Therapeutic potential of natural plant substances for the control of avian infectious bronchitis virus

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Abstract

Avian infectious bronchitis (IB) is one of the foremost avian pathologies and is caused by a coronavirus with worldwide distribution. In addition to its infectivity, the appearance of new variants and strains makes this pathology particularly difficult to control, which represents a threat to the poultry sector. Natural substances have long been used as therapeutic sources and boosters for vaccines since vaccines alone do not provide complete protection due to continuous mutations of the infectious bronchitis virus (IBV). To explore other remedies to fight IBV, several researchers have turned to using medicinal plants and extracting active ingredients with antiviral properties. The quest for alternative therapies is particularly relevant given the Sars-CoV-2 outbreak, a human coronavirus that shares similarities with IBV, such as its mutation rates and health complications. This article is a systematic review of natural products used against IBV. Thirteen natural products, including plants, essential oils, and mixtures, were identified in this study, and all showed antiviral activity against IBV, with variable effectiveness. Although the efficacy of these active substances has been established and demonstrated by scientific methods, further and more in-depth studies should be carried out to confirm the results, determine the mechanisms of action, and validate their use as a treatment or adjuvant.

Keywords: Coronavirus, IBV, plants, antiviral, poultry

INTRODUCTION

In the wake of the Sars-CoV-2 pandemic, developing effective anti-coronavirus drugs has become crucial for facing the global impact of the virus. Indeed, Covid-19 has proven to be hard to combat, creating a situation of urgency amongst researchers and scientists striving to find an effective antiviral treatment (Touil *et al.*, 2023). While there has been real progress in antiviral drug development (Rhazzar *et al.*, 2025), researchers used results from other coronavirus strains to gain insights and further their studies, such as Avian Infectious Bronchitis Virus (IBV) in poultry.

Virus infections are a concern in the poultry industry worldwide due to the considerable economic losses they entail. Some avian viruses are high priority and economically important, such as avian influenza virus (AIV) and IBV (Barjesteh *et al.*, 2020). The fight against infectious diseases, particularly IBV, has become a research challenge that aims to develop effective remedies to mitigate their effects and thus limit their spread (Fellahi *et al.*, 2015).

IBV is a highly contagious respiratory tract virus of poultry, that belongs to the Coronaviridae family, and which causes morbidity, mortality, and reduced feed conversion, leading to considerable damage in the poultry industry (Cavanagh, 2005). Coronaviruses replicate in the cytoplasm of infected cells and have a genome of approximately 27-32 kb. Virions contain four structural proteins: the spike (S), the membrane (M), the envelope (E), and the nucleo-protein (N) (Siddell, 1994; Lai and Cavanagh, 1997; Britton *et al.*, 2005; Casais *et al.*, 2005; Ren *et al.*, 2009). The IBV genome, which has the gene order replicase-S-3-M-5-N, contains two non-structural genes, 3 and 5, that express three (3a, 3b, and 3c) and two (5a and 5b) small gene products, respectively (Casais *et al.*, 2005).

Mutation and genetic recombination result in various strains, such as the Massachusetts type (Mass.), the Arkansas type (Ark.), or the 793B type (4/91). A report established by Jungherr and his colleagues in 1956 showed the absence of cross-protection between Massachusetts (discovered in 1941) and Connecticut (discovered in 1951) pathogenic strains; this was proof of the existence of several IBV serotypes (Cavanagh and Naqi, 2003). On this basis, it is established that vaccination is the key preventive measure for countering infectious bronchitis (IB). Its application in most chicken farms has decreased large-scale outbreaks of IB (Ren *et al.*, 2009), but live attenuated vaccines used for preventing this viral infection give little immunity against cross-serotypes (Cavanagh, 2005; Gelb *et al.*, 2005; Liu *et al.*, 2006). Indeed, the frequency of IBV serotype mutations makes vaccines partially effective against different strains (Zhang *et al.*, 2022). Although the chicken's innate responses eliminate invading viruses or allow the adaptive immune system to establish an effective antiviral response, viruses are multifactorial and complex (Barjesteh *et al.*, 2020).

While vaccination remains the mean used to prevent diseases and fight against infections, known to be very active in certain countries, the antigenic variation between strains of the virus poses a challenge to their identification, which explains the failure of some vaccines against the infection. Indeed, cross-protection between strains is highly variable, and the emergence of new strains is a serious issue, as the protection offered by vaccines also varies (Brochu Morin *et al.*, 2018; Regragui *et al.*, 2025).

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Currently, no antiviral drugs to treat IBV are commercialized, and farmers still rely on proper husbandry practices and available vaccines, live-attenuated and inactivated, to prevent the virus from spreading. However, despite protocols in place to control IBV outbreaks, farms still record high mortality and morbidity rates, prompting researchers to seek other solutions and alternatives for IBV treatment. Several studies have focused on the search for new natural substances allowing the control of IBV, due to their perceived advantages. These include their safety, synergistic effects, and cost-effectiveness, making them sustainable resources compared to synthetic drugs. Historically, traditional medicine relied on natural products to treat various diseases, including viral infections, suggesting that these substances may be effective in IBV infections.

The objective of this review is to gather and compare information on natural extracts and active compounds with antiviral bioactivity and establish a comprehensive description based on performance and efficacy, methods of extraction or synthesis, the active agent involved, availability of raw material, ease of large-scale application, and possibly the cost of its use. This review summarizes existing research and offers a comprehensive overview by identifying literature gaps and suggesting directions for future research.

METHOD

The selected articles for this synthesis were classified by the chronology of publication. Their results highlight the effectiveness of therapeutic agents against IBV, in particular, the active principles extracted from plants, derived either through organic synthesis or by distillation. The agents are designated by plant extract, vegetable essential oils, or mixtures.

The search was conducted using PubMed (www.ncbi.nlm.nih.gov/pubmed/) and Google Scholar (https://scholar.google.com/) databases. The following keywords were used: (coronavirus, avian and IBV) and (natural treatments, extracts, essential oils, plants, and herbal medicine). The selected articles focused on the antiviral activity of different plant parts and essential oils. Only articles up to and including 2024 were considered, and the selection of these articles was based on relevance, with a focus on recent and up-to-date studies, and methodology, meaning articles with a comprehensive overview, clinical trials, field studies, and indeed, efficacy.

RESULTS

Antiviral activity of plant extracts

Garlic, *Allium sativum*

Allium sativum, from the Liliaceae family, has been widely used for thousands of years for its medicinal properties. It has been recognized for its potential to inhibit different strains of bacteria, fungi, and viruses (Singh and Singh, 2008). *A. sativum* has been extensively studied *in vitro* , in animal and human clinical trials, and in epidemiological evaluations for its multiple medicinal effects. The medicinal part of the plant is the garlic bulb, used, fresh, dehydrated, or steam-distilled oil (Tattelman, 2005).

A. sativum oil has antitumor, antioxidant, and antimicrobial properties against gram-positive and gram-negative bacteria, such as *Salmonella typhimurium* and *Escherichia coli* (Singh and Singh, 2008).

The effect of *A. sativum* was evaluated by performing an experiment on Specific Pathogen Free (SPF) embryonated eggs inoculated with aqueous *A. sativum* extract and two strains of IBV, 4/91 (acute) being the vaccine strain and M41 (subacute) the field strain. Groups inoculated with *A. sativum* extract 8 hours after exposure to IBV were evaluated to determine the phase of infection during which the *A. Sativum* affects the virus. The results indicated that *A. sativum* extract may affect the virus in the replication phase. It appears from this study that *A. sativum* extract has variable effects, with greater efficacy on sub-acute strains. However, as a treatment after exposure to the virus, the effect is significant on both sub-acute and acute strains, as it has reduced the mean embryo index (EI) in both strains, with p-values<0.5, showing statistical significance (Table 1) (Mohajer Shojai *et al*., 2016).

The round tiger nut, *Cyperus rotundus L.*

Cyperus rotundus L. of the Cyperaceae family is considered a noxious weed to crops but is valued for its therapeutic effects, including anti-inflammatory, antimicrobial, antioxidant, antidiabetic, and antispasmodic properties (Singh *et al*., 2016). The parts of *C. rotundus* used are its tuber, leaves, seeds, rhizomes, and oil, as they contain the major active components: flavonoids, terpenoids, and essential oil (Das *et al*., 2015).

A recent study combined the aqueous extract of the roots of *C. rotundus L.* and silver nitrate ($AgNO_3$) to synthesize green silver nanoparticles (Figure 1). After determining the maximum nontoxic concentration (MNTC)

Table 1: *A. sativum* effect on IBV strains *in ovo* at dilution of 10^{-3}

Groups	IBV strain	Mean EI	P value
1	4/91	3420	<0.05
2	4/91	3675	<0.05
3	M41	1588	<0.05
4	M41	1903	<0.05
5	Non-treatment	3804	-

1: IBV-infected group (4/91, no A. sativum extract). 2: 4/91+A. sativum extract treatment 8 hours after exposure. 3: IBV-infected group (M41, no A. sativum extract). 4: M41+A. sativum extract treatment 8 hours after exposure. 5: Non-treatment control (no IBV and no A. sativum extract)

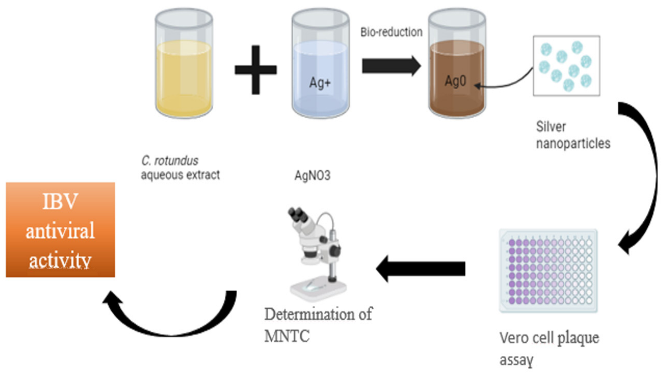

Figure 1: C. rotundus silver nanoparticles synthesis

by microscopically observing Vero cells' morphology 12 hours post-incubation, Vero cells were exposed to different concentrations of the aqueous extract and the corresponding nanoparticles before and following infection. The results showed antiviral activity of green silver nanoparticles in both pre-exposure and post-exposure cases to IBV (undetermined strain). However, the antiviral effect was greater for treated cells after virus infection (Figure 2). This study suggested an interaction of *C. rotundus* nanoparticles with the proteins of the outer envelope of IBV (Abo-El-Yazid *et al.*, 2022).

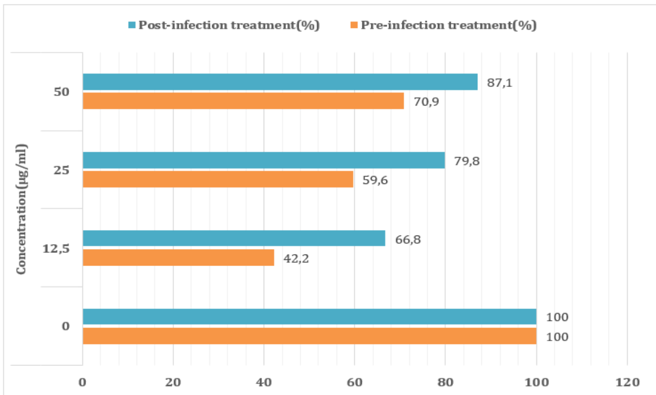

Figure 2: Antiviral activity of C. rotundus silver nanoparticles on IBV

Weeping Forsythia, *Forsythia suspensa* Vahl

Forsythia suspensa Vahl, from the Oleaceae family, is a shrub native to China whose fruits have been widely used in Asia for treating gonorrhea, erysipelas, inflammations, pyrexia, and ulcers. Its leaves have also been added to tea as a nutritional supplement (Qu *et al.*, 2010). *F. suspensa* has both antibacterial and antioxidant activities. A 2007 study also demonstrated the antibacterial and antioxidant activities due to its compounds forsythiaside (forsythoside-A) and forsythine (Qu *et al.*, 2008).

The antiviral activity of forsythoside A against the M41 and Beaudette field strains of IBV has been evaluated in vitro in chicken embryo kidney (CEK) cells and baby hamster kidney (BHK) cells, as well as *in vivo* on chicken. In CEK cell cultures, results showed that treatment before infection inhibited the production of IBV. In addition, the antiviral effect was confirmed by real-time RT-PCR. However, when administered after infection, forsythoside-A did not decrease the infectivity of the virus. These results prove that forsythoside-A inhibits IBV infection *in vitro* in a dose-dependent manner (Li *et al.*, 2011).

On BHK cell cultures, pretreatment with forsythoside A inhibited IBV replication in a dose-dependent way and reduced inflammation, apoptosis, and autophagy induced by the viral infection (Xu *et al.*, 2023). A 2020 *in vivo* study demonstrated both respiratory symptoms and activities of IBV-infected chickens were improved following forsythoside-A treatment (Wang *et al.*, 2021). Furthermore, a synergistic effect of phylligenin and baicalin, extracted from *F. suspensa* and *Scutellaria baicalensis*, respectively, have proven to be effective against IBV by improving respiratory tract flora and metabolic disorder in broilers (Table 2) (Feng *et al.*, 2024).

Chinese skullcap, *Scutellaria baicalensis*

Scutellaria baicalensis, a flowering plant from the Lamiaceae family, has been famous in traditional medicine. Its roots were known for their numerous pharmacological

Table 2: Overview of *F. suspensa* antiviral activity against IBV

Study	Active compound	Strain	Key findings	Mechanism of action	Effectiveness
In vitro on CEK cells	forsythoside A	M41 field strain	Inhibition of IBV dose-dependently: none for 0.16 mM and 0.32 mM, partial for 0.64 Mm.	Undetermined	- Pre-infection: not effective - Post-infection: effective
In vitro on BHK cells	forsythoside A	Beaudette field strain	-Inhibition of IBV replication -Attenuation of inflammation, apopstosis and autophagy.	-Inhibition of PI3K/Akt/NF-κB signaling pathway.	Effective
In vivo on chicken	forsythoside A	M41 field strain	-Reduced infection rates. -Recovery up to 86.67% for high (80 mg/kg/d) and medium dose (40 mg/kg/d) treatment.	-Increase of CD3+, CD4+ and CD8+T-lymphocyte levels. -Maintenance of IL-2 IL-4 and IFN- α concentrations.	- Prevention: effective - Treatment: effective
In vivo on chicken	-Philligenin (*F. suspensa* extract) -Baicalin (*Scutellaria baicalensis* extract)	M41 field strain	-Improvement of growth and respiration, reduction of viral load and histopathological changes and modulation of metabolism and respiratory microbiota (24 mg/ml and 450 mg/ml).	-Promotion of G3BP1 expression andante-IBV antibody level.	Synergistic effect

properties, including antioxidant, antiviral, and antimicrobial effects (Zhao *et al.*, 2016). One of its main active compounds is baicalin, a flavonoid used to prevent IBV infection. In addition to its synergistic effect with phylligenin (extract of *F. suspensa*) against IBV (Feng *et al.*, 2024), a recent study tested its efficacity on a nephropathogenic strain (NIBV) *in vivo*. IBV-infected chicken treated intranasally with 10 mg/kg of baicalin showed improved spleen health and alleviation of NIBV infections overall. Specifically, baicalin inhibited mitophagy induction and promoted innate immunity by activating type 1 IFN response (Tian *et al.*, 2023).

Chinese pepper, *Houttuynia cordata*

Hottuynia cordata is an herbaceous plant of the Saururaceae family, rich in oil, flavonoids, and alkaloids (Bauer *et al.*, 1996). Pharmacological studies have indicated that the components of the essential oil of *H. cordata* possess anti-inflammatory, antibacterial, and antiviral activities. Furthermore, flavonoids had antineoplastic, antioxidant, and antimutagenic effects, while alkaloids were proven to have potent antiplatelet and cytotoxic properties (Fu *et al.*, 2013).

The inhibitory effect of *H. cordata* on Beaudette and HH06 IBV strains was achieved by using its essential oil with sodium chloride solution (Yin *et al.*, 2011). As the Beaudette strain didn't cause any mortality, the HH06 IBV strain was used in all experiments (Table 3). *In vitro*, the mixture showed equal efficacy on Vero and CEK cells. RT-PCR analysis targeting the S1 gene suggested partial inactivation of the virus. *In ovo, H. cordata* prevented embryo mortality of SPF eggs, and *in vivo*, the virus had lower infectivity in chickens but did not provide complete protection. Likewise, using *in vitro* test, *H. cordata* failed to protect virus-infected cells. As a result, *H. cordata* can be combined with other drugs or vaccines to get a better result (Yin *et al.*, 2011).

Table 3: Antiviral activity of *H. cordata* on IBV

Study	Effectiveness on IBV
In vitro on CEK and Vero cells	-More than 90% inhibition rate. -Decrease of apoptotic cells by more than 90%.
In ovo on SPF eggs	100% protection rate.
In vivo on SPF chicken	More than 50% protection rate.

Perforated St. John's Wort, *Hypericum perforatum*

Hypericum perforatum is an angiosperm plant of the Clusiaceae family. The main active constituents are hyperforin and hypericin (Barnes *et al.*, 2001). Several pharmacological activities have been documented, including wound healing and antimalarial effects, as well as antioxidant, anticancer, antiviral, antibacterial, and antifungal properties. St. John's Wort is also used to treat psychosomatic disorders, seizures, depression, dementia, and anxiety (Velingkar *et al.*, 2017; Mullaicharam and Halligudi, 2018).

The predominant phytochemicals of *H. perforatum* are synthesized and stored in glands and secretory pockets located at the level of its aerial parts and are accumulated exclusively in the flowers (Patočka, 2003; Velingkar *et al.*, 2017).

The antiviral activity of *H. perforatum* on the M41 field IBV strain was evaluated using ethyl acetate extract. In vitro testing on CEK cells showed a dose-dependent reduction in viral mRNA levels following treatment with the ethyl acetate extract and inhibition of IBV-induced apoptosis. *In vivo*, the infected group treated with a gradual increase in extract dose showed decreased excretion of tracheal cilia and epithelial cells and reduced inflammatory serous fluid exudation. Results revealed fewer inflammatory cells and erythrocytes and reduced mRNA expression levels in the trachea and kidneys of chicks treated with ethyl acetate. Therefore, the extract from *H. perforatum* has a strong antiviral effect on IBV (Chen *et al.*, 2019). Another study investigating the activity of hypericin on the M41 IBV strain in CEK cells inhibited the virus-induced apostosis and reduced reactive oxygen species (ROS) generation induced by IBV (Table 4) (Chen *et al.*, 2019).

Ginseng, *Panax ginseng*

Panax ginseng is a Korean plant from the Araliaceae family. The main active ingredients of *P. ginseng* are the ginsenosides extracted from the roots, leaves, and flowers (Gillis, 1997). The pharmacological effects are improvement of brain functions, inhibition of pain, prevention of cancer, and strengthening of the immune system (Choi, 2008).

The therapeutic activity of *P. ginseng* against the H120 vaccine strain was evaluated in chickens by combining ginseng stem-leaf saponins (GSLS) with selenium (Se) and administrating it as an adjuvant to the vaccine (Ma *et al.*, 2019). The results showed a strengthening of the im-

Table 4: Mechanism of action of *H. perforatum* active compounds on IBV

Study	Main component	Mechanism of action
In vitro on CEK cells	Ethyl acetate extract	-Up-regulation of type I interferon (IFN). -Down-regulation of IL-6 and TNF- α via NF- κB pathway.
In vivo on chicken	Ethyl acetate extract	-Decrease of mRNA levels of IL-6, TNF- α, NF- Kb. -Increase of mRNA levels of MDA5, IFN- α and IFN- β.
In vitro on CEK cells	Hypericin	-Apoptosis: Decrease of mRNA levels of Fas, FasL, JNK, Bax, Caspase 3, Caspase 8. Increase of Bcl-2 expression. -Reduction of ROS generation.

mune response through rapid and lasting production of antibodies, proliferation of lymphocytes and cytokines in the blood, and a rise of neutralizing antibodies (Ma *et al*., 2019).

Cholistani Plants

Different plants native to the Cholistan desert in Pakistan are renowned for their medicinal benefits, including their antiviral effect, especially against IBV. These plant species include *Oxystelma esculentum, Achyranthes aspera, Salsola baryosma, Neuroda procumbens,* and *Ochthochloa compressa* (Aslam *et al*., 2010) (Shahzad *et al*., 2020).

The antiviral effects of Cholistani Plants were tested against IBV using different extracts, namely n-hexane, ethyl acetate, and n-butanol extracts. The results showed that 5 n-hexane extracts out of 11, 10 ethyl acetate extracts out of 11, and 5 n-butanol extracts out of 11 were active against IBV. *O. esculentum, A. aspera, S. baryosma, N. procumbens,* and *O. compressa* are the five plants whose extracts were effective in controlling IBV (Table 5) (Figure 3) (Shahzad *et al*., 2020).

Elderberry, *Sambucus nigra*

Sambucus nigra L., a shrub in the family Adoxaceae, is recognized for its antioxidant, anti-inflammatory, antiallergic, antiviral, anti-urolithiasis, and antidiabetic pharmacological effects. All parts of the plant (flowers, fruits, leaves, bark, and roots) proved effective. However, the most commonly used parts are the flowers and fruits, which are included in the composition of many food products and pharmaceutical and cosmetic preparations (Młynarczyk and Walkowiak-Tomczak, 2017).

S. nigra is known for its antiviral activity against influenza A and B viruses. Treatment of IBV Beaudette strain in Vero cells with the extract of *S. nigra* at an early stage of infection inhibited the virus. Observation by electron microscopy revealed that the extract of *S. nigra* disrupts the structure of the IBV virion, as compromised envelopes and the presence of membrane vesicles were observed, likely rendering it non-infectious. The hypothesis is that the polyphenols, with antiviral properties, present at high levels in the plant are the source of this inhibition (Chen *et al*., 2014).

Enantia chlorantha:

Enantia chlorantha is a high tree mainly found in tropical Africa (Cameroon, Gabon, and Congo) and undergrowth of the rainforest from the south of Nigeria to Angola. Palmatine, the main component of the protoberberine extract from this tree, is a natural non-flavonoid alkaloid that has proven effective against IBV. *In vitro*, palmatine inactivated IBV (undetermined strain). It inhibited the adsorption, penetration, and intracellular replication in CEK cells by increasing pro-inflammatory cytokines *IL-1β* and *TNF-α* expressions and inducing type 1 interferon production (Figure 4) (Zhang *et al*., 2024).

Table 5: Effectiveness of various cholistani plant extracts against IBV using HA titer values

Plant / HA titer	*O. esculentum*	*N. procumbus*	*A. aspera*	*S. baryosma*	*S. surattense*	*S. icolados*	*O. compress*	*H. recurvum*	*P. antodotale*	*S. fructicosa*	*H. salicornum*
n-hexane	0	0	0	0	64	512	64	64	64	512	2048
ethyl acetate	0	0	0	0	0	0	32	2048	8	32	0
n-butanol	8	64	0	0	128	256	8	128	128	64	2048

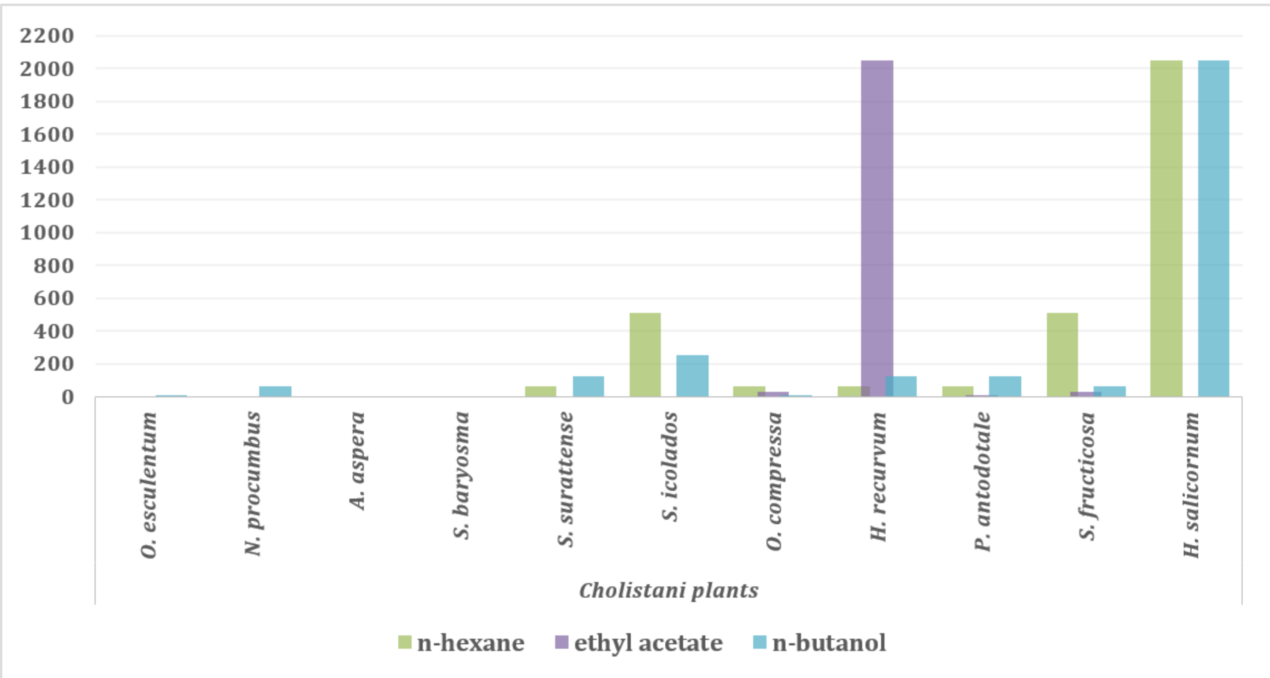

Figure 3: HA titers of active components of various cholistani plants

Mixed Herbal Extract

Certain plants of the families Myricaceae, Pinaceae, Primulaceae, Polygonaceae, and Anacardiaceae naturally contain a flavonoid, myricetin, that has various pharmacological powers: antioxidant, antiviral, antibacterial, anticancer, and anti-inflammatory (Agraharam *et al.*, 2022).

After treating IBV-infected Vero cells (undetermined strain) with myricetin, the duration of expression of the antiviral effect in these cells was shorter than in virus-infected not-treated cells, which means that the myricetin may play a role in inhibiting the immune evasion mechanism of IBV and enhancing the innate immune response against IBV infection. The results showed that myricetin targeted the Papain-Like protease (PLpro) of IBV by inhibiting the deubiquitination of its activity and modulating NF- κB and IRF7 pathways (Peng *et al.*, 2022).

Antiviral activity of mixtures

The mixtures are blends of plant extracts of different natures, natural active ingredients, essences, or essential oils which have therapeutic properties for controlling IBV.

Mixture AuraShield L

AuraShield L is a blend of citrus extract, maltodextrin, sodium chloride, lactic acid, and citric acid. The *in vitro* experiment was conducted in Hep-2 cells using the B1648 IBV strain. Pretreatment with AuraShield L reduced plaque count by over 80%, while post-treatment led to a 52% reduction in virus titer. The *in vivo* experiment in chickens using the same strain showed the AuraShield L mixture inclusion in drinking water reduced viral load in the trachea and lungs of broiler chickens by more than 80%. It also reduced clinical signs, such as rales, sneezing, coughing, and dyspnea, registering a 93.7% survival rate in the treated group vs. 66.6% in the untreated one. The mechanism of action might involve an increase in IgA levels and short-chain fatty acid production in both the trachea and lungs (Balta *et al.*, 2020).

Mixture QR448(a)

QR448(a) is an antiviral product composed of a mixture of oleoresins and essential plant oils (produced by Quigley Pharma, 2008), recommended for the transmission prevention of IBV in chickens.

The effect of QR448(a) on IBV was tested *in vitro* in Vero cell culture and embryonated eggs using the Beaudette strain and in chickens using the M41 and Ark. strains (Jackwood *et al.*, 2010).

For Vero cell culture, the minimum dose against the Beaudette strain of IBV was 1×10^3 at $1 \times 10^{0,8}$ $TCID_{50}$/ml.

In vivo experiences in IBV-infected chickens showed the efficacy of administering QR448(a) by intranasal inoculation and spraying two hours before the challenge, whereas water administration had little effect regardless of the treatment period. Therefore, QR448(a) reduced IBV titer below an infectious threshold (Jackwood *et al.*, 2010).

Treatment with QR448(a) protected chickens up to 4 days after treatment from the expression of clinical signs of disease, but not against infection, and reduced IBV transmission over 14 days, as stated by Quigley Pharma.

QR488(a)-induced transmission reduction could control the spread of IBV in the herd and likely decrease or prevent the persistence of the Arkansas virus. Since QR448(a) is effective against the pathogenic M41 strain of IBV, it may also limit the transmission of field pathogenic strains of IBV in commercial broilers (Jackwood *et al.*, 2010).

Antiviral activity of essential oils

Essential oils are a complex mixture of volatile organic compounds naturally synthesized by plants and extracted through low-pressure steam distillation. Their structure includes a range of chemical compounds, including phenolics, terpenoids, aldehydes, ketones, ethers, and epoxides. These components confer efficacy against bacterial, fungal, and viral pathogens, as well as the potential to enhance the immune system (Zhang *et al.*, 2022).

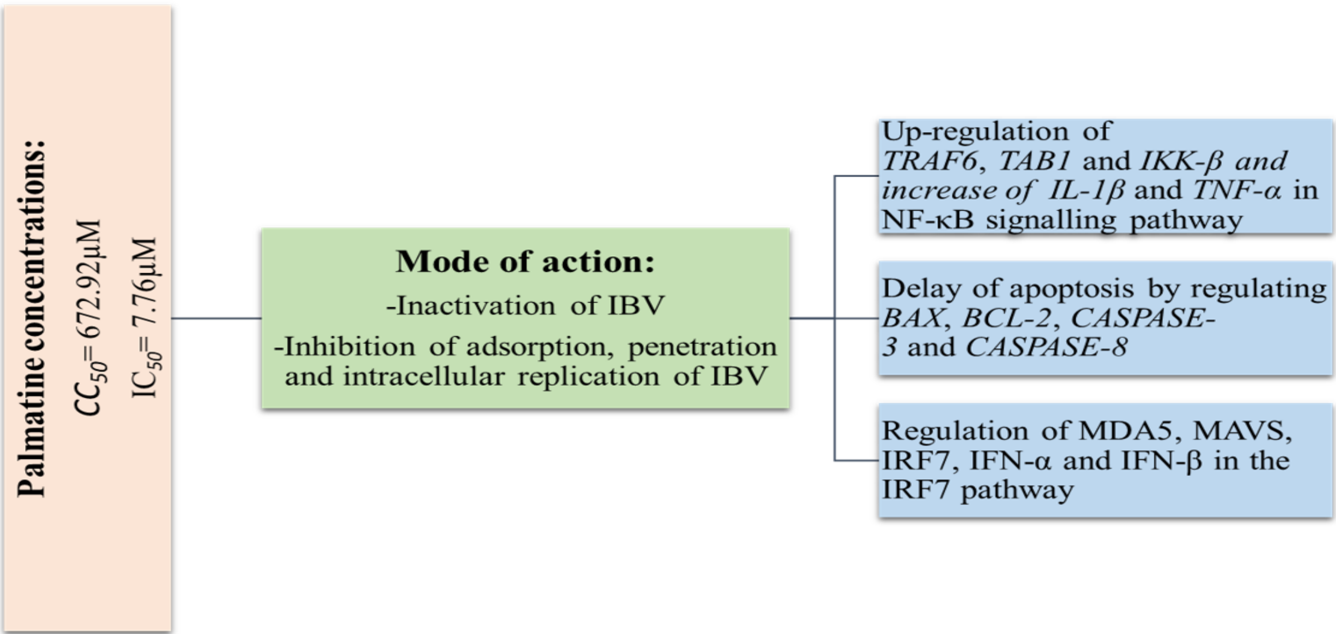

Figure 4: Palmatine's mechanisms and pathways against IBV in CEK cells

CC_{50}= Cytotoxic Concentration 50; IC_{50}= Inhibitory Concentration 50

Mixture of Cinnamaldehyde and Glycerol Monolaurate

The antiviral effect against IBV *in vivo* of a mixture of cinnamaldehyde (CA) extracted from cinnamon and glycerol monolaurate (GML) extracted from coconut oil was studied by Zhang *et al.* (2022). The choice of these compounds was due to their documented antiviral properties. Among the virtues of CA is its beneficial effect on nutrition, metabolism, and growth of broilers and enhancement of their immune function (Bravo *et al.*, 2011). On the other hand, GML is a natural compound considered a safe and effective alternative to antibiotics; it promotes growth and has antimicrobial, antiviral, and anti-inflammatory properties (Bravo *et al.*, 2011; Minjie Zhao *et al.*, 2020).

The experiment used broiler chickens to evaluate the effectiveness of the mixture of plant essential oils (PEO) made of CA and GML *in vivo* (Figure 5). All the groups received nasal-ocular 10^8 50% tracheal organ culture infection dose ($TOC\text{-}ID_{50}$), except the blank group (uninfected and untreated). Results relating to antiviral therapy against the M41 IBV strain showed out of three doses tested (PEO-L: 0.5 ml/l; PEO-M:1 ml/l and PEO-H: 2 ml/l), 1ml/l registered the highest cure rate of 92.3%, reduced symptoms in chickens and decreased mRNA expression levels and IL-6 levels, while increasing IL-4 and IFN-γ anti-inflammatory factors. Therefore, the mixture of CA and GML could significantly inhibit viral proliferation and improve immune function for a dose of 1 ml/l in drinking water in chickens (Zhang *et al.*, 2022).

Mixture of L-menthol and cineole

The therapeutic actions of essential oils on respiratory infection in broiler chickens were tested by Barbour *et al.* (2008), using a mixture of eucalyptus and peppermint essential oils. Its active ingredient, 1,8-cineol, known as eucalyptol, is used to reduce respiratory infections of viral or bacterial origin (Barbour *et al.*, 2008).

Eucalyptus spp. of the Myrtaceae family is an aromatic tree native to Australia with wide adaptation, particularly *Eucalyptus globulus*, a species introduced to multiple environments for the production of paper pulp, notably in Europe around the Mediterranean, in North Africa, and Latin America. A study on the chemical composition of the essential oils of eight species of eucalyptus, as well as the evaluation of their antibacterial, antifungal, and antiviral activities, showed that the species *E. cinerea* is the richest in 1,8-cineol and that its antimicrobial activity is the highest (Elaissi *et al.*, 2012).

Eucalyptus is toxic to humans and animals in general. It contains cyanogenic glycosides that can release hydrocyanic acid and block cellular respiration (Gleadow and Møller, 2014). However, cineole eucalyptus oil is safe for adults; systemic toxicity may result from ingestion or topical application of quantities above recommended doses (Darben *et al.*, 2007).

Mentha piperita is an aromatic plant from the Lamiaceae family. The leaves are generally destined for culinary purposes, while the essential oil is used to flavor confectionery, toothpaste, and medicine. Pure peppermint oil is almost colorless and is rich in menthol.

The mixture of L-menthol and cineole was administered to immuno-compromised broiler chickens exposed to *Mycoplasma gallisepticum*, the live strains B1 and LaSota Newcastle of the vaccine against the disease, the virus infectious bursal disease and Mass. IBV strain. The obtained results showed that this mixture was able to reduce morbidity 2 days after the IBV challenge and to reduce specific lesions such as abdominal airsacculitis, enteritis, splenomegaly, bursal congestion, and liver hypertrophy

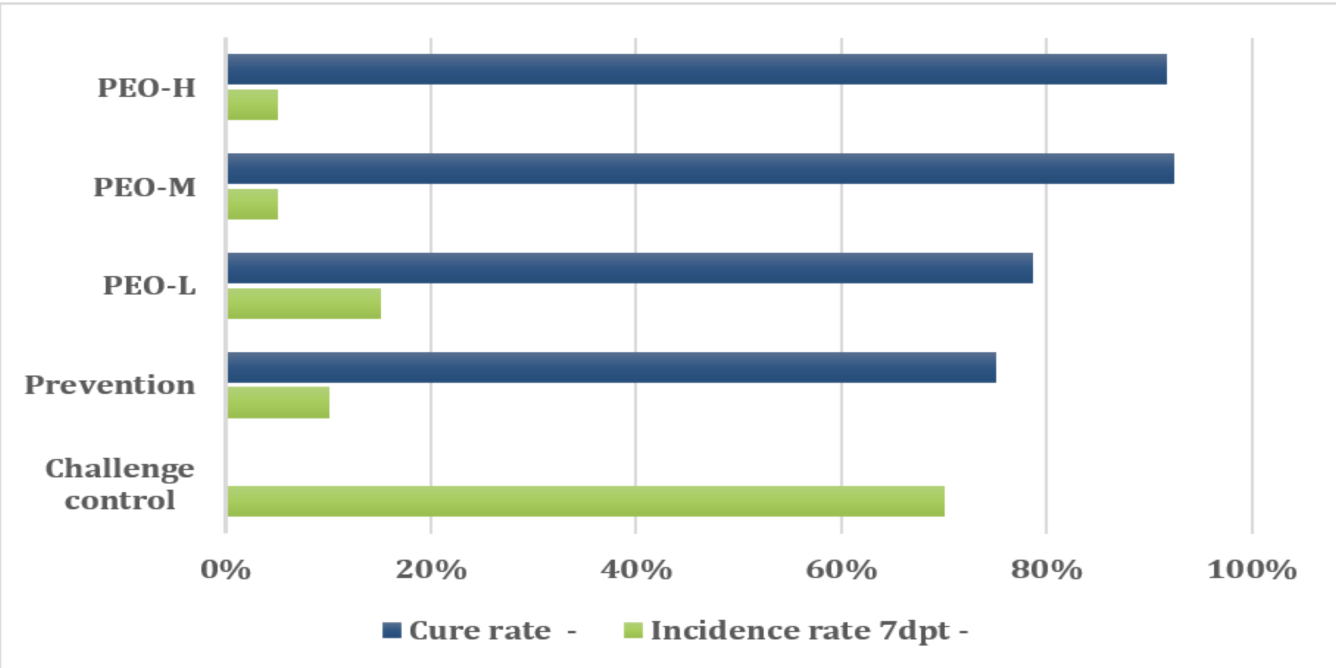

Figure 5: Antiviral activity of PEO against IBV in vivo

Challenge control: uninfected and untreated; Prevention: treated with 1.5 ml/l of PEO 5 days before challenge; PEO-L: 0.5 ml/l; PEO-M: 1ml/l; PEO-H: 1.5 ml/l; 7dpt: 7 days post-treatment; 5dpc: 5 days post-challenge

(Barbour *et al.*, 2008). The effective dose for IBV treatment was 0.25 ml/l of drinking water. In addition, treatment with this mixture improved the feed consumption index and the conversion coefficient (Barbour *et al.*, 2008).

DISCUSSION

In the articles consulted, the plants used are frequently native species, which gives the therapeutic trials a regional character specific to the strains of IBV identified locally. Other plants are cosmopolitan species whose tests are more extended geographically. Also, many plant-derived antiviral compounds have been, fully or partially, characterized as bioactive compounds, able to directly or indirectly reduce viral bronchial infection in vitro or in vivo. The most commonly used test methods to assess IBV infectivity include cell viability assays and quantitative RT-PCR.

Our review focused on the action of plants against IBV strains, emphasizing the most significant mechanisms of action. The modes of action that emerged from our review showed that plants such as *A. sativum* and coconut oil extract (GML) were effective during the replication phase. Others, however, affected the virus envelope, either by altering its proteins or by inactivating or disrupting the viral membrane, such as *C. rotondus, S. nigra*, or the QR448 mixture. *P. ginseng, F. suspense,* and *H. perforatum*, as well as the Aurashield blend targeted lymphocytes and reduced pro-inflammatory cytokines levels. Myricetin inhibited the deubiquitinating activity of the papain-like protease, restoring the ubiquitin modification and promoting an innate response.

The molecular mechanisms of some natural products against IBV have been partially elucidated, while for others, they remain unknown. The variety of research protocols has made comparing results across studies challenging. Furthermore, *in vitro* results do not always translate to *in vivo* effectiveness because of variations in metabolism, absorption, and immune responses. *In vivo* experiments also face challenges due to the lack of mechanism insights, the influence of health conditions, and the route of treatment administration, all of which can affect absorption. Notably, recent *in ovo* experiments using thymoquinone—a bioactive compound extracted from *Nigella sativa*—demonstrated significant antiviral activity against IBV, supporting its potential as a natural antiviral agent (Regragui *et al.*, 2025). These findings contribute to the growing body of evidence highlighting the efficacy of plant-derived compounds in controlling IBV infections *in vivo*.

Some experiments used field strains, mainly the M41, while others used vaccine strains, such as H120 and 4/91. Vaccine strains are attenuated or inactivated versions of the pathogen and using them in a controlled setting decreases the risk of causing an outbreak. Because vaccine strains are standardized, their consistency reduces variability in results and ensures reproducibility across different studies and laboratories. On the other hand, using field strains in experimentation provides real-world relevance. It also allows researchers to evaluate products' effectiveness against a wide range of viral variants present in the field. Experimenting with field strains ensures realistic outcomes, as researchers can predict how the treatment will act on viruses circulating in the field, thus finding practical solutions and a better understanding of the effectiveness of treatments in natural environments.

While testing various antiviral natural products may seem advantageous, the variability in their composition could lead to inconsistent results. Factors such as the source and geography of the plant change active compound levels and influence biochemical profiles. Moreover, the extraction methods of active components from plants can affect their composition. Due to this inherent variability, achieving standardized protocols proved complicated, making it challenging to draw reliable conclusions about their efficacy.

This variability has also been observed in antibacterial studies using essential oils, such as those derived from cinnamon and oregano, which showed significant effects against avian *Escherichia coli* strains (Khribch *et al.*, 2018).

Similarly, essential oils from plants like *Plectranthus amboinicus* have demonstrated antimicrobial potential, reinforcing the interest in natural substances for managing infectious diseases in poultry (Hassani *et al.*, 2012).

However, all the studies showing efficacy against IBV provided a strong basis for implementing strategies against all coronaviruses, as natural products used to reduce respiratory inflammation caused by IBV or to modulate the immune response could yield promising results in managing Sars-CoV-2 infections and addressing the virus complications.

CONCLUSION

It emerges from this research that certain plants tested revealed promising properties against strains of IBV, particularly the inhibiting action of the infection by their different extracts. The results from this synthesis confirm the importance of plant selection for the adjustment of their bioactivity according to a set of criteria: i) Plant availability; ii) Adequate technique for extraction of the active ingredient; iii) Identification of bioactive molecules and the stage of their antiviral performance and iv) Potential and cost of their large-scale application. However, the lack of statistical analysis in many studies prevents drawing conclusive results about the efficacy of natural products, and remedying this requires more thorough research. That is why establishing standardized protocols that ensure repeatability and reproducibility is essential for having reliable findings.

Therefore, the application of a therapeutic protocol highlighting these active substances, or the possibility of combining them and using them as adjuvants, proves to be a necessary and promising alternative for broad efficacy against IBV. With further research, these results could be extrapolated to other coronaviruses, especially human ones such as MERS-CoV, SARS-CoV, and COVID-19.

REFERENCES

Abo-El-Yazid Z.H., Ahmed O.K., El-Tholoth M., Ali M.A.S. (2022). Green synthesized silver nanoparticles using *Cyperus rotundus* L. extract as a potential antiviral agent against infectious laryngotracheitis and infectious bronchitis viruses in chickens. *Chemical and Biological Technologies in Agriculture*, 9: 55.

Agraharam G., Girigoswami A., Girigoswami K. (2022). Myricetin: a Multifunctional Flavonol in Biomedicine. *Current Pharmacology Reports,* 8: 48–61.

Aslam A. , Mirza Imran Shahzad S.P., Ashraf H., Naz N., Zehra S. S., Zahid Kamran, Qayyum A., Mukhtar M.M. (2010). Evaluation of Antiviral Potential of Different Cholistani Plants against Infectious Bursal Disease and Infectious Bronchitis Virus. *Pakistan Veterinary Journal. Animals*, 36: 302-306.

Balta I., Stef L., Pet I., Ward P., Callaway T., Ricke S.C., Gundogdu O., Corcionivoschi N. (2020). Antiviral activity of a novel mixture of natural antimicrobials, *in vitro*, and in a chicken infection model *in vivo*. *Scientific Reports*, 10:16631.

Barbour E.K., Yaghi R.H., Shaib H.A., Tayeb I.T., Sleiman F.T. (2008). Evaluation of an essential oil in treatment of immunosuppressed-coinfected broilers. *Journal of Sustainable Agriculture*, 2: 212–218.

Barjesteh N., O'Dowd K., Vahedi S.M. (2020). Antiviral responses against chicken respiratory infections: Focus on avian influenza virus and infectious bronchitis virus. *Cytokine*, 127: 154961.

Barnes J., Anderson L.A., Phillipson J.D. (2001). St John's wort (*Hypericum perforatum* L.): a review of its chemistry, pharmacology and clinical properties. *Journal of Pharmacy and Pharmacology,* 53: 583–600.

Bauer R., Pröbstle A., Lotter H., Wagner-Redecker W., Matthiesen U. (1996). Cyclooxygenase inhibitory constituents from *Houttuynia cordata*. *Phytomedicine*, 2: 305–308.

Bravo D., Utterback P., Parsons C.M. (2011). Evaluation of a mixture of carvacrol, cinnamaldehyde, and capsicum oleoresin for improving growth performance and metabolizable energy in broiler chicks fed corn and soybean meal. *Journal of Applied Poultry Research*, 20: 115–120.

Britton P., Evans S., Dove B., Davies M., Casais R., Cavanagh D. (2005). Generation of a recombinant avian coronavirus infectious bronchitis virus using transient dominant selection. *Journal of Virological Methods*, 123: 203–211.

Brochu Morin, M.-E. Chénier S., Bergeron N. (2018). La bronchite infectieuse aviaire au Québec. *Bulletin Zoosanitaire*.

Casais R., Davies M., Cavanagh D., Britton P. (2005). Gene 5 of the avian coronavirus infectious bronchitis virus is not essential for replication. *Journal of Virology*, 79: 8065–8078.

Cavanagh D. (2005). Coronaviruses in poultry and other birds. *Avian Pathology*, 34: 439–448.

Cavanagh D., Naqi S.A. (2003). Infectious Bronchitis. In H. J. Barnes J.R. Glisson A.M. Fadly L.R. McDougald D.E. Swayne Y., M. Saif (Eds.), Diseases of Poultry (11^{th} ed., pp. 101–119). Iowa State University Press.

Chen C., Zuckerman D.M., Brantley S., Sharpe M., Childress K., Hoiczyk E., Pendleton A.R. (2014). *Sambucus nigra* extracts inhibit infectious bronchitis virus at an early point during replication. *BMC veterinary research*, 10: 24.

Chen H., Feng R., Muhammad I., Abbas G., Zhang Y., Ren Y., Huang X., Zhang R., Diao L., Wang X., Li G. (2019). Protective effects of hypericin against infectious bronchitis virus induced apoptosis and reactive oxygen species in chicken embryo kidney cells. *Poultry Science*, 98: 6367–6377.

Chen H., Muhammad I., Zhang Y., Ren Y., Zhang R., Huang X., Diao L., Liu H., Li X., Sun X., Abbas G., Li G. (2019). Antiviral activity against infectious bronchitis virus and bioactive components of *Hypericum perforatum* L. *Frontiers in Pharmacology*, 10: 1272.

Choi K.T. (2008). Botanical characteristics, pharmacological effects and medicinal components of Korean Panax ginseng C A Meyer. *Acta Pharmacologica Sinica,* 29: 1109–1118.

Darben T., Cominos B., Lee C. (2007). Topical eucalyptus oil poisoning. *Australasian Journal of Dermatology*, 39: 265–267.

Das B., Pal D., Haldar A. (2015). A review on *Cyperus rotundus* as a tremendous source of pharmacologically active herbal medicine. *International Journal of Green Pharmacy,* 9: 4.

Elaissi A., Rouis Z., Salem N.A.B., Mabrouk S., Ben Salem Y., Salah K.B.H., Aouni M., Farhat F., Chemli R., Harzallah-Skhiri F., Khouja M.L. (2012). Chemical composition of 8 eucalyptus species' essential oils and the evaluation of their antibacterial, antifungal and antiviral activities. *BMC Complementary and Alternative Medicine*, 12: 1–15.

Fellahi S., Ducatez M., El Harrak M., Guérin J.L., Touil N., Sebbar G., Bouaiti ElA., Khataby K., Ennaji M.M., El-Houadfi M. (2015). Prevalence and molecular characterization of avian infectious bronchitis virus in poultry flocks in Morocco from 2010 to 2014 and first detection of Italy 02 in Africa. *Avian Pathology*, 44: 287–295.

Feng H., Zhang J., Zhang K., Wang X., Guo Z., Wang L., Li J. (2024). Synergistic anti-infectious bronchitis virus activity of Phillygenin combined Baicalin by modulating respiratory microbiota and improving metabolic disorders. *Poultry Science*, 103:2.

Fu J., Dai L., Lin Z., Lu H. (2013). *Houttuynia cordata* Thunb: A Review of Phytochemistry and Pharmacology and Quality Control. *Chinese Medicine*, 04: 101–123.

Gelb J., Weisman Y., Ladman B.S., Meir R. (2005). S1 gene characteristics and efficacy of vaccination against infectious bronchitis virus field isolates from the United States and Israel (1996 to 2000). *Avian Pathology*, 34: 194–203.

Gillis N. (1997). Punax ginseng Pharmacology: A Nitric Oxide Link? *Biochemical Pharmacology*, 54: 1-8.

Gleadow R.M., Møller B.L. (2014). Cyanogenic glycosides: Synthesis, physiology, and phenotypic plasticity. *Annual Review of Plant Biology*, 65: 155–185.

Hassani M.S., Zainati I., Zrira S., Mahdi S., Oukessou M. (2012). Chemical Composition and Antimicrobial Activity of *Plectranthus amboinicus* (Lour) Spring. Essential Oil from Archipelago of Comoros. *Journal of Essential Oil Bearing Plants*, 15: 637–644.

Jackwood M.W., Rosenbloom R., Petteruti M., Hilt D.A., McCall A.W., Williams S.M. (2010). Avian coronavirus infectious bronchitis virus susceptibility to botanical oleoresins and essential oils *in vitro* and *in vivo*. *Virus Research*, 149: 86–94.

Khribch J., Smara N., Laghrib F., Nassik S., Zrira S., El Houadfi M., Oukessou M. (2018). Comparative study of the antibacterial activity of cinnamon and origan essential oils and their primary components on avian *Escherichia coli* strains. *International Journal of Advanced Research*, 6: 373–381.

Lai M.M.C., Cavanagh D. (1997). The molecular biology of coronaviruses. *Advances in virus research*, 48: 1-100.

Li H., Wu J., Zhang Z., Ma Y., Liao F., Zhang Y., Wu G. (2011). Forsythoside A inhibits the avian infectious bronchitis virus in cell culture. *Phytotherapy Research*, 25: 338–342.

Liu S., Chen J., Han Z., Zhang Q., Shao Y., Kong X., Tong G. (2006). Infectious bronchitis virus: S1 gene characteristics of vaccines used in China and efficacy of vaccination against heterologous strains from China. *Avian Pathology*, 35: 394–399.

Ma X., Bi S., Wang Y., Chi X., Hu S. (2019). Combined adjuvant effect of ginseng stem-leaf saponins and selenium on immune responses to a live bivalent vaccine of Newcastle disease virus and infectious bronchitis virus in chickens. *Poultry Science*, 98: 3548–3556.

Minjie Zhao Z.J., Haiying Cai, Yang Li, Qiufen Mo, Lingli Deng, H.Z., Liu T., Hui Zhang, Jing X. Kang, Feng F. (2020). Modulation of the gut microbiota during high-dose glycerol monolaurate-mediated amelioration of obesity in mice fed a high fat diet. *Mbio*, 11: 10–1128.

Młynarczyk K., Walkowiak-Tomczak D. (2017). Bioactive properties of elderflowers (*Sambucus nigra* L.). *World Scientific News*, 2: 115–119.

Mohajer Shojai T., Ghalyanchi Langeroudi A., Karimi V., Barin A., Sadri N. (2016). The effect of *Allium sativum* (Garlic) extract on infectious bronchitis virus in specific pathogen free embryonic egg. *Avicenna journal of phytomedicine,* 6: 4.

Mullaicharam A.R., Halligudi N. (2018). St John's wort (*Hypericum perforatum* L.): A Review of its Chemistry, Pharmacology and Clinical properties. *International Journal of Research In Phytochemical And Pharmacological Sciences,* 1:1.

Patočka J. (2003). The chemistry, pharmacology, and toxicology of the biologically active constituents of the herb *Hypericum perforatum* L. *Journal of Applied Biomedicine*, 1: 61–70.

Peng S., Fang C., He H., Song X., Zhao X., Zou Y., Li L., Jia R., Yin Z. (2022). Myricetin exerts its antiviral activity against infectious bronchitis virus by inhibiting the deubiquitinating activity of papain-like protease. *Poultry Science*, 101: 626.

Qu H., Zhang Y., Wang Y., Li B., Sun W. (2008). Antioxidant and antibacterial activity of two compounds (forsythiaside and forsythin) isolated from *Forsythia suspensa*. *The Journal of Pharmacy and Pharmacology,* 60: 261–266.

Regragui R., Arbani O., Touil N., Oukessou M., Bouzoubaa K., El Houadfi M., Fellahi S. (2025). Surveillance and coinfection dynamics of infectious bronchitis virus and avian influenza H9N2 in Moroccan broiler farms (2021-2023): phylogenetic. *Viruses*, 17: 786.

Regragui, R., Oukessou, M., Touil, N., Rhazzar, Z., & Bouzoubaa, Khalid Fellahi, S. (2025). Antiviral efficacy of thymoquinone, a bioactive compound of Nigella sativa, against avian infectious bronchitis virus. *Brazilian Journal of Biology*, 85, Accepted for publication.

Ren X., Yin Æ.J., Ma Æ.D. (2009). Characterization and membrane gene-based phylogenetic analysis of avian infectious bronchitis virus Chinese strain HH06. *Virus Genes*, 38: 39-45.

Rhazzar Z., Regragui R., Touil N., El Fahime E., Oukessou Mohamed Fellahi S., Nyabi O., Gala J.-L., Ennibi K. (2025). *In vitro* Potential Antiviral SARS-CoV-2 activity of Thymoquinone, the main *Nigella sativa* component. *Reasearch Journal of Pharmacy and Technology*, 18(4).

Shahzad M. I., Anwar S., Ashraf H., Rani Aslam Z., Saba N., Kamran Z., Ali, Arshad (2020). Antiviral activities of Cholistani plants against common poultry viruses. *Tropical Biomedicine,* 37:4.

Siddell S.G. (1994). The Small-Membrane Protein. *The Coronaviridae*, 181-189. Boston, MA: Springer US.

Singh N., Sharma A., Bharadwaj R. (2016). Ethno-Pharmacotherapeutic Activities of *Cyperus Rotundus, International Journal of Multidisciplinary Approachand Studies*, 3: 186-194.

Singh V.K., Singh K. (2008). Pharmacological Effects of Garlic (*Allium sativum* L.). *Annual Review of Biomedical Sciences*, 10: 6–26.

Tattelman E. (2005). Health Effects of Garlic - American Family Physician. www.aafp.org/afp.

Tian G., Huang C., Li Z., Feng C., Zhuang Y., Liu P., Hu G., Gao X., Guo X. (2023). Baicalin mitigates nephropathogenic infectious bronchitis virus infection-induced spleen injury via modulation of mitophagy and macrophage polarization in Hy-Line chick. *Veterinary Microbiology,* 286: 109891.

Touil N., Touzani C.D., Benaissa E.M., Kasouati J., Rhazzar Z., El Annaz H., El Mrimar N., Neffah L., Abi R., Tagajdid R., El Kochri S., Ducatez M., Akhouad Y., Reggad A., El Kassimi Z., Zrara A., Bssaibis F., El Fahime E., Amine I. L., Ennibi K. (2023). Neutralising antibodies against SARS-CoV-2 give important information on Covid-19 epidemic evolution in Rabat, Morocco, March 2020-February 2021. *African Health Sciences*, 23: 400–405.

Velingkar V.S., Gupta G.L., Hegde N.B. (2017). A current update on phytochemistry, pharmacology and herb–drug interactions of *Hypericum perforatum*. *Phytochemistry Reviews,* 16: 725–744.

Wang X., Li X., Wang X., Chen L., Ning E., Fan Y., Wang H., Chen T., Wang W. (2021). Experimental study of Forsythoside A on prevention and treatment of avian infectious bronchitis. *Research in Veterinary Science*, 135: 523–531.

Xu J., Yin P., Liu X., Hou X. (2023). Forsythoside A inhibits apoptosis and autophagy induced by infectious bronchitis virus through regulation of the PI3K/Akt/NF-κB pathway. *Microbiology Spectrum*, 11(6).

Yin J., Li G., Li J., Yang Q., Ren X. (2011). *In vitro* and *in vivo* effects of *Houttuynia cordata* on infectious bronchitis virus. *Avian Pathology*, 40: 491–498.

Zhang W., Chen Y., Zhang H., Su T., Wang J., Zhang Y., Song X. (2024). Antiviral effect of palmatine against infectious bronchitis virus through regulation of NF-κB/IRF7/JAK-STAT signalling pathway and apoptosis. *British Poultry Science*, 65: 119–128.

Zhang Y., Li X.Y., Zhang B.S., Ren L.N., Lu Y.P., Tang J.W., Lv, D., Yong L., Lin L.T., Lin Z.X., Mo Q., Mo M. L. (2022). *In vivo* antiviral effect of plant essential oils against avian infectious bronchitis virus. *BMC Veterinary Research*, 18(1).

Zhao Q., Chen X.Y., Martin C. (2016). *Scutellaria baicalensis*, the golden herb from the garden of Chinese medicinal plants. *Science Bulletin*, 61: 1391–1398.