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In Ovo Evaluation of Antiviral Potential of Selected Medicinal Plant Extracts Against Newcastle Disease Virus

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ABSTRACT

Newcastle Disease (ND) is economically noteworthy and infectious viral disease affecting domestic poultry and several wild species. A virus named Newcastle Disease Virus (NDV) is responsible for the disease. It is non-segmented, negative-sense, single-stranded RNA virus belonging to the species Avian orthoavulavirus 1. Presently, disease control is primarily achieved through vaccination, and therapeutic options to eradicate the disease is limited. In the current study, antiviral potential of selected medicinal extracts, i.e., *Zingiber officinale* (ZO), *Allium cepa* (AC), *and Allium sativum* (AS), was performed against velogenic strain of NDV. Embryonated chicken egg assay was performed to assess the antiviral activity. A working 4 Hemagglutinaing unit (HA) virus suspension was prepared by diluting 1 ml of virus holding allantoic fluid with 31 mL of normal saline to attain the prerequisite HA concentration. Different concentrations of plant extracts along with virus were inoculated with embryonated eggs and viral replication was assessed using the haemagglutition (HA) assay, whereas embryo viability and mortality was determined to evaluate toxicity. The results displayed that none of the tested concentrations of AS inhibited viral replication. In contrast, AC extract confirmed inhibition of viral replication up to a concentration of 250 µg/mL without causing toxicity to the embryos. Likewise, ZO extract exhibited inhibitory effects on viral growth up to 1000 µg/mL, while no embryo mortality was observed. The results confirm the potential of ZO and AC extract as source of bioactive compounds for development of alternative antiviral strategies against NDV.

INTRODUCTION

Newcastle disease (ND) is one the major infectious disease causing significant economic losses in the poultry industry worldwide (Boroomand et al., 2023, Wang et al., 2024). A member of the genus Avulavirus within the family Paramyxoviridae is responsible for the disease, which is a single-stranded, non-segmented RNA, enveloped virus, which resembles in genome configuration to Avian Paramyxoviridae serotype-1 (APMV-1). It is highly contagious affecting a wide range of wild and domestic bird species (Amoia et al., 2023, Durkwa et al., 2025).

Since ancient times, medicinal plants have been used for the treatment and prevention of diseases and endure an imperative source of therapeutic agent. More than 25% of presently used drugs are derivative directly or indirectly from plant sources (Newman and Cragg, 2020). The World Health Organization (WHO) has likewise highlighted the significance of traditional medicinal plants, as a large proportion of world population relies on natural medications for primary healthcare, mainly in developing countries (Wong et al., 2025). Diverse pharmacological activities including antiviral properties have been reported by a wide variety of bioactive secondary metabolites of plant extracts. These metabolites include but not limited to flavonoids, tannins, phenolic compounds, terpenoids. Several mechanisms such as blocking entry into host cells, inhibiting viral genome replication, modulating host response and interfering with viral protein synthesis have been reported in literature (Guo et al., 2022, Mani et al., 2020). In this context, antiviral

potential of plant extract has been continuously explored against a wide range of DNA and RNA viruses. Notwithstanding, association the outcomes among different studies are often difficult becuase of variation in experimental models, extraction method of plant extract, plant source and viral stains under investigation (Arumugam et al., 2025a). Furthermore, emergence of new viral diseases and development of resistance to conventional antiviral drugs have exaggerated the exploration from natural resources (Tian and Wang, 2023).

A broad spectrum of biological activates has been reported for the commonly consumed food ingredients such as AS, AC, and ZO (Onyiba, 2022, Rajendrasozhan, 2024a, Pebam et al., 2022). Many bioactive compounds such as organosulfur, phenolic, and flavonoids compounds, exhibiting antioxidant, anti-inflammatory, antiviral, and antimicrobial activities, have been documented (Melguizo-Rodríguez et al., 2022, Rajendrasozhan, 2024b). For instance, randomized and experimental studies investigating extract of AS have demonstrated significant immunomodulatory, antiviral, and anti-inflammatory properties. In AS, allicin, ajoene, diallyl disulphide, and diallyl sulphide are found major bioactive organosulfur compounds displaying inhibitory effects against several non-enveloped and enveloped viruses comprising influenza, human rhinovirus, and herpes simplex virus. They interfere with viral replication by disrupting viral envelope proteins and hindering viral entry into host cells. Furthermore, modulating immune response by decreasing pro-inflammatory cytokines such as tumor necrosis factor-α and C-reactive proteins have been revealed by garlic extract. Also, the extract can supress nuclear factor-$_k$B signalling pathways and boosts immune cell activity, which auxiliary adds towards antiviral defence mechanism. Similarly, extract of AC contains quercetin, surfur-containing compounds, and flavonoids, which contribute to its therapeutic effects. Similarly, ginerols, shogaols, zingerone, and paradols present, the major active constituents of ginger, are responsible for inhibition of viral attachment and internalization into host cells, thus reducing viral replication.

Based on their virulence, the NDV stains are classified into three main pathotypes, such as lentogenic, virogenic, and mesogenic. Among them, Velogenic strains are extremely lethal and many cause mortality rates of up to 90% in susceptible poultry flocks, while mesogenic strain causes moderate disease characterized principally by neurological and respiratory symptoms. The lentogenic strains, however, are mildly pathogenic and are frequently used as L attenuated vaccines (Desingu et al., 2021, Elbestawy et al., 2023). Inspite of availability of vaccines, outbursts of Newcastle disease stay to occur, stressing the need for alternative therapeutic and preventive strategies. In this regard, rare studies have evaluated the efficacy of natural plant extracts against NDV, particularly using *in ovo* experimental model. Therefore, the present study was designed to investigate the antiviral activity and safety profile of aqueous extracts of AS, AC, and ZO against a velogenic strain of NDV using embryonated chicken eggs. These findings may contribute to the development of cost-effective, safe, and economical strategies for controlling NDV.

MATERIALS AND METHODS

Plant Collection, Identification and Extraction

Bulbs of AC and AS, and rhizomes of ZO were collected form District Lahore, Pakistan. The materials were identified and authenticated at the Herbarium of Govt College University, Lahore, and a sufficient quantity of each sample was preserved for further experimental use. The plant material was thoroughly washed with distilled water to remove adhering impurities or soil, and was air-dried under shade to prevent degradation of bioactive constituents. The dried materials were crushed using a sterile laboratory blender (Philips, Netherlands) to gain paste-like consistency. The minced samples were afterwards placed in a desiccator (Pyrex, USA) and were allowed to dry overnight to remove residual moisture. The 100 g of plant material was macerated in 300ml of distilled water using sterile reagent glass bottles of 500 ml capacity and the bottle was properly labelled. The bottle was placed in a vibrator for 24 h, and plant material was allowed to macerate. After 24 h, the macerated extracts were filtered using Whatman No 1 filter paper (Whatman, UK) to remove suspended particles. The extract was further sterilized by passing them through 0.22 µm syringe filter (Millipore, USA) under aseptic conditions to eliminate potential contamination. The sterility of the extract was confirmed by spreading 1 ml of each extract onto nutrient agar plates (Oxoid, UK). The plates were left undisturbed for two minutes to allow absorption of the extract, and the excess extract was discarded. The inoculated plates were incubated for 24 h at 37^{0}C (Memmert, Germany), after which the plates were examined for any bacterial growth. The herbal extracts were kept at 40^{0}C for 90 hours in incubator for drying to obtain solid material.

Source of Embryonated Eggs

Nine- to ten days old embryonated eggs were acquired from Big Bird Hatchery (Big Bird Pvt. Ltd. Lahore) and incubated at 37^{0}C in an egg incubator (70% humidity). The eggs were examined by candling to distinguish D embryos from L ones, and total of two hundred and seventy viable embroyonated eggs were selected for the study.

Virus Source and Identification

The Newcastle disease virus (NDV) was obtained from the WTO-Quality Operation Laboratory, University of Veterinary and Animal Sciences, Lahore. Hemagglutination inhibition (HI) test was used to confirm identity of the virus. Adopting the previous method, HI test was performed to determine the 4HA activity of the test virus (Allan and Gough, 1974).

Haemagglutination (HA) Assay

0.5 mL of normal saline was dispensed into 1-12 wells of rows A, B, C, and D. 0.05mL of virus suspension was added to first well, followed by two-fold serial dilutions across the plate. Subsequently, 0.05mL of 1% chicken RBC suspension was added to each well. The plates were gently tapped and incubated at 20^{0}C for 60 minutes to allow RBCs to settle. After tilting the plate, HA activity was determined by observing agglutination patterns, and 4HA titre was calculated.

Haemagglutination Inhibition (HI) Assay

Wing vein of adult broiler was used for collection of 5 ml blood form broiler chicken and the blood was stored in EDTA-containing tubes. The blood was centrifuged at 1500 rotation per minute (rpm). The plasma was discarded using sterile plastic dropper, and normal saline was added to attain final volume of 5 mL. The washing procedure was repeated thrice and the RBCs were re-suspended to prepare 1% working suspension of RBCs. The HI assay was performed according to guidelines of world organisation for animal health (OIE, 2009). Briefly, 0.025mL of phosphate buffer saline (PBS) was put into each well of 96 well microtiter plate. 0.025mL of standard serum was added to the first well and serial two-fold dilution were prepared upto the tenth well. Afterwards, 0.025mL of antigen containing 4 haemagglutinating units (4HA) of NDV were added to each well and incubated at room temperature for 30 minutes. Thereafter, 0.025ml of 1% chicken RBC suspension was added and plates were gently shaken and incubated for 45-60 minutes. Agglutination was assessed by tilting the plate and relating with control wells. The highest serum dilution displaying complete inhibition of haemagglutination was noted as the HI titre.

Experimental design

A total of 90 embryonated chicken eggs were allocated to eighteen groups for each plant extract with five eggs per group. Among them, eight groups were designated for antiviral activity for the extract of AS (named as AV1–AV8), AC (designated as BV1-BV8), and ZO (mentioned as CV1–CV8). Likewise, eight groups were assigned for cytotoxicity studies designated as AC1–AC8, BC1–BC8, CC1–CC8 respectively for plant extracts of AS, AC, and ZO respectively. The positive control mentioned recieved 4HA NDV virus only and were designated as AP. The negative control recieved normal saline and mentioned as AN. Eggs in the treatment groups were inoculated with 4HA NDV along antimicrobials (i.e., Gentamycin 100 mg/ml, Penicillin 500000 IU/ml, Amphotericin B 250 µg/ml, Streptomycin 500 mg/ml), and different concentrations of plant extract ranging from 31.25-4000 µg/mL. A drug control groups treated with different concentration of a standard antiviral drug, ribovirin were also incorporated. For this purpose, 400 mg of the drug was dissolved in 200 mL of sterile deioned distilled water with throughout mixing to ensure complete dissolution. From the stock solution (2mg/ml), a series of working concentrations were obtained through appropraite dilutions in order to achieve final concentrations of 20 µg/mL, and 40 µg/mL. To 20 eggs, above mentioned doses of ribovirin was also inoculated for antiviral and cytotoxicity assay and were designated as RV1-RV2, and RC1-RC2 respectively. For inoculation, a small hole was made above the air sac of embryonated egg using a sterile syringe needle. Afterwards, the opening was sealed with sterile wax. The eggs were properly marked for identification. Afterwards, the eggs were placed in trays with broad end upwards and incubated at 37^0C with 60-70 % relative humidity. The eggs were turned three times daily. After 72 h of incubation, viral replication in the antiviral activity groups was evaluated using the spot haemagglutination test (SHT), while embryo viability in the cytotoxicity group was assessed by candling.

The SHT was performed by placing one drop of allantoic-amniotic fluid onto glass slide and adding one drop of chick RBCs suspension. The contents were lightly mixed and observed for agglutination. Slide presenting visible agglutination were recorded as positive, and vice versa.

RESULTS

No bacterial growth was observed on nutrient agar plates at 37^0C incubation for 24 h, confirming the sterility of the herbal extracts. According to outcome from HA assay, the working 4HA virus suspension was prepared by diluting 1 mL of virus containing allantois fluid (AAF) with 31 mL of normal saline to reach necessary 4HA concentration for all subsequent experiments. As haemagglutination activity was noticed in all treatment groups, it proved that none of the concentration of AS extract had prevented viral replications. Furthermore, all test concentrations were non-toxic, as no embryo mortality was seen in the groups of AC1-AC8 (Table 1). The results from AC indicated successful inhibition of virus replication upto concentration of 250 µg/mL without causing harm to the embryo (Table 2). For ZO, it inhibited the viral growth until 1000 µg/mL, whereas no embryo mortality was observed at all the concentrations (Table 3). For drug control treated with ribovirin, 20 µg/mL presented anti- NDV activity, while the higher concentration of 40 µg/mL resulted in embryo death (Table 4). Henceforth, 20 µg/mL was reflected as a non-toxic concentration with effective antiviral activity.

Table 1

Toxic and Antiviral Effect of Allium Sativum on Newcastle Disease Virus Replication.

Egg Number	Negative control (NS)		Positive control (virus)		Antiviral Activity (Herbal extract of AS + virus)															
	Viab.	HA	Viab.	HA	C1 4000µg/ml		C2 2000µg/ml		C3 1000µg/ml		C4 500µg/ml		C5 250µg/ml		C6 125µg/ml		C7 62.5µg/ml		C8 31.25µg/ml	
					HA	Viab.	HA	Viab.	HA	Viab.	HA	Viab.	HA	Viab.	HA	Viab.	HA	Viab.	HA	Viab.
1.	L	-ve	D	+ve	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D
2.	L	-ve	D	+ve	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D
3.	L	-ve	D	+ve	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D
4.	L	-ve	D	+ve	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D

5.	L	-ve	D	+ve	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D
Mortality Ratio	0%		100%		100%		100%		100%		100%		100%		100%		100%		100%	

Egg Number	Negative control (NS)		Positive control (virus)		Cytotoxic Activity (Herbal extract of AS + Normal saline)							
	Viab.	HA	Viab.	HA	C1 4000µg/ml	C2 2000µg/ml	C3 1000µg/ml	C4 500µg/ml	C5 250µg/ml	C6 125µg/ml	C7 62.5µg/ml	C8 31.25µg/ml
					Viab.	Viab.	Viab.	Viab.	Viab.	Viab.	Viab.	Viab.
1.	L	-ve	D	+ve	L	L	L	L	L	L	L	L
2.	L	-ve	D	+ve	L	L	L	L	L	L	L	L
3.	L	-ve	D	+ve	L	L	L	L	L	L	L	L
4.	L	-ve	D	+ve	L	L	L	L	L	L	L	L
5.	L	-ve	D	+ve	L	L	L	L	L	L	L	L
Mortality Ratio	0 %		100%		0 %	0 %	0 %	0 %	0 %	0 %	0 %	0 %

Table 2

Toxic and Antiviral Effect of Allium Cepa on Newcastle Disease Virus Replication.

Egg Number	Negative control (NS)		Positive control (virus)		Antiviral Activity (Herbal extract of AC + virus)													
	Viab.	HA	Viab.	HA	C1 4000µg/ml		C2 2000µg/ml		C3 1000µg/ml		C4 500µg/ml		C5 250µg/ml		C6 125µg/ml		C7 62.5µg/ml		C8 31.25µg/ml	
					HA	Viab.	HA	Viab.	HA	Viab.	HA	Viab.	HA	Viab.	HA	Viab.	HA	Viab.	HA	Viab.
1.	L	-ve	D	+ve	-ve	L	-ve	L	-ve	L	-ve	L	-ve	L	+ve	D	+ve	D	+ve	D
2.	L	-ve	D	+ve	-ve	L	-ve	L	-ve	L	-ve	L	-ve	L	+ve	D	+ve	D	+ve	D
3.	L	-ve	D	+ve	-ve	L	-ve	L	-ve	L	-ve	L	-ve	L	+ve	D	+ve	D	+ve	D
4.	L	-ve	D	+ve	-ve	L	-ve	L	-ve	L	-ve	L	-ve	L	+ve	D	+ve	D	+ve	D
5.	L	-ve	D	+ve	-ve	L	-ve	L	-ve	L	-ve	L	-ve	L	+ve	D	+ve	D	+ve	D
Mortility Ratio	0 %		100 %		0 %		0 %		0 %		0 %		0 %		100 %		100 %		100 %	

Egg Number	Negative control (NS)		Positive control (virus)		Cytotoxic Activity (Herbal extract of AC + Normal saline)							
	Viab.	HA	Viab.	HA	C1 4000µg/ml	C2 2000µg/ml	C3 1000µg/ml	C4 500µg/ml	C5 250µg/ml	C6 125µg/ml	C7 62.5µg/ml	C8 31.25µg/ml
					Viab.	Viab.	Viab.	Viab.	Viab.	Viab.	Viab.	Viab.
1.	L	-ve	D	+ve	L	L	L	L	L	L	L	L
2.	L	-ve	D	+ve	L	L	L	L	L	L	L	L
3.	L	-ve	D	+ve	L	L	L	L	L	L	L	L
4.	L	-ve	D	+ve	L	L	L	L	L	L	L	L
5.	L	-ve	D	+ve	L	L	L	L	L	L	L	L
Mortility Ratio	0 %		100%		0 %	0 %	0 %	0 %	0 %	0 %	0 %	0 %

Table 3

Toxic and Antiviral Effect of Zingiber Officinale on Newcastle Disease Virus Replication.

Egg Number	Negative control (NS)		Positive control (virus)		Antiviral Activity (Herbal extract of AC + virus)													
	Viab.	HA	Viab.	HA	C1 4000µg/ml		C2 2000µg/ml		C3 1000µg/ml		C4 500µg/ml		C5 250µg/ml		C6 125µg/ml		C7 62.5µg/ml		C8 31.25µg/ml	
					HA	Viab.	HA	Viab.	HA	Viab.	HA	Viab.	HA	Viab.	HA	Viab.	HA	Viab.	HA	Viab.
1.	L	-ve	D	+ve	-ve	L	-ve	L	-ve	L	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D
2.	L	-ve	D	+ve	-ve	L	-ve	L	-ve	L	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D
3.	L	-ve	D	+ve	-ve	L	-ve	L	-ve	L	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D
4.	L	-ve	D	+ve	-ve	L	-ve	L	-ve	L	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D
5.	L	-ve	D	+ve	-ve	L	-ve	L	-ve	L	+ve	D	+ve	D	+ve	D	+ve	D	+ve	D
Mortility ratio	0 %		100 %		0 %		0 %		0 %		100 %		100 %		100 %		100 %		100 %	

Egg Number	Negative control (NS)		Positive control (virus)		Cytotoxic Activity (Herbal extract of AC + Normal saline)							
	Viab.	HA	Viab.	HA	C1 4000µg/ml	C2 2000µg/ml	C3 1000µg/ml	C4 500µg/ml	C5 250µg/ml	C6 125µg/ml	C7 62.5µg/ml	C8 31.25µg/ml
					Viab.	Viab.	Viab.	Viab.	Viab.	Viab.	Viab.	Viab.
1.	L	-ve	D	+ve	L	L	L	L	L	L	L	L
2.	L	-ve	D	+ve	L	L	L	L	L	L	L	L
3.	L	-ve	D	+ve	L	L	L	L	L	L	L	L

4.	L	-ve	D	+ve	L	L	L	L	L	L	L	L
5.	L	-ve	D	+ve	L	L	L	L	L	L	L	L
Mortility Ratio	0 %		100%		0 %	0 %	0 %	0 %	0 %	0 %	0 %	0 %

Table 4

Toxic and Antiviral Effect of Ribovirin on Newcastle Disease Virus Replication.

Egg Number	Antiviral Activity (Ribovirin + virus)				Toxicity (Ribovirin + NS)	
	RV1 20 µg/ml		RV2 40µg/ml		CV1 (20 µg/ml)	CV2 (40µg/ml)
	HA	Viab.	HA	Viab.	Viab.	Viab.
1.	-ve	L	-ve	D	L	D
2.	-ve	L	-ve	D	L	D
3.	-ve	L	-ve	D	L	D
4.	-ve	L	-ve	D	L	D
5.	-ve	L	-ve	D	L	D
Mortility Ratio	0 %		100 %		0 %	

DISCUSSION

Medicinal herbs are being used for the cure of diseases and it has been documented in history of human civilization. Substantial attention has been directed worldwide towards the treatment and prevention of viral infections, mainly due to the limitations of currently available antiviral drugs (Xu et al., 2022, Thomas et al., 2021). Besides, the synthetic antiviral drugs are often own noteworthy adverse effects and rapid emergence of resistance (Ma et al., 2021). Henceforth, there is escalating concern in finding alternative antiviral agents, particularly those derived from natural resources.

The present study explored the antiviral potential of AS, AC, and ZO against Newcastle disease virus (NDV) using an embryonated egg model. The findings clearly displayed that AC and ZO possess meaningful antiviral activity without causing toxicity to the developing embryos, whereas AS did not demonstrate inhibitory effects at the tested concentrations. These results add to the growing body of evidence supporting medicinal plants as promising antiviral agents, especially at a time when conventional antiviral drugs face challenges such as toxicity, high cost, and the rapid emergence of resistance (Ma et al., 2021, Tian and Wang, 2023). The sterility was ensured at the start which further supported our confidence in the outcomes, as contamination may impede both the embryo survival and viral replication. Similarly, the usage of a standardized viral dose (4HA units) enhanced the reliability of association between treatments, considering that the NDV relies on hemagglutinin-neuraminidase proteins to bind host cells and initiates infection. Here, AS did not show antiviral activity, despite its well-established fame as a natural antiviral agent. Organosufur compounds such as ajoene, diallyl sulfides, allicin, exist in garlic extract, have shown broad spectrum activity against viruses like rhinovirus, herpes simplex virus, and influenza.

reputation as a natural antiviral agent. Garlic is known to contain organosulfur compounds such as allicin, ajoene, and diallyl sulfides, which have shown activity against viruses like influenza, herpes simplex virus, and rhinovirus (Melguizo-Rodríguez et al., 2022). The absence of antiviral effect at the dose may be elucidated by the instability of these compounds, mainly allicin, which easily undergoes degradation during aqueous extraction and processing (Irianto et al., 2025).

Nevertheless, AC exhibited robust antiviral activity by inhibiting NDV replication up to 250 µg/mL without any observed embryo toxicity. Thus, the extract possesses active compounds capable of interfering with viral processes while maintaining the safety of the extract for the host. This fact may be due to rich flavonoid contents of onion such as quercetin, along with phenolic and sulfur-containg compounds. These molecules have already established proves on inhibiting entry to virus into host cells, inhibiting viral enzymes such as RNA polymerase, and disrupt viral protein synthesis (Arumugam et al., 2025b). Additionally, their anti-oxidant properties may aid diminishing oxidative stress, which is often accompanying with enhanced viral replication. Antiviral effect of AC has been reported against adenovirus, influenza virus, and other RNA virus, thus further supporting its potential as natural antiviral agent (Singh et al., 2025).

ZO extract also exhibited the antiviral activity, although at relatively higher concentrations (upto 1000 µg/mL). The bioactive compounds inside ginger such as shogaol, papadols, gingerol, and zingerone, have been widely reported to possess anti-inflammatory and antiviral properties. They have shown up to disrupt the viral envelope, blocking virus-host interactions, and impeding viral replication processes. Recent studies have also underlined the immunomodulatory effects of ginger, including stimulating antiviral cytokines and decreasing the inflammatory responses.

These compounds may act by disrupting the viral envelope, blocking virus–host interactions, and inhibiting viral replication processes. Recent studies have also emphasized the immunomodulatory effects of ginger, including stimulation of antiviral cytokines and reduction of inflammatory responses (Pebam et al., 2022, Rajendrasozhan, 2024b). The effect at higher dose as compared to onion may reflect differences in compound potency or bioavailability in aqueous extracts.

The validation of results was performed by using ribavirin as a reference antiviral drug. Although the drug showed effective anti-NDV activity at lower concentration of 20

µg/mL, but higher doses ensued in embryo mortality. These results highlight its narrow therapeutic window, which is a known limitation of many synthetic antivirals. The drug inhibits viral RNA-dependant RNA polymerase and induces mutations in viral genome, however its toxicity at higher doses limits its practical application.

Ribavirin works by inhibiting viral RNA-dependent RNA polymerase and inducing mutations in viral genomes, but its toxicity at higher doses limits its practical application (Shi et al., 2025). However, the plant extracts have exhibited a more favourable safety profile, reinforcing the idea that natural products may offer safer alternative with multi-target mechanisms tha lessens the probability of resistance.

Despite of encouraging outcomes, the use of crude aqueous extracts may not fully represent the antiviral potential of purified bioactive compounds, and the embryonated egg model does not completely mimic real life dynamics of poultry infections. Hence, the future research must focus on the characterized and specific active compounds responsible for antiviral activity, as well as investigating their mechanistic reasoning of antiviral activity. Also, studies using live poultry model, and cell culture system will be essential to optimize dose, confirm efficacy, and evaluate practical applicability of the compounds for controlling NDV infections.

CONCLUSION

The present study demonstrated via *in ovo* model that the selected plant extracts own promising antiviral potential against NDV infection. Among the extract, ZO and AC exhibited significant inhibitory effects on viral replication without bringing harmfulness in embroynated chicken eggs, thus underlining their safety and therapeutic relevance. However, AS did not express the therapeutic potential under the experimental conditions, possibly becuase of instability of its active constituents during extraction or the experiment. The findings, henceforth, emphasize the potential of natural derivatives as alternative antiviral agents, particularly in the context of limitations associated with conventional drugs, such as their side effects and resistance. Nevertheless, validation of the results is necessary in an *in vivo* model. Overall, this study supports the investigation of medicinal plants as safe and cost-effective approaches for controlling NDV infection in poultry.

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