Research Article

An *in ovo* investigation on antiviral activity of *Cannabis sativa* extracts against Newcastle Disease Virus (NDV)

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Abstract

Background: Newcastle disease is an acute, highly contagious and rapidly spreading viral disease of domestic poultry and other wild species of birds. The disease is caused by a single stranded, non-segmented, negative sense RNA virus, also known as Avian paramyxovirus 1 (APMV-1) and currently have no means of treatment except vaccination. **Objective**: Aim of present study was carried out to ascertain the antiviral activities of *Cannabis sativa* plant extracts against velogenic strain of Newcastle Disease Virus (NDV). **Material and methods:** Phytochemical study was done for the screening of various chemical constituents. Antiviral activity was assessed on a velogenic strain of Newcastle disease virus using embryonated chicken eggs assay. Toxicity study was carried out through determination of EID_{50}. **Results:** The findings of this study revealed the presence of bioactive compounds such as alkaloid, flavonoid, tannins, phenol, steroids, glycoside, saponins and triterpenoid in *Cannabis sativa* extracts. The findings on the toxicity of *Cannabis sativa* plant extracts showed that the extracts were relatively non-toxic. However, minute toxicity was observed in lower concentrations of 20 and 40mg/ml of petroleum ether seed, ethyl-acetate seed, ethyl-acetate leaves and in methanol seed extracts. Methanol seed and leaves extracts had the highest antiviral activity against NDV as embryo mortality was not observed during the study at various extract concentrations. There is significant antiviral activity of *Cannabis sativa* plant extracts against Newcastle disease virus ($p<0.05$). **Conclusion:** Thus, *Cannabis sativa* plant extracts are viable therapeutic agents that could serve as leads in the development of new pharmaceuticals research activities that brings about breakthrough in the treatment of NDV.

***Keywords*:** Newcastle disease, *Cannabis sativa*, phytochemicals, antiviral, poultry

Introduction

A central tenet underlying the use of plant preparations is that plants contain many bioactive phytochemical compounds used for myriad of pharmacological purposes (Maria, 2016). One of the earliest used plants in history is *Cannabis sativa* as fossil record data reveals the regular use of plant extracts by humans for therapeutic purposes (Alfred, 2013; Federica et al., 2018). From that point, the development of traditional medical system incorporating plants as a means of therapy can be traced back only as far as record documents (Nunez-Selles et al., 2007). Many plant parts are used as medicine for humans and livestock; whole seeds and seed oil are eaten by human, seeds and leaves are fed to animals, seeds oil and stalks are burned for fuel. Whole plants, leaves and wood have environmental uses, bark, fiber and seeds are also of ritual importance (Ullah, 2011; Esra et al., 2012).

Newcastle disease (ND) is a transmissible and notifiable disease caused by a single stranded, non-segmented, negative sense RNA virus, also known as Avian paramyxovirus 1 (APMV-1) (Haque et al., 2010). The virus of the genus *Avulovirus,* subfamily Paramyxovirinae and of the family Paramyxoviridae, is an acute, highly contagious rapidly spreading viral disease of domestic poultry and other wild species of birds of all ages (Iran et al., 2013; Aamir et al., 2014). It is considered to be among the most important viral diseases of poultry worldwide and a major

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constraint to successful poultry production in developing countries (Diel et al., 2012; Namdeo et al., 2015; Lawal et al., 2015). Newcastle Disease Virus (NDV) had been reported endemic in many developing countries of Africa such as Kenya (Njagi et al., 2010); Cameroon (Chaka et al., 2013; Mai et al., 2014); Tanzania (Salum et al., 2002); Ethiopia (Chaka et al., 2013); Egypt (Mohammed et al., 2011) and Nigeria (Okwor et al., 2010; Salihu et al., 2012). The disease is primarily transmitted via inhalation or ingestion of virus shed in feces and respiratory secretions by infected birds for variable lengths of time (Leighton and Heckert, 2007; CFSPH, 2016). Some virus isolates have been found to be transmitted through the egg to the hatching chick (Roy and Venugopalan, 2005). NDVs are capable of infecting humans and infection typically results in conjunctivitis and/or influenza-like symptoms, including fever, headache, and malaise (CFSPH, 2016). Exposure to a large amount of virus is necessary, thus human infections are most common in individuals working on poultry farms or in slaughterhouses (Goebel et al., 2007). Some of the clinical signs that may be associated with ND are respiratory distress, diarrhoea, cessation of egg production, depression, edema of head, face, wattle, nervous sign and death (Namdeo et al., 2015). These clinical signs and symptoms vary widely in the birds infected with NDV and depend on factors such as the virus strain, host species, age of the birds, activities of other pathogens, environmental stress and the immunity status of the host (Sadiq et al., 2011; Gogoi et al., 2015). Due to the detrimental effect of viral diseases to poultry production and the entire global economy, hence the need for this research to determine the antiviral activities of *Cannabis sativa* plant extracts against Newcastle disease virus (NDV).

Materials and methods

Study Area

The study was carried out at the Department of Viral Research, National Veterinary Research Institute Vom, Plateau State. The plant extraction and phytochemical screening was conducted at the Department of Chemistry, Bayero University Kano.

Ethical approval

The ethical approval was obtained from National Head Quarter, National Drug Law Enforcement Agency (NDLEA) Abuja, Nigeria.

Collection and processing of the plant sample

One kilogram (1kg) of dried leaf and seed of *Cannabis sativa* (Indian hemp) were obtained from Taraba State Command, National Drug Law Enforcement Agency (NDLEA), Jalingo, Taraba State. The samples were confirmed at the Department of Plant Biology, Bayero University Kano. The samples were grounded into powder using mortar and pestle as described by Mukhtar and Tukur (1999).

Extraction of plant materials

A procedure by Redfern et al. (2014) was followed for extraction using Soxhlet apparatus (Pyrax Company, United Kingdom). Petroleum ether, ethyl-acetate and methanol solvents were used for the extraction. One hundred and eighty grams (180g) of the powdered plant leaf and seed material was filled in cotton sacked material and introduced inside the Soxhlet extractor. Following this, five hundred miles (500ml) of ethyl-acetate was also introduced into the soxhlet extractor. The side arm was lagged with glass wool, the solvent was heated using hotplate, the condenser on the isomantle beginning to evaporate moving through the apparatus to condenser, the condenser thin dripped into the reservoir containing the plant materials. As the level of solvent reached the siphon, it poured back into the flask and the circle continued until the plant material colour turned to colourless, and the extracted material poured into the beaker. The process was done for each of the solvents, with the same gram of the plant material. The extracts were left to air dry for the solvent to evaporate and the fraction was later obtained.

Phytochemical screening

Phytochemical screening was carried out using method of Edeoga et al. (2005). A 0.1g of each of leaf and seed extract of *Cannabis sativa* was separately dissolved into 10ml of petroleum ether, ethyl acetate and methanol solvents which were introduced into the beaker which served as a working solution.

Test for Alkaloid

One mile (1ml) of the seed and leaf extract was introduced into four test tubes. A drop of Wanger reagent was added to each of the test tubes. Formation of brown/reddish precipitate is considered positive indicating the presence of alkaloid.

Test for Flavonoids

One mile (1ml) of the seed and leaf extract was introduced into four test tubes. A drop of sodium hydroxide (NaOH) solution was added into each testtubes. Formation of tence yellow precipitate is considered positive indicating the presence of flavonoids.

Test for Tannins

One mile (1ml) of the seed and leaf extract was introduced into four (4) test tubes. A drop of chloroform was added to each extract. A drop of hydrochloric acid, acetic acid and

concentrated sulphuric acid were also added into the extracts.The formation of green colour was observed for the presence of tannins.

Test for Saponins

One mile (1ml) of the seed and leaf extract was introduced into four (4) test tubes. A drop of ordinary water was added into the each testtube. Pros formation was observed for the presence of saponins.

Test for Glycoside

One mile (1ml) of the seed and leaf extract was introduced into four (4) test tubes. A drop of glacial acetic acid, furic chloride and concentrated sulphuric acid were carefully added to each of the texttubes to form a lower layer for the formation of reddish brown colour at the surface.

Test for Phenol

One mile (1ml) of the seed and leaf extract was introduced into four (4) test tubes. Two (2) drops of ferric chloride solution was added to observe the formation of bluish black colour at the surface, indicating the presence of phenol.

Test for Steroid/Triterpenoid

One mile (1ml) of the seed and leaf extract was introduced into four (4) test tubes. A drop of acetic anhydrate was added, boiled and cooled. A drop of concentrated sulphuric acid was added from the side of the testtubes, and observed the formation of brown ring at the junction of the two layers. Green colouration of the upper layer is the presence of steroid and the formation of deep red colour in the lower layer indicate the presence of triterpenoid.

Collection of Virus and 9-day old embryonated chicken eggs for the antiviral assay

A velogenic strain of Newcastle disease virus (NDV) was obtained from the Department of Viral Research, National Veterinary Research Institute (NVRI) Vom, while embryonated chicken eggs were obtained from poultry division, National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria. The following antibiotics and antifungals; penicillin, streptomycin, gentamycin and amphotoricin B were added to the phosphate buffer (pH 7.2) which was allowed to dissolve completely in order to prevent further contamination from bacteria and fungi. The procedure was carried out aseptically in the biosafty cabinet using hand gloves, facemask and laboratory coat. The embryonated chicken eggs were disinfected using 70% alcohol. The embryonated eggs were candled and the inoculated sites were marked with pencil, one by the side of the egg and the other at the upper site of the egg. In order to expel air, the eggs were placed in egg racks for the procedure (Chollom et al., 2013).

Toxicity Assay

This was carried out to check the toxicity of the extracts on the 9-day old chicken embryos according to Chollom et al. (2013). Nine day old embryonated chicken eggs were divided into seven groups of five. Group one (1) to five (5) were swabbed with 70% alcohol using cotton wool, and was punched with the egg puncher and 0.2mls of the extract at the concentration of 100mg/ml, 80mg/ml, 60mg/ml, 40mg/ml and 20mg/ml in that order were introduced into the eggs through allantoic cavity. The needle was then withdrawn from the eggs and the hole was sealed with molten wax. Group six (6) was inoculated with 0.2mls of phosphate buffer saline (PBS) diluents control. Group seven (7) was not inoculated with any extract (negative control). The eggs were sealed with molten and were incubated at 37^0C for 24hrs. This was done for each of the extract, the embryo survival was observed after 24 hrs, 48hrs and 72 hrs, and the result was recorded.

Determination of EID_{50} of the Virus

This was carried out to determine the concentration of dilution to which the virus has infected 50% of the embryos. The egg infective dose (EID_{50}) of the virus was determined according to the method of Young et al. (2002). Sterile testtubes were labeled $10^{-1} – 10^{-10}$. A fold serial dilution was carried out. A 0.9mls of sterile phosphate buffer saline (PBS) was added to each of the testtubes. A 0.1ml of NDV was added to the first testtube (10^{-1}) and was mixed thoroughly from the content of the first testtube; 0.1ml was transferred to the second testtube (10^{-2}) and was mixed thoroughly. This dilution continued till the last testtube content of (10^{-10}), when 0.1ml of the last testtube was discarded into the discard jar.

The embryonated eggs were divided into eight (8) group of five (5) for the inoculation of the dilutions. Group one was inoculated through allantoic cavity with 0.2mls of the content of 10^{-1}, group two was inoculated with 0.2mls of the content of 10^{-2}. Same inoculation was done to other groups with corresponding dilution of the virus. Group six (6) was inoculated with phosphate buffer saline (PBS), group seven (7) was inoculated with the virus only and group eight (8) was inoculated with phosphate buffer and virus. Another group of five eggs were not inoculated with anything. The infected eggs were sealed and incubated in humid chamber at 37°C for 24hrs. The inoculated eggs were candled after 24hrs for unspecific dead while percentage mortality after 48hrs to 72hrs was recorded.

Haem agglutination test

This was carried out to confirm the presence of the virus in the virus suspension through the method of Young et al. (2002). A

drop of 1% chicken red blood cell was mixed thoroughly with a drop of virus stock on a tile, which was gently rocked for a visible agglutination indicating viral activity. This was done for the remaining eggs and the observation was recorded.

Preparation of Inoculum (Virus/extract mixture)

A 1:2 v/v dilution of the EID_{50}/0.1ml of virus with predetermined extract concentration was made to put extract final concentration in the virus/extract mixed at 100mg/ml, 80mg/ml, 60mg/ml, 40mg/ml and 20mg/ml. The virus/extract mixture was kept at 40°C for 1hour to react.

Inoculation of eggs/ Antiviral Assay

Chollom et al. (2013) was followed. Nine day-old embryonated chicken egg was divided into nine groups of five and labeled according to the extract concentration. A set of plastic egg tray was thoroughly cleaned with Virkon®. The eggs were swabbed with 70% alcohol in cotton wool and were transfered into a clean tray. The swabbed eggs were placed in the biosafety cabinet and were punched with egg puncher, and immediately inoculated with the extract mixture/virus through the allantoic cavity. Group 1 – 5 were inoculated with 0.2mls of virus/extract mixture at the final concentration of 100mg/ml, 80mg/ml, 60mg/ml, 40mg/ml and 20mg/ml in that order. Group 6 was inoculated with 0.2ml of $100EID_{50}$/0.1ml standard NDV (virus control). Group seven (7) was inoculated with 0.2ml of extract suspension at 100mg/ml concentration (extract control). Group eight (8) was inoculated with 0.2ml of phosphate buffer saline (PBS) (diluent control). Group nine (9) had eggs that were not inoculated with anything (uninoculated control). The eggs were sealed with molten wax and incubated at 37°C. Embryo survival was observed daily for 24rs, 48hrs and 72hrs, for unspecific and mortality, few eggs from selected group left to hatch. Allantoic fluid from treated eggs was collected for spot test to detect the presence of NDV in the allantoic egg fluids.

Spot haemagglutinaion test

The method used by Chollom et al. (2013) was adopted. This was done to confirm the presence of NDV in the eggs. Dead embryo that had been chilled was brought out from the refrigerator and was kept at room temperature for 30minutes. The eggs were swabbed and placed in biosafety cabinet, the shell of each egg was opened to reveal the air space and a pipette was used to dispense a drop of 1% washed red blood cells on the white tiles, a wire loop was thoroughly flamed and was used to pick a drop of the allantoic fluid which was mixed with a drop of blood, the tiles were gently rocked for visible agglutination, indicating viral activity. This was done for every egg and the observation was recorded.

Statistical Analysis

The results obtained after bioassay were subjected to analysis of Variance (ANOVA). Data for the screening of activity of *Cannabis sativa* extracts against Newcastle disease virus isolates from poultry were determined by the analysis of variance using SPSS (version 16) and GraphPad Instat3.0 statistical softwares. However the EID_{50} was calculated from the mean survival of the brine shrimp larvae using Microsoft excel.

Results

Physical properties and phytochemical constituents of *C. sativa* plant extracts

Extracts of *C. sativa* were tested for their physical properties (weight, percentage yield, physical appearance before and after evaporation) as showed in table 1. The result revealed that the weight yield of the extracts ranged from 14.1g to 94.2g. The yield of the extracts were in the order, PES (94.2g), EAL (87.3g), MEL (50.4g), PEL (47.2g), MES (26.9g) and EAS (14.1g). There were changes in the consistency of the plant extracts after the evaporation of extracts as compared to the appearance of the samples before the evaporation of extracts.

Phytochemical components were screened in the extracts of

Table 1. Physical properties, weight and percentage of *C. sativa* plant extracts

Plant Extracts	Physical Appearance		Weight (g)	Percentage Yield (%)
	Before evaporation of extract	After evaporation of extract		
PES	Green oily liquid	Green oily solid	94.2	52.3
PEL	Green liquid	Green gummy solid	47.2	26.2
EAS	Yellowish oily liquid	Yellowish oily solid	14.1	7.8
EAL	Dark green oily liquid	Dark green gummy solid	87.3	48.5
MES	Brownish oily liquid	Brownish oily solid	26.9	14.4
MEL	Green liquid	Dark green gummy solid	50.4	28.0

Key: PES = petroleum ether seed extract, PEL = petroleum ether leaves extract, EAS = ethyl-acetate seed extract, EAL = ethyl-acetate leaves extract, MES = methanol seed extract, MEL = methanol leaves extract

C. sativa. The result revealed that in the the petroleum ether seed extract (PES), only glycoside and triterpenoids phytochemicals were present. Similarly, in methanol seed extract (MES), only two (tannins and triterpenoids) of the eight screened bioactive compounds were found. As presented in the Table 2 below, methanol leaves extract (MEL) was observed to have more bioactive contents than other extracts of the same plant. The phytochemicals constituents of ethyl-acetate seed extract (EAS) were tannins, steroids, saponins and triterpenoid while the phytochemicals constituents of petroleum ether leaves extract (PEL) were steroids, glycoside and triterpeniod. The result also showed that tannins, steroids, glycoside and triterpenoid were observed in ethyl-acetate leaves extract (EAL) (Table 2).

Table 2. Phytochemical constituents on *C. sativa* plant extracts

Phytochemical components	Plant Extracts					
	PES	EAS	MES	PEL	EAL	MEL
Alkaloid	−	−	−	−	−	+
Flavonoid	−	−	−	−	−	+
Tannins	−	+	+	−	+	+
Phenol	−	−	−	−	−	+
Steroids	−	+	−	+	+	−
Glycoside	+	−	−	+	+	+
Saponins	−	+	−	−	−	+
Triterpenoid	+	+	+	+	+	−

Key: − = Negative, + = Positive, PES = petroleum ether seed extract, PEL = petroleum ether leaves extract, EAS = ethyl-acetate seed extract, EAL = ethyl-acetate leaves extract, MES = methanol seed extract, MEL = methanol leaves extract.

Toxicity study of *C. sativa* extracts on Newcastle Disease Virus (NDV)

The result of the toxicity assay conducted on the various extracts of *C. sativa* showed that the extracts were relatively non-toxic. However, minute toxicity was observed in lower concentrations of petroleum ether seed extract (PES), ethyl-acetate seed extract (EAS), ethyl-acetate leaves extract (EAL) and in methanol seed extract (MES). In EAS, there were 40% mortality of embryonated eggs at the concentration of 20mg/ml. In addition, EAL extract was

Table 3. Toxicity properties of *C. sativa* plant extracts on Newcastle Disease Virus (NDV)

Extract(s)	Concentration (mg/ml)	Number of Eggs	Number of eggs with dead embryo after 48hrs	Number of eggs with live embryo after 48hrs	% Mortality at 72hrs
PES	100	5	0	5	0
	80	5	0	5	0
	60	5	0	5	0
	40	5	0	5	0
	20	5	1	4	20
PEL	100	5	0	5	0
	80	5	0	5	0
	60	5	0	5	0
	40	5	0	5	0
	20	5	0	5	0
EAS	100	5	0	5	0
	80	5	0	5	0
	60	5	0	5	0
	40	5	0	5	0
	20	5	2	3	40
EAL	100	5	0	5	0
	80	5	0	5	0
	60	5	0	5	0
	40	5	1	4	20
	20	5	0	5	0
MES	100	5	0	5	0
	80	5	0	5	0
	60	5	0	5	0
	40	5	0	5	0
	20	5	1	4	20
MEL	100	5	0	5	0
	80	5	0	5	0
	60	5	0	5	0
	40	5	0	5	0
	20	5	0	5	0
Dc	0.2mls	5	0	5	0
Nc	–	5	0	5	0

Keys: PES = petroleum ether seed extract, PEL = petroleum ether leaves extract, EAS = ethyl-acetate seed extract, EAL = ethyl-acetate leaves extract, MES = methanol seed extract, MEL = methanol leaves extracts, Dc = Diluent control, Nc = Negative control

observed to cause 20% mortaliy of embryonated eggs at the concentration of 40mg/ml. Toxicity was not observedin either petroleum ether leaves extract (PEL) and methanol leaves extracts (MEL). The result of the toxicity assay of *C. sativa* plant extracts is shown in table 3.

Egg Infective Dose (EID_{50}) of NDV

Egg Infective Dose (EID_{50}) of NDV was carried out to determine the concentration of dilution to which the virus has infected 50% of the embryos. From the result observed in Table 4.4, the $EID_{50}$was found to be at dilution 10^{-4}. This was the concentration at which the Newcastle disease virus (NDV) affected about 54% of the embryos.

Antiviral activity of *C. sativa*

The result of the antiviral activity of *C. sativa* plant extractson NDV showed that methanol leaves extract (MEL) had the highest antiviral activity against NDV. From the result shown in table 5, at the various concentrations and varying incubation timeof MEL, there was no observed motality in the egg embryos. This was followed by methanol seed extract (MES) and ethyl-acetate seed extract (EAS) that both induced a 20% mortality rate at 20mg/ml extract concentration after 72hours of incubation. Petroleum seed extract (PES) was observed to have the least antiviral activity against NDV by exacting 40% and 60% mortality rate at 40 and 20mg/ml concentrations of PES respectively. Petroleum ether leaves extract (PEL) and ethyl-acetate leaves extract (EAL) both exacted an antiviral mortality rate of 40% on the virus at 20mg/ml concentration after 72 hours of incubation.

In addition, the result of the spot haemagglutinaion test conducted to confirm the presence of NDV in the inoculated eggs showed varying degree of agglutination at varying extract concentration in petroleum ether seed and leaves extracts, and in ethyl-acetate seed and leaves extracts. However, presence of agglutination which indicates viral activity was not observed in methanol seed and leaves extracts (Table 5).

Discussion

The findings on the physicochemical constituents of *C. sativa* indicated that the yield of extracts recovered varies with the type of solvent used. The petroleum ether produced a higher yield, followed by ethyl-acetate, while methanol yielded the least amount of extract. Beside the type of solvent used, variations in extraction methods were also showed to contribute to such kind of observations. The phytochemical analysis conducted on *C. sativa* leaves and seeds extracts revealed the presence of bioactive compounds such as alkaloid, flavonoid, tannins, phenol, steroids, glycoside, saponins and triterpenoid. These compounds corresponded with those reported by Aslam et al. (2009). However, variations in extraction methods resulted to some variation in the identified bioactive compounds. This is observed in the present study as methanolic extract of the leaves contain more phytochemicals than those found in ethyl-acetate and petroleum ether plant extracts. This result correspond with the report of Nataranjan et al. (2003) who reported that different plant extracts give rise to varying yield and phytochemical constituents. More so, the variation between the various extracts in phytochemical constituents resulted from the extraction ability of a particular component which appear to depend on extraction medium (solvent) polarity and the ratio of solute to solvent as well as increase in temperature (Simon et al., 2015). The presence of secondary metabolites in plants produces some biological

Table 4. EID_{50} of Newcastle Disease Virus (NDV) at 72hours

Virus Dilution	Number of Dead	Number of Alive	Cumulative Dead	Cumulative Alive	Proportion dead total	% Mortality
10^{-1}	4	1	19	1	19/20	95
10^{-2}	4	1	15	2	15/17	88
10^{-3}	4	1	11	3	11/14	79
10^{-4}	2	3	7	6	7/13	54
10^{-5}	1	4	5	10	5/15	33
10^{-6}	1	4	4	14	4/18	22
10^{-7}	1	4	3	18	3/19	16
10^{-8}	1	4	2	22	2/24	8.3
10^{-9}	1	4	1	26	1/27	3.7
10^{-10}	0	5	0	31	0/31	0

Table 5. Antiviral activity of *C. sativa* plant extracts on Newcastle Disease Virus (NDV)

Extracts	Concentration (mg/ml)	Number of Eggs	Mortality			Spot Test		% Mortality
			24hrs	48hrs	72hrs	+ve	–ve	
PES	100	5	0/5	0/5	0/5	0	5	0
	80	5	0/5	0/5	0/5	0	5	0
	60	5	0/5	0/5	0/5	0	5	0
	40	5	0/5	1/5	1/4	2	3	40
	20	5	0/5	1/5	2/4	3	2	60
PEL	100	5	0/5	0/5	0/5	0	5	0
	80	5	0/5	0/5	0/5	0	5	0
	60	5	0/5	0/5	0/5	0	5	0
	40	5	0/5	0/5	0/5	0	5	0
	20	5	0/5	1/5	1/4	2	3	40
EAS	100	5	0/5	0/5	0/5	0	5	0
	80	5	0/5	0/5	0/5	0	5	0
	60	5	0/5	0/5	0/5	0	5	0
	40	5	0/5	0/5	0/5	0	5	0
	20	5	0/5	0/5	1/5	1	4	20
EAL	100	5	0/5	0/5	0/5	0	5	0
	80	5	0/5	0/5	0/5	0	5	0
	60	5	0/5	0/5	0/5	0	5	0
	40	5	0/5	1/5	1/4	2	3	40
	20	5	0/5	1/5	1/4	2	3	40
MES	100	5	0/5	0/5	0/5	0	5	0
	80	5	0/5	0/5	0/5	0	5	0
	60	5	0/5	0/5	0/5	0	5	0
	40	5	0/5	0/5	0/5	0	5	0
	20	5	0/5	0/5	1/5	0	5	20
MEL	100	5	0/5	0/5	0/5	0	5	0
	80	5	0/5	0/5	0/5	0	5	0
	60	5	0/5	0/5	0/5	0	5	0
	40	5	0/5	0/5	0/5	0	5	0
	20	5	0/5	0/5	0/5	0	5	0
Vc	0.1ml	5	0/5	0/5	0/5	5	0	100
Ec	100mg	5	0/5	0/5	0/5	0	5	0
Dc	0.2ml	5	0/5	0/5	0/5	0	5	0
Nc	-	5	0/5	0/5	0/5	0	5	0

Key: Vc = Virus control, Ec = Extract control, Dc = diluent control, Nc = Negative control

activity in man and animal and is responsible for their use as herbs. According to Patel (2010), plant metabolites also serve as defensive mechanism to protect the plant against infection by microorganisms, predation by insect and herbivores while some give plants their characteristic odors, flavors and are responsible for their pigments. In some cases, the activity has been associated with specific compounds or classes of compounds. These active constituents can be used to search for bioactive lead compounds that could be used as precursors for the synthesis of more useful drugs (Ogbonnia et al., 2008).

The result of the toxicity assay conducted on the methanol, petroleum ether and ethyl-acetate seed and leaves extracts of *Cannabis sativa* showed that the extracts were relatively non-toxic. However, minute toxicity was observed in lower concentrations of 20 and 40mg/ml of petroleum ether seed, ethyl-acetate seed, ethyl-acetate leaves and in methanol seed extracts. In support of the present study, Auwal et al. (2012) who conducted an *in vivo* study to determine the acute toxicity potential of some plant extracts observed that petroleum ether extracts were more toxic than methanolic extracts which corresponds to the findings of the current study. The minute toxicity observed in the current study could be as a result of less cannabinoid content; a compound known for the toxicity potential of *cannabis* plant (Brian and Thomas, 2019).

The Egg Infective Dose (EID_{50}) of NDV was carried out to determine the concentration of dilution to which the virus has infected 50% of the embryos. From the result observed in the present study, the EID_{50} was found to be at dilution 10^{-4}. The result observed in this study was slightly different from the result obtained by Chollom et al. (2013). This could be as a result of variations in the inoculation procedure in the the growth medium. In addition, the result of the spot haemagglutinaion test conducted to confirm the presence of NDV in the inoculated eggs showed varying

degree of agglutination at varying extract concentration in petroleum ether seed and leaves extracts, and in ethyl-acetate seed and leaves extracts. However, presence of agglutination which indicates viral activity was not observed in methanol seed and leaves extracts (Table 5).

The antiviral potential of *C. sativa* plant extracts on Newcastle Disease Virus (NDV) showed that methanol leaves extract (MEL) had the highest antiviral activity against NDV. The result of the present study revealed that at the various concentrations and varying incubation time of MEL, there was no observed motality in the egg embryos. Considering the high antiviral activity of the methanol leaves extracts, *C. sativa* can be used for the treatment of viral diseases especially in birds after further scientific examination. This research work holds novelty in its application for the treatment of Newcastle disease in poultry birds.

Conclusion

The present findings revealed the presence of bioactive compounds such as alkaloid, flavonoid, tannins, phenol, steroids, glycoside, saponins and triterpenoid in *Cannabis sativa* extracts. The findings on the toxicity of *Cannabis sativa* plant extracts showed that the extracts were relatively non-toxic. However, minute toxicity was observed in lower concentrations of 20 and 40mg/ml of petroleum ether seed, ethyl-acetate seed, ethyl-acetate leaves and in methanol seed extracts. Methanol seed and leaves extracts had the highest antiviral activity against NDV as embryo mortality was not observed during the study at various extract concentrations. There is significant antiviral activity of *Cannabis sativa* plant extracts against Newcastle disease virus ($p < 0.05$). Thus, *Cannabis sativa* plant extracts are viable therapeutic agents for the treatment of Newcastle disease.

Recommendation

Considering the high antiviral activity of *Cannabis sativa* methanolic leaves extract against Newcastle disease, the researcher recommends that *Cannabis sativa* plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities that brings about breakthrough in the treatment of NDV. Further studies should be conducted to isolate the compound responsible for the biological activities and elucidate the structure for drug development.

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Conflicts of interest

The authors have no conflict of interest to be declared.

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