0.97 (3H, *s*, C-29) and 0.92 (3H, *s*, C-30); one methyl doublets that appeared at δ 0.88 (3H, *d*, J = 6.6 Hz, C-23) and one proton attached to carbon bearing hydroxyl group attachment (C-3) appeared as doublet of triplets at δ 3.32 (1H, *dt*, J=10.8, 4.4 Hz, C-3).

The comparisons between experimental and literature ^{13}C NMR and ^{1}H NMR chemical shift values for carbons and protons of compound 2 are shown in Table 3 and 4 respectively.

Antiviral evaluation

Maximum non-toxic concentration of isolated compounds in ECEs

0.2 mg/ml was the highest concentration of compound **1** and **2** which did not interfere the normal growth of embryos in ECEs. This was revealed by continuous embryo growths in eggs, 4 days after inoculation. Therefore, 0.2 mg/ml was considered as the maximum non-toxic concentration and selected for later experiments.

Time for embryo deaths

Times of embryo deaths in NDV infected eggs are shown in Table 5. During the 4 days of observation, no embryo deaths were observed in group 1 and 4. However, one day after NDV inoculation, embryo deaths were observed in group 2, 3 and 5; by two days, the mortality rate in each of these groups was 100%.

Table 1. ^{13}C NMR chemical shifts (600 MHz, CD_2Cl_2, δ_C in ppm) for 3β-Friedelanol (1)

Position	Experimental δ	Literature δ (Salazar *et al*., 2000; Chang *et al*., 2012)	Position	Experimental δ	Literature δ (Salazar *et al*., 2000; Chang *et al*., 2012)
1	16.4	16.2	16	36.7	36.5
2	35.8	35.7	17	30.3	30.0
3	73.1	73.1	18	43.4	43.2
4	49.8	49.6	19	36.1	35.9
5	37.7	37.5	20	28.6	28.7
6	42.3	42.1	21	33.4	32.7
7	18.1	17.9	22	39.8	39.7
8	53.7	53.6	23	11.9	12.0
9	38.9	38.2	24	16.8	16.8
10	61.9	61.8	25	18.6	18.6
11	35.9	35.6	26	19.0	19.0
12	30.5	31.0	27	20.5	20.5
13	38.4	38.7	28	32.1	32.5
14	40.2	40.0	29	35.3	35.4
15	32.9	33.2	30	32.4	32.2

Table 2. ^{1}H NMR chemical shifts (600 MHz, CD_2Cl_2, δ_H in ppm) for 3β-Friedelanol (1)

Position	Splitting pattern, number of Hydrogen	Experimental δ	Literature δ (Salazar *et al*., 2000; Chang *et al*., 2012)
C-3	*br,* 1H	3.70	3.73
C-23	*d*, J=7.0 Hz, 3H	0.92	0.91
C-24	*s*, 3H	0.95	0.93
C-25	*s*, 3H	0.86	0.83
C-26	*s*, 3H	1.02	0.98
C-27	*s*, 3H	0.99	0.95
C-28	*s*, 3H	0.99	0.97
C-29	*s*, 3H	0.94	0.92
C-30	*s*, 3H	1.18	1.14

Table 3. ^{13}C NMR chemical shifts (600 MHz, $CDCl_3$, δ_C in ppm) for 3α-Friedelanol (2)

Position	Experimental δ	Literature δ (Salazar *et al*., 2000; Chang *et al*., 2012)	Position	Experimental δ	Literature δ (Salazar *et al*., 2000; Chang *et al*., 2012)
1	19.8	19.6	16	35.8	36.1
2	36.9	36.7	17	29.9	30.1
3	72.4	72.2	18	43.0	42.9
4	53.4	53.2	19	36.3	36.1
5	37.3	37.4	20	28.4	28.1
6	41.6	41.4	21	32.3	32.4
7	18.1	17.8	22	39.5	39.3
8	53.2	53.0	23	10.1	9.9
9	38.5	38.7	24	14.8	14.6
10	60.3	60.1	25	18.4	18.1
11	35.6	35.3	26	20.4	20.1
12	30.2	30.6	27	18.9	18.6
13	39.9	39.7	28	30.8	31.8
14	38.3	38.3	29	32.0	32.1
15	32.6	32.8	30	35.2	35.1

Table 4. ^{1}H NMR chemical shifts (600 MHz, $CDCl_3$, δ_H in ppm) for 3α-Friedelanol (2)

Position	Splitting pattern, number of Hydrogen	Experimental δ	Literature δ (Salazar *et al*., 2000; Chang *et al*., 2012)
C-3	*dt*, J=10.8, 4.4 Hz, 1H	3.32	3.41
C-23	*d*, J=6.6 Hz, 3H	0.88	1.01
C-24	*s*, 3H	0.75	0.78
C-25	*s*, 3H	0.79	0.81
C-26	*s*, 3H	0.96	0.98
C-27	*s*, 3H	0.99	1.01
C-28	*s*, 3H	1.15	1.18
C-29	*s*, 3H	0.97	0.97
C-30	*s*, 3H	0.92	1.17

Table 5. Time of embryo deaths after NDV inoculation of ECEs and incubation for 4 days

Treatment	Group	Time of embryo death (day)				
		0	1	2	3	4
Un-inoculated	Group 1	0	0	0	0	0
NDV only	Group 2	0	2	4		
NDV + 80% DMSO	Group 3	0	1	5		
NDV + 80% DMSO + 3β -Friedelanol (1)	Group 4	0	0	0	0	0
NDV + 80% DMSO + 3α -Friedelanol (2)	Group 5	0	2	4		

Mean embryo weights

The data about the effect of the test compounds on the weights of NDV infected embryos are given in Table 6. Weights of embryos from ECEs in group 2, 3 and 5 were measured at a time where death occurred while embryo weights in group 1 and 4 were measured at day 4.

The mean embryo weights in the positive control groups were significantly lower ($p < 0.05$) than that of the negative control. Group 4 treated with 3β-Friedelanol (1), the mean embryo weight, was not significantly different ($p > 0.05$) from that of the negative control. Nevertheless, group 5 treated with 3α-Friedelanol (2), a significant decrease ($p < 0.05$) in mean embryo weight compared to negative control was observed.

Haemagglutination inhibition titres

Haemagglutination (HA) titres of NDV infected chicken embryos after treatment with compound 1 and 2 are shown in Table 7. No viruses were detected in the allantoic fluids of NDV inoculated ECEs treated with 3β-Friedelanol (1). Thus, compound 1 was capable of reducing HA titre to zero. The HA titres of 16 were detected in both group 3 and 5. However, the highest HA titre of 32 was recorded in group 2.

Table 6. Mean embryo weights after NDV inoculation of ECEs and incubation for 4 days

Treatment	Group	Mean weight (M±SEM)	P - value
Un-inoculated	Group 1	6.6±0.27	
NDV only	Group 2	2.4±0.31	0.000002
NDV + 80% DMSO	Group 3	2.4±0.32	0.000002
NDV + 80% DMSO + 3β -Friedelanol (1)	Group 4	6.5±0.21	0.72
NDV + 80% DMSO + 3α -Friedelanol (2)	Group 5	2.3±0.23	0.00003

Table 7. Haemagglutination virus titres in ECEs after NDV inoculation and incubation for 4 days

Treatment	Group	HA virus titre
Un-inoculated	Group 1	0
NDV only	Group 2	32
NDV + 80% DMSO	Group 3	16
NDV + 80% DMSO + 3β -Friedelanol (1)	Group 4	0
NDV + 80% DMSO + 3α -Friedelanol (2)	Group 5	16

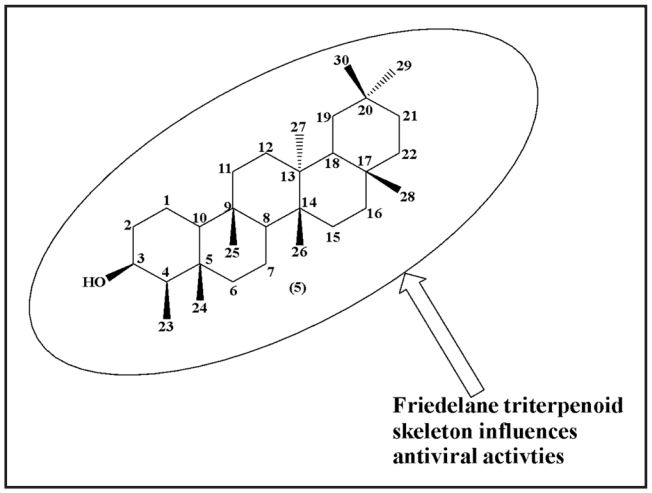


Figure 5. Friedelane triterpenoid skeleton.

DISCUSSION

Previous phytochemical screening of extracts from *S. glaucescens* have shown to contain Terpenoids, steroids, tannins, coumarins, glycosides and phenolic compounds (Mabiki *et al*., 2013a). So far, only euphol (3) and β-sitosterol (4) have been isolated from this plant (Nyigo *et al*., 2016). Therefore, the present study reports the isolation of 3β-Friedelanol (1) and 3α-Friedelanol (2) from the leaves of S. *glaucescens* for the first time. However, literature search revealed that these compounds have also been isolated from other plant species previously (Queiroga *et al*., 2000; Salazar *et al*., 2000; Van Kiem *et al*., 2004; Yang *et al*., 2010; Chang *et al*., 2012; Sharker *et al*., 2012; Mokoka *et al*., 2013; Islam *et al*., 2014; Nigussie, 2020).

Traditionally, *S. glaucescens* is used for treatment of asthma, HIV, TB, leprosy, sores, wounds and worms in humans and healing of east coast fever (ECF) in cattle and ND in poultry (Mabiki *et al*., 2013a, 2013b, 2013c). Its leaves extract have been previously confirmed to have anti-NDV activity (Mabiki *et al*., 2013c). However, it is hard to find information regarding the identification of bioactive compounds responsible for its anti-NDV activity.

Therefore, this study reports for the first time anti-NDV activity 3β-Friedelanol (1) isolated from the leaves of *S. glaucescens*.

The study of anti-NDV activity in ECEs was done based on embryo weights and the viral load. That is, a compound was considered active if it inhibited viral replication in the embryo cells by allowing embryo growth and if it reduced the viral load in the allantoic fluid of ECEs which prevents the death of embryo.

Invasion of NDV into embryo cell is aided by their functional spikes glycoprotein present on their envelope while their replication inside the cytoplasm of embryo cells is the mechanism of causing disease (Bakari *et al*., 2012; Mabiki *et al*., 2013c). Therefore, antiviral agents is expected to specific inhibit viral replication without affecting normal embryo cell growth (Mabiki *et al*., 2013c). In this study, 0.2 mg/ml was the maximum concentration of 3β-Friedelanol (1) and 3α-Friedelanol (2) which was not found to interfere the normal embryo cell growths.

The death of all embryos observed in group 2, 3 and 5 within 2 days after NDV inoculation clearly showed that, the virus was highly virulent. However, treatment of ECEs with 3β-Friedelanol (1) in group 4 maintained the survival of the embryos as shown in Table 5. The continuous growth of embryos in this group was revealed by organs formation and increase in body weight. The increase in body weight was not significant ($p > 0.05$) different from un-inoculated ECEs as shown in Table 6. This indicates that 3β-Friedelanol (1) could potentially affected the viral replication. Additionally, the results of HA test to quantify the amount of NDV in the allantoic fluids, treatment of ECEs with 3β-Friedelanol (1) in group 4 diminished the viral load to zero as shown in Table 7, this further suggests the compound holds anti-NDV activity.

Although 3β-Friedelanol (1) and 3α-Friedelanol (2) are epimeric friedelanol triterpenoids, the study observed the variation in activity against NDV; while 3β-Friedelanol (1) showed activity, 3α-Friedelanol (2) was not active.

In seeking to establish anti-NDV structure activity relationship (SAR) of 3β-Friedelanol (1); several studies on SAR of 3β-Friedelanol (1) and 3α-Friedelanol (2) against other single-strand ribonucleic acid (ssRNA) genomic enveloped viruses structurally similar with NDV have been conducted (Chang *et al*., 2012; Diniz *et al*., 2021).

From the studies above, similar variation in antiviral activity between the two compounds was observed, that is, compound 1 was active while compound 2 inactive. These studies discovered that friedelane triterpenoid skeleton (5) as shown in Figure 5 is the potential structural requirement for antiviral activity. Therefore, activity of 3β-Friedelanol (1) was favored by the friedelane skeleton, while 3α-Friedelanol (2) was affected by its inverted orientation at C-3 proton.

Basing on these matches of variation in antiviral activities of these compounds described above, it can also be reasonably suggested that, anti-NDV activity of 3β-Friedelanol (1) might be influenced by its friedelane skeleton while 3α-Friedelanol (2) could be affected its inverted orientation at C-3 proton. However, SAR study of these compounds against NDV may be done in the future.

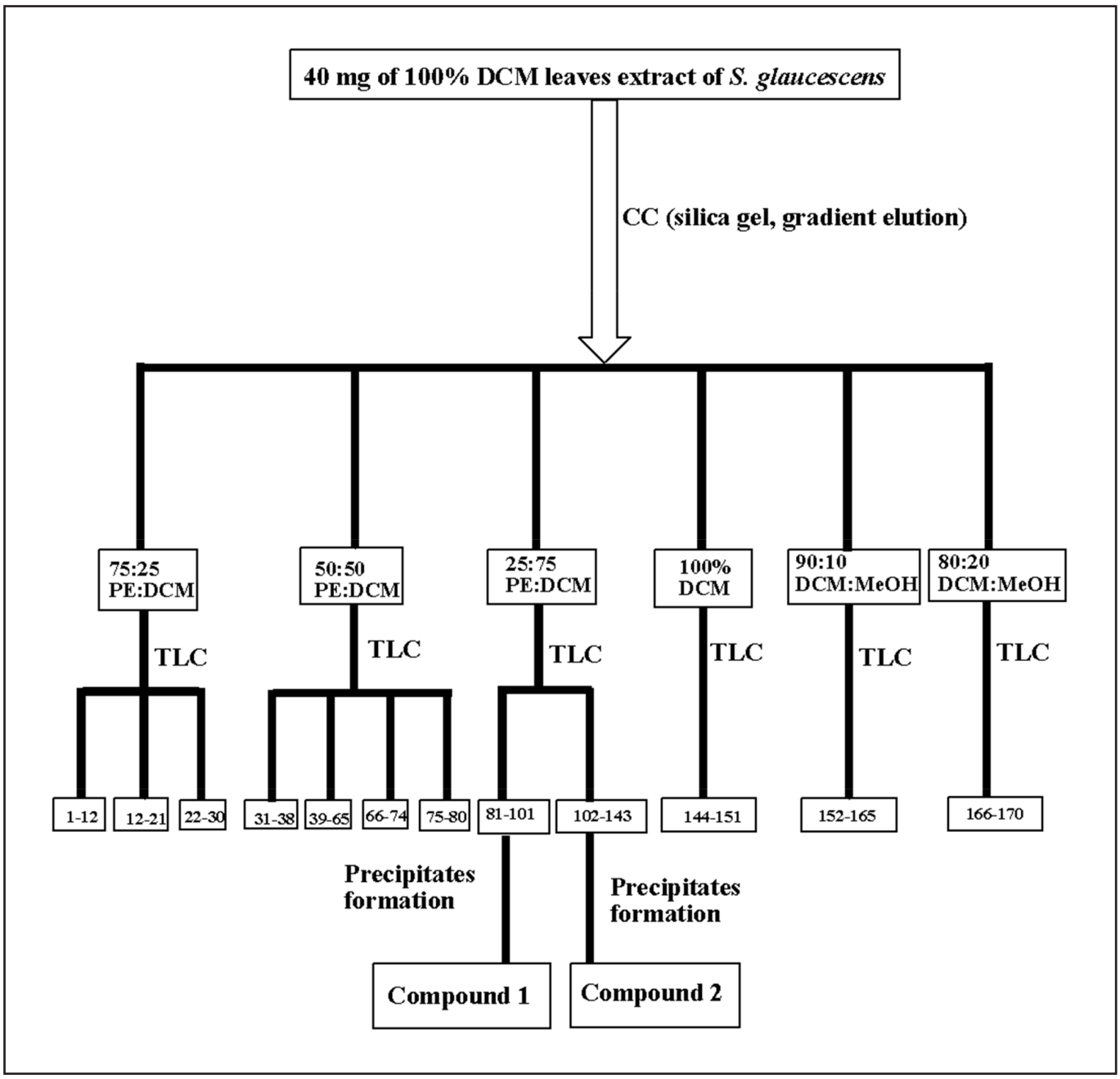


Figure 6. Scheme of isolation of compound 1 and 2 from 100% DCM leaves extract of *Synadenium glaucescens*.

In conclusion, presence of friedelane triterpenoid, 3β-Friedelanol (1) in the leaves of *S. glaucescens* can rationalize its earlier described anti-NDV activity and further confirms the claimed traditional use of the plant in the treatment of ND in poultry. Therefore, its leaves extract may be considered for development of anti-NDV herbal formulation. Additionally, 3β-Friedelanol could either serve as a drug or lead compound for synthesis of anti-NDV drugs. However, future studies on possible toxicity of these compounds are recommended.

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Declaration of conflict interest

The authors declare no conflict of interests.

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