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Herbal medicine additives as powerful agents to control and prevent avian influenza virus in poultry – a review

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Abstract

The complicated epidemiological situation of Avian Influenza Viruses (AIV) caused by continuous emergence of new subtypes with failure of eradication, monitoring and vaccination strategies opens the door to alternative solutions to save the status quo and prevent new disasters for the poultry industry. Using of synthetic antiviral drugs such as neuraminidase and haemagglutinin inhibitors has been limited due to
development of drug resistance and expensive commercial application. One of the most promising alternatives is herbal products and botanicals. This review presents a comprehensive and specialized view of in vivo studies of herbal plants in poultry species. Many herbal extracts as Nigella Sativa oil, Astragulus, Cochinchna Momordica and Sargassum pallidum polysaccharides proved very effective as adjuvants for AIV vaccines. Another beneficial role of herbs is enhancement of host response to vaccination with further better prevention of infection and easier control. For enumeration not inventory, this is best achieved with the use of virgin coconut oil, Echinacea purpura, Ginseng stem-and-leaf saponins (GSLS), Astragulus polysaccharides (APS), Myrtus Communis oil, Garlic powder, Turmeric, Thyme and Curcumin. This review aimed to evaluate the most of the in vivo studies performed on poultry species as a step and a guide for scientists and field practitioners in establishment of new effective herbal-based drugs for prevention and control of AIV in poultry.

Key words: Eco-treatment, Avian influenza, botanicals, poultry, immunity
Avian Influenza Viruses (AIV) had been clearly established as one of the main causes of severe economic losses due to high mortality and severe decrease of egg production in poultry farms worldwide and, human infection with AIV led to thousands of deaths among human population especially those with direct contact with the poultry industry (Rodrigo and Martins, 2012; Dhingra et al., 2018). AIA viruses are very contagious among birds and some of these viruses can sicken and even kill certain domesticated bird species including chickens, ducks, and turkeys. Infected birds can shed avian influenza A viruses in their saliva, nasal secretions, and feces. Susceptible birds become infected when they have contact with the virus as it is shed by infected birds. They also can become infected through contact with surfaces that are contaminated with virus from infected birds. Infection of poultry with HPAI viruses can cause severe disease with high mortality. AIA viruses are classified into low pathogenic avian influenza (LPAI) A viruses, and highly Pathogenic avian influenza (HPAI) A viruses (Pantin-Jackwood and Swayne, 2009; Dharmayant et al., 2018). HPAI virus strains of subtype H5N1 caused several outbreaks in various countries of Southeast Asia, such as Cambodia, China, Indonesia, India, Bangladesh, Thailand, Viet Nam, Laos, and Myanmar. It led to severe disease and high mortality (mortality rates with flock often above 50%) in both human and animal. Currently, HPAIV H5N1 has become endemic in domestic poultry, and resulted in death or slaughtering of 250 million birds (OIE, 2010). In fact, the use of antiviral drug families such as neuraminidase and haemagglutinin inhibitors to fight influenza viruses in humans limits their application in animals and poultry to reduce the development of drug resistance as much as possible. Moreover, commercial application of those drugs seems to be very expensive and unaffordable by many countries (Abdelwhab and Hafez, 2012). Since long time herbal and traditional plants had been used to prevent and control many diseases and health problems on a small scale such as in heavy metals toxicity (Khafaga
et al., 2019), ectoparasites (Abbas et al., 2018), reproductive and renal toxicity (Khafaga and Bayad, 2016a,b), heat stress (Khafaga et al., 2019), and viral disease (Oyuntsetseg et al., 2014; Mahmood et al., 2018; Sun et al., 2018). People around the world are now aware of the advantageous use of natural derived products such as microalgae (Subhani et al., 2018; Abdelnour et al., 2019; Abd El-Hack et al., 2019), rare earth elements (Abdelnour et al., 2019), and botanicals (Mahmood et al., 2018; Shah et al., 2018) over synthetic drugs and chemicals in terms of lower cost, toxicity and adverse effects and very low resistance (Karimi et al., 2015). As a result, Herbal medicine is gaining more importance in the anti-influenza research owing to their widespread availability and easy application in the diet (Abd El-Hamid et al., 2018). Hundreds of studies evaluated the efficacy of different forms of herbs either extract, part of or whole plant blends against Avian Influenza in vitro focusing on the efficacy and safety of these preparations. Based on these data, further in vivo studies were conducted on mice models and different poultry species. In fact, the commercial application of herbal plants in prevention and control of AIV are dependent on in vitro but more importantly on in vivo studies.

Therefore, the aim of this review is to present and evaluate all in vivo studies done on poultry species as a step and a guide for scientists, field practitioners and pharmaceutical corporations in developing new effective herbal-based drugs for prevention and control of AIV in poultry.

**Vaccine inadequacy**

Global efforts join hands for prevention and control of avian influenza outbreaks through monitoring, eradication, biosecurity and vaccination strategies especially in developing countries. Although vaccine implementation in many countries resulted in
promising eradication of the disease at the beginning, it usually followed by failure due to antigenic drift of AIV (Lee et al., 2004; Busani et al., 2009; Capua et al., 2009).

There are several factors contribute to the inadequacy of vaccination as a stand-alone tool for AI prevention including: (1) inactivated vaccines are specific to only one subtype of AI Poor vaccine application (Suarez and Schultz-Cherry, 2000), (2) vaccine interference with maternally-derived antibodies neutralized their action (mainly in the first week of age) (De Vriese et al., 2010; Kim et al., 2010; Sarfati-Mizrahi et al., 2010; Maas et al., 2011), (3) immunosuppressive diseases facing birds and affecting their response to vaccination (Hao et al., 2008; Sun et al., 2009; Hegazy et al., 2011), (4) Difficulties in diagnosis of field infection within vaccinated flocks (Suarez, 2005), (5) high mutability rate of the virus induced by vaccine pressure (Lee et al., 2004; Boni, 2008; Escorcia et al., 2008; Cattoli et al., 2011a; Cattoli et al., 2011b; Park et al., 2011; Lee et al., 2012; Lee et al., 2013), (6) variable host response to vaccination among different species and breeds of poultry (Martelli et al., 2005; Philippa et al., 2005; Tian et al., 2005; Bertelsen et al., 2007; Kapczynski and Swayne, 2009; Koch et al., 2009; Lecu et al., 2009; Cagle et al., 2011), (7) Inappropriate application of cold chain principle in many countries with resultant loss or decrease of vaccine efficacy (Abdelwhab and Hafez, 2012).

**Probable antiviral mechanism of Botanicals**

Great number of herbal extracts and botanicals has been investigated for their antiviral property. Generally, herbal preparations had potential strengthen impact on immune system, which resulted in fighting ability against invading viral infectious (Ganjhu et al., 2015; Sun et al., 2018). Several reports concluded that immunomodulatory properties of various plant extracts is mediated via enhancement of pro-inflammatory cytokines production such as IL-6 and IL-12. IL-12 is released from activated monocytes, macrophages, and dendritic cells; and then it stimulate release of
IFN-γ, enhance Th-1 responses, and promote the activity of CD^8^ cytotoxic T cell, thereby playing a pivotal role in controlling viral infection (Trinchieri, 2003). In addition, various studies concluded association between IL-6 production from macrophages and response against virus replication (Velazquez-Salinas et al., 2019), in addition to its role in viral infection clearing from the physiological system (Paludan, 2001). Therefore, herbal products/botanicals that exhibit immunomodulatory properties can play pivotal role in phagocytic promotion, which is essential to attenuating viral infection, replication and spread (Qureshi et al., 2017). Type I interferons (IFN-α & IFN-β) are cytokines eliciting antiviral, antiproliferative, and immunomodulatory effects (Cheng et al., 2007).

Concerning anti-influenza plants and their mechanism of antiviral action, the most of the studied plants were concluded to function either by inhibiting viral hemagglutinin or neuraminidase activity. In addition, inhibition of viral nucleoprotein RNA levels and polymerase activity are key to antiviral action for other herbal products and botanicals (He et al., 2011).

**Uses of Botanicals facing avian influenza viruses**

Figure 1 presents the different types of herbal plants and their role in improvement of chicken humoral immunity. A summary for major findings related to uses of botanicals facing avian influenza viruses is presented in Table 1.

**Adjuvant effects**

*Sargassum pallidum polysaccharides (SPP) (Turner)*

The efficacy of SPP as adjuvant in inactivated vaccines of NDV, AIV and Infectious bronchitis virus (IBV) in chickens was tested by Li *et al.* (2012). In that study, the vaccines containing SPP 10, 30 and 50 mg SPP/ml were compared with the traditional oil adjuvant vaccines. Serum antibody titers against the three viruses significantly
increased at the dose 30 mg/ml. Moreover, the CD4 content and T lymphocyte multiplication were enhanced in all treated groups.

*Astragalus Membranaceous polysaccharides* (*Astragalus*), *Epimedium polysaccharides* (*EPS*) (*Barrenwort, Horny goat weed*) and *sulfated APS* (*sAPS*)

The effect of APS, EPS and sAPS on the immune-responsiveness in chickens were evaluated. The best results of lymphocyte multiplication and enhancement of antibody titers against AIV and Newcastle Disease Virus (NDV) were obtained in the group of Sulfated EPS (sEPS). So, the adjuvant effect of APS–sEPS on NDV and AIV vaccines, at dose rate of 150, 100 and 50 mg/kg were further studied and the results indicated that improvement of antibody titers against AIV and NDV, beside, lymphocyte proliferations were best achieved at 100 mg/kg concentration (Guo *et al.*, 2012).

*Nigella sativa oil* (*Black caraway, Black cumin*)

Mady *et al.* (2013) used nigella sativa oil as an adjuvant during preparation of DNA vaccine based on the HA1 gene from Egyptian virus A/chicken/Egypt/1055/2010 (H5N1) and subcloned into plasmid containing Cytomegalovirus (CMV) immediate-early enhancer/promoter region, a â-globin/IgG chimeric Intron and neomycin PClneo mammalian expression vector. The H5-DNA vaccine with nigella sativa oil adjuvant induced potent cell mediated immune response in SPF chickens reached up to 86% phagocytic percent and 0.5 lymphocyte proliferation at 14 days post vaccination.

*Morinda citrifolia, L.* (*Cheese fruit, Indian mulberry, Great morinda, Beach mulberry, or Noni*)
Morinda citrifolia, L., particularly its fruit contains several chemical constituents like scopoletin, polysaccharide, proxeronine, proxeroninase, and dammacanthal which are soluble in different solvents and some of them have adjuvant activity. A study done by Sasmito (2012) tested the efficacy of M. citrifolia fruit extracts as adjuvant in 28-week-old Lohmann laying chickens vaccinated with avian influenza (H5N2) vaccine. N-hexane, ethanolic and aqueous extract of M. citrifolia (prepared in capsules) were administered once a day and divided into 3 subgroups, with three different dose levels (29 mg, 58 mg, and 116 mg/chicken/day); results of this study proved that administration of M. citrifolia fruit aqueous extract at dose level of 58 mg/chicken/day had a better capability to enhance serum production of specific immunoglobulin (IgY) and IgG against H5N2 vaccine.

Enhancement of immunity to vaccination

Pleurotus ostreatus wastes (Oyster mushroom)

Effect of dietary supplementation with two different levels of oyster mushroom powder (10, and 20 g/kg) on humoral immune responses of Ross 308 male broiler chicks to NDV and AIV compared to a prebiotic inclusion (1 g/kg A-Max®, a Mannan-oligosaccharides (MOS)) was investigated; results all over the entire experimental period (1-42 d) showed that birds in prebiotic-supplemented group had the highest body weight and the lowest feed conversion ratio compared to the other treatments. However, no significant change was reported in ND and AI antibody responses of chicks at any level of supplements (Toghyani et al., 2012). Similarly, another study by Fard et al. (2014) indicated that feed supplementation with 1% and 2% mushroom wastes led to slight increase in influenza antibody titers in chickens. These findings counter the fact that mushrooms contain immune-stimulant compounds like glycosides, polysaccharides, volatile oils, alkaloids, selenium and organic acids (Yang and Feng, 1998; Vetter and
Lelley, 2004; Willis et al., 2007), which are able to stimulate the antioxidant system with subsequent promotion of immunity.

**Virgin coconut oil (VCO) (Coconut Palm Oil)**

Another experiment was conducted by Yuniwarti et al. (2012) who investigate the impact of VCO at four different levels (0, 5, 10, 15 mL/kg feed) for four weeks, birds were divided into eight groups (4 AI-vaccinated and 4 unvaccinated groups). The result showed an increment in number of lymphocytes and Th-CD4 in AI vaccinated birds received 10 mL per kg diet than unvaccinated chicks received the same level of VCO. However, decrease in these numbers was reported in chicks given 15 ml VCO/kg diet; this increase may attribute to increased proliferation of lymphocytes through formation of phospholipid and stimulation of IL-2 receptors by VCO; that VCO-stimulated increase in T lymphocyte would therefore increase T-helper cells with subsequent stimulation of antibody production from B lymphocyte cells. On the other hand, reduction in number of lymphocyte in chicks given 15 ml VCO/kg diet may attribute to the alteration of lipid structure and membrane fluidity, with subsequent decrease in IL-2 receptor sensitivity and inhibition of lymphocyte proliferation.

**Echinacea purpurea (Eastern purple coneflower, Hedgehog coneflower, or Purple cone flower)**

Two hundred one-day old male broiler chickens (Ross 308) were given vitamin E (150 mg/kg diet), 0.1% aqueous Echinacea, and levamisole (15 mg/kg BW), the hemagglutination inhibition (HI) titers were estimated against both NDV and H9N2 vaccines after 21 and 42 days; results showed that treatment didn’t affect antibody titers against AI, ND, total anti-SRBC (at 21 day of age) and IgM (at 21 and 42 days of age)
(Miran et al., 2010). Similarly, Landy et al. (2011) used different forms and levels of Echinacea purpurea L. (EP) in one-day old broiler chicks (Ross 308), namely dried aerial part powder of E. purpurea (5 g and 10 g /kg diet) either continuously or intermittently with 3-days of application and 11 days free of application, or ethanolic extract (0.25 g / kg diet), chicks exhibited non-significant response against AI vaccination, however NDV vaccination increase production of antibodies in chicks given E. purpurea continuously at level (5 g/kg fed).

In contrast, inclusion of 0.1% and 0.5% Echinacea in the diet of broiler chickens for 2 weeks led to significant difference in the antibody titer against influenza vaccine as compared to controls chicks. In ELISA test, better impact was obtained in dose level of 0.5% than 0.1%. Therefore, Echinacea may be helpful in promoting influenza vaccine and control avian influenza virus (Najafzadeh et al., 2011). In another trial, the effect of dietary supplementation of E. purpurea root powder in broiler diet (0.1% and 0.5%) for short term (one week) and long term (six weeks) was studied. The results revealed that six-week consumption of E. purpurea induced marked alteration (p < 0.05) in feed conversation ratio, number of lymphocytes and heterophils, total counts of white blood cells (WBCs), and antibody titers against NDV and AIV, suggesting that long term feeding of E. purpurea may enhance the immune response and feed conversion in broiler chicks (Dehkordi et al., 2011).

**Rosmarinus officinalis L. (Rosemary)**

Rostami et al. (2018) studied the effect of supplementation of different levels of rosemary (Rosmarinus officinalis L.) powder (RP) and vitamin E (VE) on humoral immune response of broiler chicks throughout complete production cycle of 42-day. A total number of 270 1-day-old male chicks were vaccinated with commercially available
inactivated AIV and NDV vaccines, and live IBV vaccine, and they were supplemented with RP (0, 0.5, or 1.0%) and VE (0, 100, or 200 mg/kg). Both RP and/or VE had no significant impact (P > 0.05) on antibody titers and lymphoid tissues weight. However, significant effect (P < 0.05) on level of plasma globulin was obtained with supplementation of RP and VE combination. Thus, it may be concluded that dietary inclusion of RP and VE able to enhance broilers humoral immunity, but it not enough to improve antibody titers against specific virus during broiler production cycle.

_Eucalyptus globulus_ (Southern blue-gum, Tasmanian blue-gum, or Blue gum) and Peppermint (Mentha × piperita, Mentha balsamea Wild) essential oils

The effects of the essential oils of eucalyptus and peppermint on humoral and/or cell mediated immunity were investigated in AIV and NDV vaccinated chicks. The obtained results showed that volatile oils-supplemented chicks exhibited an increment in HI titers against ND and AI vaccines in comparison with untreated control chicks. Also, significant (P<0.05) improvement of macrophages phagocytic activity was reported at 14, 28 and 42 days of age in essential oil-supplemented chicks compared to control group (Awaad et al., 2010).

_Ginseng stem-leaf saponins_ (GSLS) (Panax ginseng, Chinese ginseng Asian ginseng, or Korean ginseng)

Zhai et al. (2011) studied the impact of oral administration of (5 mg/kg of BW) ginseng stem-and-leaf saponins (GSLS) for 7 days on the humoral immune responses of chickens against inactivated AI vaccines; also, they investigated the enhanced response of serum antibody against AI vaccination. Another study was illustrated the effect of GSLS and Astragulus polysaccharides (APS) on the immune response to a bivalent ND-AI
inactivated vaccine in SPF chickens immunosuppressed by cyclophosphamide (Cy). Chicks were immunosuppressed by intramuscular injection of Cy (100 mg/kg BW) for consecutive 3 days, following that, three groups received 2.5, 5 and 10 mg/kg BW GSLS and one group received 200 mg/L of APS in drinking water for 7 days. After that treated and control groups were injected with a bivalent ND-AI inactivated vaccine. The results showed that oral administration of GSLS prior to immunization induce recovery of IgA+ cells, intestinal intraepithelial lymphocytes (iIELs) as well as splenocyte proliferation; also, the specific antibody response against ND-AI vaccine was enhanced in Cy-immunosuppressed chickens. So, GSLS could be used as a potential agent to positively modulate the vaccination in immunosuppressed birds (Yu et al., 2015). Further study on the relation between astragalus and ginseng polysaccharides (APS, GPS) and the improvement of chicken immune response to H5N1 vaccine was done by Abdullahi et al. (2016). Three concentrations of APS and GPS were used (100, 200, and 400 mg/kg) from day 12 after hatch while H5N1 vaccine was subcutaneously injected at day 15. Results revealed that all the polysaccharide groups had significant increase in the antibody levels and the expression of cytokines ($P < 0.05$) in the APS and GPS groups compared to control ones.

Based on the positive impact of GPS on increased major histocompatibility complex (MHC) and cytokine expression in chicken embryo fibroblast (CEF) before and during H9N2 infection, Kallon and Abdullahi (2015) investigated the potential of Panax ginseng polysaccharide (GPS) humoral immunization against H9N2 in chickens. GPS improved the antibody titers of GPS-treated groups (5 mg/kg, 10 mg/kg and 20 mg/kg) 7 and 14 days post H9N2 AIV infection and inactivated H9N2 vaccine. These obtained results showed that the pretreatment of CEF could enhance the anti-viral activity of GPS; also, that GPS able to improve the early humoral immunity in young chicks.
Cochinchina Momordica (Bitter melon; Bitter apple; Bitter gourd; Bitter squash; Balsam-pear)

The role of Cochinchina Momordica, a Chinese traditional medicine plant, in the improvement of immune response of chicken against avian influenza vaccine (H5N1) was studied. Two weeks old chicks were vaccinated with H5N1 vaccine either alone or in combination with different levels of ECMS (5, 10, 20, 40 and 80 μg/dose). Results indicated that all ECMS- supplemented birds showed numerical increase in the levels of antibody, however chicks supplemented with 10 and 20 μg/dose exhibited significant ($P<0.05$) improvement of total IgG on day 28, as compared to control birds. Adjuvant effect was also confirmed through immunizing chickens with 20 μg/dose ECMS suggesting the potential ability of ECMS to enhance immune responses in chickens (Rajput et al., 2007).

Coriander seed powder (Cilantro or Chinese parsley)

Dietary supplementation of coriander powder (2% from broiler diets) for 42 days was insufficient to induce any significant modulation of antibody titers against AI as measured at 1, 35, and 42 days of age. The author attributed this nonsignificant effect to strict biosecurity measures observed throughout the experimental period (Hosseinzadeh et al., 2014).

Myrtus Communis Oil (MCO) (The common myrtle)

Supplementing the basal diet of Ross 308 broiler chickens with three different levels of MCO (100, 200, 300 mg/Kg) or Flavophospholipol antibiotic (FPL) (600 mg/Kg) led to significant improvement ($P<0.05$) in average body weight gain, feed intake, and feed conversion ratio in comparison with the control chicks. Moreover, it increased the
antibody titers against AIV and NDV, although 200 mg/Kg concentration of MEO had significant better effect (Mahmoodi et al., 2014). In the same line, Goudarzi et al. (2016) reported that the dietary supplementation of MCE in broiler basal diet improve the antibody titers against AIV and NDV.

_Aloe vera (AV) (Indian Aloe, Chinese Aloe, Barbados Aloe, True Aloe, Burn Aloe)_

Supplementation of different levels of AV (0.5%, 0.75% and 1%) in drinking water as a candidate antibiotic alternative growth promotor (AGP), and their effect on growth performance and immune system of broilers was studied. Although inclusion of AV at level 1% induced greater antibody titers against SRBC as compared with other groups (p<0.05), all treatments had no effect on antibody titer against AIV (Shokraneh et al., 2016). In another work, one-day-old, Ross chicks supplemented with aloe vera (3% in drinking water), garlic powder (3% in diet), and combination of aloe vera (1.5% in drinking water) and garlic powder (1.5% in diet). No significant variation was reported between control and treated chicks in NDV and AIV antibody titers; however, high expression of both viruses' antibody titers was noticed on 18 and 28 days in combined AV-garlic group (Fallah, 2014).

_Allium sativum powder (Garlic)_

The ability of 1 and 3% fresh garlic powder in broiler diet to promote the immune response against AIV H9N2 vaccine in broiler chicks was evaluated. The results showed that neither treatments nor removal of garlic had any effect on antibody titers to H9N2 vaccine, suggesting failure of dietary garlic to stimulate chickens humoral response against AIV vaccine (Jafari et al., 2009). In addition, no significant enhancement in the humoral antibodies against AIV and NDV was observed in the age of 18 and 28 days in
broiler chickens with diets supplemented with 2 and 4 g/kg cinnamon or garlic powder (Toghyani et al., 2011). On contrary, Eid and Iraqi (2014) concluded that dietary inclusion of garlic powder (100, 150, or 200 g/ton) induced marked significant impact (P<0.001) on NDV and AIV antibodies titers, however, diet supplemented with 200 g/ton of garlic powder had the most better positive effect.

*Mannanoligosaccharides (MOS) (Mannan) and Humate (HU)*

The antibody titers against AIV in response to dietary inclusion of different levels of MOS and HU (0.1, 0.2 and 0.3%) were evaluated in broiler chicks. The MOS supplementation led to marked increment in AIV antibody titers after four, five, and six weeks of age. All MOS concentrations besides 0.3% HU were effective in stimulating action of humoral immunity against AIV vaccine. Generally, results of such investigation concluded much better effect of MOS over HU in production of antibody against AIV (Tohid et al., 2011).

*Curcuma domestica Val. powder (Turmeric)*

Nouzarian et al. (2011) investigated the effect of dietary inclusion of various levels of turmeric powder (3.3, 6.6 and 10 g/kg) on growth performance, blood biochemical parameters, and humoral immunity in male broiler chicks. No significant impact was reported for turmeric powder on NDV and AIV antibody titer. Moreover, supplementation of turmeric powder in broiler diet was insufficient to induce significant enhancement of parameters of growth performance except for feed efficiency.

On the other hand, the effect 50% ethanol extract of turmeric as immunomodulator in layer chicken one week before and after AI vaccination was studied. The results revealed significant increase of total heterophils after vaccination while the treatment and
vaccine didn’t significantly affect the total basophils number (p>0.05). So, it can be concluded that curcumin was effective in increasing innate immunity, in terms of heterophil count (Widhowati et al., 2018).

Curcuma heyneana Val (Herbal Formula of Viranur, Turmeric .) and Phyllanthus niruri L. (Phyllanthus)

The effects of that Herbal Formula (Viranur, Turmeric and Phyllanthus) on the immunity of chickens was illustrated in terms of histopathological changes, all the chickens were vaccinated with ND vaccine at 7th day of age followed by AI vaccine at 14th day. One group (KB) received herbal solution containing 5 g turmeric and 25 g phyllanthus in drinking water and the other one (KC) received herbal solution containing 36 g the herbals formula Viranur and 25 g phyllanthus (Phyllanthus niruri L.) for four weeks. The weight index of thymus, spleen, and Fabricius bursa thirty days after AI vaccination had no significant alteration (P > 0.05) between control and treated groups, although the treatment groups had higher weight index. The histopathological findings revealed nonsignificant difference in the spleen in both control and treatment groups, lymphocyte increase in lymphoid follicles of the bursa of Fabricius in the group KC and more widening in the cortex than medulla of the thymus in the group KB and KC. In conclusion, the previously mentioned herbals can stimulate lymphocyte activity with subsequent immunomodulatory effect (Hartati et al., 2015).

Curcuma heyneana Val. (Turmeric ) and Thymus vulgaris powders (German thyme, Common thyme, garden thyme or thyme)

Investigation of the effects of dietary supplementation of turmeric, thyme powders and their combination on growth performance, blood biochemical parameters and immune
response of broiler chicks was done by Fallah and Mirzaei (2016). In this study, the diet of commercial Ross 308 chickens was provided with 5 g/kg turmeric powder, 5 g/kg thyme powder and 2.5 g/kg turmeric powder + 2.5 g/kg thyme powder, respectively in addition to control diet (no supplement). The results suggested that addition of different concentrations of turmeric and thyme powders increased antibody titers against NDV and AIV besides increasing (p>0.05) chicks body weight in comparison to the control chicken at 42 days of age.

*Curcuma longa (Curcumin) and Xanthophyll, Zeaxanthin (Lutein)*

The impact of curcumin and lutein dietary inclusion on immunity and pigmentation was compared in lipopolysaccharide (LPS)-stimulated Arbor Acres broiler chicks. Broiler basal diets were supplemented with curcumin (CRM) or lutein (LTN) at dose level of 200 mg/kg diet for 42 days. At 16, 18, and 20 days of age, 50% of chicks in each group received abdominal injection of LPS (250 mg/kg of BW) or of NaCl (0.9%) at equal volume. Results revealed significant elevation in NDV and AIV antibody titers in CRM-supplemented chicks over chicks in other groups at 20 and 30 days; however, curcumin supplementation stimulate proliferation of B and T lymphocyte at day 21 in both LPS- and non-LPS-induced chicks. Moreover, at 42 days, curcumin was able to stimulate proliferation of B lymphocyte in non-LPS-induced chicks. Therefore, it could be concluded that dietary inclusion of lutein in broiler chicks induce better pigmentation efficiency, however curcumin-treated chicks showed enhanced immune status (Rajput et al., 2013).

*Thymus vulgaris (German thyme, Common thyme, thyme, or garden thyme)*
The impact of dietary inclusion of thyme powder on growth performance, immune responses, hematological and biochemical parameters in broiler chicks were investigated. In that study, chicks were treated with antibiotic (flavophospholipol) and thyme powder (5 and 10 g/kg). Inclusion of antibiotic and 5 g/kg of thyme led to significant increment (P<0.05) in body weight, while no significant effect was reported in all treatment on hematological parameters including hemoglobin value, hematocrit values, and count of red and white blood cells. Moreover, non-significant (P>0.05) alteration was reported for immunity indicators in all treated chicks indicating that dietary inclusion of 5 g/kg of thyme powder could improve growth performance similar to the favorable effect of antibiotic growth promoter without any deleterious effect on immune status and serum biochemical parameters (Toghyani et al., 2010). Similarly, the relation between drinking Thyme Essence (DTE) (Zero, 0.10, 0.15 and 0.20 mL/L) and antibody titers of AI, ND, IBV and IBD in broiler chickens was studied. At 21 and 42 days of age, the serum antibody titers did not show significant differences between different treatments (Saki et al., 2014).

In contrast, another investigation was designed to study the impact of different levels of thyme extract (0.1%, 0.15% and 0.2%) in drinking water on immune response of broiler chickens vaccinated against NDV and AIV subtype H9N2. Results indicated that the extract had no effect on antibody response against NDV vaccine, but 0.2% thyme extract induce significant increase in specific antibody response against H9N2 vaccine 14 days after vaccination compared to all groups (Talazadeh et al., 2015). Furthermore, 0.1% and 0.2% Antibiofin® (contained Thymus vulgaris) in drinking water were tested for their impact on immune response of broiler chicks against AIV subtype H9N2 vaccine. The obtained results exhibited that Antibiofin® at dose level of 0.1% and 0.2% elevate the specific antibody titer against H9N2 vaccine 14 and 28 days after vaccination compared to the control group (Talazadeh et al., 2016).
Various concentration of sweet orange (*Citrus sinensis*) peel extract (SOPE) in drinking water was tested in broiler chickens for 42 days for their efficacy on humoral immune responses. Chicks were treated with 1000 ppm and 1250 ppm of SOPE besides control negative group. In a dose-dependent manner, significant elevation (*P* < 0.05) in serum antibody titers responses to all vaccines were reported by administration of SOPE, while the relative weights of Fabricius bursa and spleen had no significant alteration (Zohreh *et al.*, 2014). Another research used various concentrations (0.25%, 0.5%, 0.75% and 1%) of dried *Citrus sinensis* peel (DCSP) for six weeks in broiler chickens. On day 42, total sheep red blood cells (SRBC) were significantly changed (*P*<0.05) while specific antibodies for AIV and NDV had no significant alteration (*P*>0.05). The lowest AI titer was obtained at 0 and 0.25% concentration, while the highest one was obtained at 0.5 concentrations (Ebrahimi *et al.*, 2015).

*Dietary treatment with nettle and ginger of one-day-old broilers (Ross 308)* at concentrations of 2 g/kg nettle powder; 4 g/kg ginger powder and 2 g/kg ginger+2 g/kg nettle powder resulted in nonsignificant difference in antibody titers against NDV, AIV and SRBC (*P*>0.05). Serum antioxidant capacity was significantly elevated by nettle or ginger (*P*< 0.05). In conclusion, nettle or ginger can improve serum antioxidant capacity of broiler chicks but cannot be used as an immunomodulator (Toghyani *et al.*, 2015).

*Origanum vulgare (Oregano essential oil, OEO)*

Oral supplementation with OEO (0.005 and 0.01%) in broiler chickens immunized against AIV and NDV revealed that the higher dose improved parameters of growth.
performance in birds in early life and up to 21 days of age. Furthermore, the best specific antibody titers against NDV and AIV-HI were obtained when the birds vaccinated with NDV and AI and supplemented with OEO. It could be concluded that dietary inclusion of OEO able to improve growth parameters, humeral and innate immunity (Galal et al., 2016).

*Oil extracted propolis (OEP) (Bee Glue)*

Taheri et al. (2005) conducted an experiment to investigate the impact of various levels of oil extracted propolis (OEP) on humoral immune response of broilers from one to seven weeks of age. Chicken received 7 different levels of OEP (0, 40, 70, 100, 400, 700 and 1000 mg/kg), and serum samples were collected twice at day 21 and 42 of age for HI and ELISA tests. Results revealed significant increase (P<0.05) in AI, ND and IBD antibody titer in response to OEP inclusion, while there was no effect on IB titers. Surprisingly, high concentration of OEP induces negative impact on broiler humoral immunity suggesting dose-dependent immune response against OEP. Further *in vivo* experiment investigated the influence of various concentrations of ethanolic extract of propolis, on humoral immunity and parameters of growth performance of chickens comparable to the antibiotic flavophospholipol.

In that study, one-day old broiler chicks (Ross 308) were received dietary supplementation of flavophospholipol (4.5 mg/kg) and ethanol extracts of propolis (50, 100, 200, and 300 mg/kg) for complete production cycle (42 days). The results revealed non-significant alteration in growth performance parameters; however, body weight and feed intake were improved (p>0.05) as compared to control chicks. Moreover, none of the supplements affected humoral immune function significantly (Gheisari et al., 2017). Controversial results were obtained when mixed-sex quail chicks were treated with 1000
and 5000 mg/kg of ethanolic extract of propolis, 1000 and 5000 mg/kg of pollen powder, 100 mg/kg of royal jelly and 22 g/L of honey in drinking water. There was significant difference \( (p < 0.01) \) in specific serum AIV antibody titers and sheep red blood cells. Also, significant difference heterophils to lymphocytes ratio were observed among treatment and control groups \( (p < 0.01) \) (Babaei et al., 2016).

*Hypericum perforatum L.* (HPE) (*Common Saint John's Wort, Perforate St John's-Wort, And St John's Wort*)

The influence of dietary supplementation of 250, 500, and 1,000 mg/kg HPE and humoral immune response to reassortant AI vaccine in chickens administered on 20\textsuperscript{th} day of age and boosted 20 days later was studied. HPE was provided 7 days after each vaccination. 500 mg/kg concentration enhanced H5 antibody titer by 9.82\% after the first vaccination and by 30.63\% after the second one. Moreover, an increase in Re-4 and Re-5 polyclonal H5 antibodies was observed after the first and second vaccinations (Jiang *et al.*, 2012).

*Mentha spicata* (*Spearmint, garden mint, common mint, lamb mint and mackerel mint*)

extract

The drinking water application of 0.2\%, 0.4\% and 0.6\% concentrations of Mentha spicata extract causes nonsignificant difference \( (P>0.05) \) between treatments in terms of relative weights of immune organs and serum antibody titers to AIV and NDV at 21 and 42 days of age (Nanekarani *et al.*, 2012).

*Tribulus terrestris L.* (*puncture vine*)
Application of 1 or 5 g/kg of puncture vine increased serum antibody titers against NDV while 1 g/kg vine showed the highest antibody titer against AIV and sheep RBCs at 28 and 31 days; respectively (Yazdi et al., 2014).

*Satureja hortensis* L. (*Savory*)

A study on the effects of *Satureja hortensis* L. on immune responses and serum biochemical indicators of broiler chicks (Ross 308) was conducted by Ghalamkari et al. (2011). In this study, 5 and 10 g/kg savory powder were fed for 42 days. Although 5 g/kg savory increased antibody titers against SRBC as compared to other groups, it failed to induce any remarkable effect on specific antibody titers against NDV and AIV at 42 days of age.

*Azadirachta indica* (Neem)

Neem is able to induce desirable impact on broilers immune responses without any reported negative effect on growth performance (Mahmood et al., 2018). To clarify this, one-day old broiler chicks were fed a diet containing 7 and 12 g of neem fruit powder/kg compared with flavophospholipol antibiotic growth promotor. Treatment with 7 g neem/kg increased antibody titers against SRBC more than 12 g neem/kg and influenza virus in comparison with the control diet and the flavophospholipol diet. At the same time, all performance parameters were enhanced at this concentration (Landy et al., 2011).

*Rhus coriaria* L. (*Sumac*) (*fruit powder*)

The addition of 3 and 7 g/kg sumac powder in broiler ration was compared with AGP (flavophospholipol). The treatments didn’t induce marked effect on antibody titers
against SRBC, NDV and AIV (P> 0.05) but heterophile to lymphocyte ratio were improved in response to 7 g/kg dose and AGP (P< 0.05) (Toghyani and Faghan, 2017).

*Radix astragali, Radix codonopis (dang shen or poor man's ginseng), Herba epimedii (Yin Yang Huo) and Radix glycyrrizae (Liquorice root / licorice)*

Liu *et al.* (2009) compared the immunomodulatory effect of Radix astragali, Radix glycyrrizae, Radix codonopis, and Herba epimediti aqueous extracts in clinical healthy chickens or mitigation of the effect of experimentally induced immunosuppression by reticuloendotheliosis virus (REV) infection either singly or in combination. Antibody titers against NDV and AIV-H5 didn’t affected by these herbal extracts in clinically healthy chickens. Although the antibody titers against NDV and AIV-H5 were increased significantly by using these four herbal extracts in REV-immunosuppressed chicks in comparison to the immunosuppressed control chicks, the titers were still significantly lower than those in chicks not infected by REV.

**Enhancement of immune response to challenge**

*Cocos nucifera (Virgin coconut oil, VCO)*

The effect of treatment of diet with 10 ml VCO/kg starting from 7th day of age in broiler chickens challenged with H5N1 either vaccinated or nonvaccinated. The results revealed that VCO supplementation led to significant increase in specific antibody titers against H5N1, lymphocyte count, CD4 count and number of CD8 after challenge with AI virus. In addition to immunological parameters, an increase in chicken viability and significant reduction of mortality was observed (Yuniwarti *et al.*, 2015).
*Echinacea purpurea* (EF) and *Sambucus nigra* (SAM) (European elderberry, European elder, Elderberry, European black elderberry, and Black elder)

The *in vivo* antiviral effect of commercial extracts of EF and SAM in broiler chickens challenged with H9N2 was investigated. EF and SAM were administered in drinking water in different groups 8 h after challenge for 7 days while in prophylaxis group of EF 5 days before the challenge for 10 days. In EF-prophylactic group, reduction of the fecal viral shedding in all days post-challenge was observed. Treatment with amantadine and SAM resulted in significant reduction in the number of tracheal positive samples as compared to untreated and EF-treated groups. So, it can be concluded that administration of EF and SAM in chickens able to reduce shedding of H9N2 virus from trachea and faeces (Karimi *et al.*, 2014).

*Mentha × piperita* (Peppermint) and *Eucalyptus globulus* (eucalyptus essential oils)

Eucalyptus and peppermint essential oils in commercial product such as Mentofin, was evaluated against several viral diseases such as bursa disease (Shah *et al.*, 2018) and influenza. Micro and macroscopic lesions of broilers challenged intratracheally by *Mycoplasma gallisepticum* (MG) and/or AIV subtype H9N2 at 1 week of age was evaluated after administration of Mentofin for 6 days post-challenge. Histopathological findings revealed a significant reduction (*P* < 0.05) in tracheal deciliation, mucosal hypertrophy, goblet cell degeneration and heterophil infiltration of birds treated with Mentofin in comparison control groups (Barbour *et al.*, 2010).

*Astragalus polysaccharide* (APS) (*Astragalus*)
Li et al. (2011) mentioned that APS is a common immune adjuvant which used widely to enhance the immune response, and induce the expression of cytokines, specific antibodies, and proliferation of lymphocyte. On the other hand, the effect of humoral immunization of APS against H9N2 infection in chickens was investigated. Owing to the enhancement of IL-4, IL-6, IL-10, LITAF, IL-12 and antibody titers to H9N2 AIV in the 1st week after APS supplementation, APS treatment was able to reduce replication of H9N2 and enhance early humoral immunity in young chicks (Kallon et al., 2013).

**NAS preparation**

The *in vivo* inhibitory effect of NAS preparation (traditional Chinese herbal medicine) on H9N2 AIV was evaluated. Supplementation of infected chickens with two different levels of NAS (0.2 or 0.1g/kg/d) for four days has prevented the shedding of H9N2 virus at the 7th day PI. However, the virus could be detected in other untreated birds. Thus, it could be concluded that NAS preparation might have the potential ability to control H9N2 shedding in infected chicks (Shang et al., 2010).

**Camellia sinensis (Green tea)**

Although oral supplementation of green tea by-products decreases viral titers against H9N2 in mice lungs at the beginning of infection, they failed to prevent disease and death in these mice. In contrary, dietary inclusion of 10 g green tea by-products/kg resulted in significant antiviral effect in chickens. Moreover, ethyl acetate-soluble and hexane-soluble fractions of green tea by-products including catechins showed strong anti-influenza activity. Also, dietary supplementation of catechins (contained in lyophilized green tea by-product extracts) reduced replication and excretion of H9N2 virus in experimentally infected chickens in a dose-dependent manner (Lee et al., 2012).
Herbal I (sambiloto, temu ireng, adas bintang, sirih merah) and herbal II (sambiloto, adas bintang, sirih merah)

Setiyono and Bermawie (2014) evaluated the efficacy of feeding two herbal formula on survivability and histopathological changes in chickens 3 weeks prior to challenge with H5N1. Overall, no chicken in treatment groups survived until day 8. The histopathological findings of respiratory system in treated chicken group revealed congestion and edema of respiratory tract epithelium, lymphoid depletion of spleen and bursa of Fabricius with spread of virus particles in tissue of respiratory and lymphoid system.

Nigella sativa seeds (Black caraway, Black cumin, Nigella, Kalojeere)

Besides the previously mentioned adjuvant effects of Nigella sativa, further study tested the efficacy of their seeds on the immune response of unvaccinated turkey to H9N2 infection. In this experiment, 1% and 3% levels of Nigella sativa seeds were used from day one of age and birds were infected with $10^6$ EID50 of the virus at the 4th week. Results indicated that treatment with Nigella sativa significantly decreased the clinical signs and viral shedding in infected birds compared with untreated infected group. In addition, significant increase in serum antibody titers against H9N2 and increased IFN-γ mRNA expression with subsequent reduction of pathogenicity of the virus was proved in birds receiving 3% level (Umar et al., 2015). The same author performed another experiment on Nigella sativa but with different levels namely 2%, 4% and 6% NS seeds. Similarly, pronounced effects on severity of clinical signs, serum antibody titers and cytokine production were observed in dose dependent manner (Umar et al., 2016a).
Thymoquinone (TQ) and curcumin (Cur)

Umar et al. (2016b) tested the effects of TQ and Cur on immune response and pathogenesis of H9N2 (A/chicken/Pakistan/10RS3039-284-48/2010) in unvaccinated turkeys. Although groups receiving either showed promising results in terms of production of specific antibodies, viral shedding, cytokine expression and suppression of viral pathogenicity, groups treated with combination of TQ and Cur showed the best result.

Pinus monophylla shell polysaccharide (PSP) (Pinon)

Prophylactic administration of PSP in chickens before infection with H9N2 virus resulted in enhanced expression of IL-6 with elevated antibody titers one week post PSP treatment. Thus, PSP administration decreases the replication of H9N2 and enhances early humoral immunity in young chicks (Xie et al., 2012).

Olea europaea leaf extracts (OLE) (Olive)

The in vivo antiviral activity of OLE was investigated against the highly pathogenic avian influenza H5N1 Egyptian virus. Although both OLE treatment 3 days pre- and post-infection or only post infection resulted in 70 % protection of birds, pre-treatment delayed the beginning of mortalities. Similarly, application of the extract only before infection characterized by delayed mortalities but with higher mortalities Saif (2015).

Conclusion

Biosecurity and effective vaccination programs remain the corner stones for controlling the pandemic AI infections; however botanicals may assist the strategies for disease prevention and control through several mentioned axes as adjuvants, enhancement of immunity to vaccination or challenge. Although, several imperative research programs
from veterinary and agricultural science must be performed specially on botanicals to develop improved control measures of AI that can be applied under different local and commercial conditions to maximize its benefits in combating this serious disease.

Conflict of interests
Authors declare no conflict of interests.

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driven by mutations in major antigenic sites of the hemagglutinin molecule analogous to those for human influenza virus. *Virology* 85: 8718-8724.


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**FIGURE LEGEND**
Different types of herbal plants and their role in improvement of chicken humoral immunity. **PSP**: *Pinus monophylla* shell polysaccharide; **TQ**: Thymoquinone; **VCO**: Virgin coconut oil; **SAM**: *Sambucus nigra*; **APS**: Astragulus polysaccharides; **SPP**: Sargassum pallidum polysaccharides; **NS**: Nigella sativa; **MC**: Morinda citrifolia, *L*; **EPS**: Epimedium polysaccharides; **Thy**: Thyme; **HPF**: Hypericum perforatum *L*.; **OEO**: Oregano essential oil; **TUR**: Turmeric; **MOS**: Mannan-oligosaccharides; **AV**: Aloe vera; **CRM**: curcumin; **MCO**: Myrtus Communis Oil; **GSLS**: Ginseng stem-and-leaf saponins; **ECMS**: Cochinchina Momordica.
Table 1. Summary for the major findings related to uses of botanicals facing avian influenza viruses.

<table>
<thead>
<tr>
<th>Herbal</th>
<th>Dose level(s)</th>
<th>Species/strain</th>
<th>Main findings</th>
<th>Ref.</th>
</tr>
</thead>
</table>
| *Sargassum pallidum* | 10, 30 and 50 mg SPP/ml | Broiler chicken | • Serum antibody titers against the NDV, AIV and (IBV) viruses were significantly increased at the dose 30 mg/ml  
• The CD4+ content and T lymphocyte multiplication were enhanced in all treated groups. | Li *et al.* (2012) |
| *Astragalus Membranaceous polysaccharides (APS)*  
*Epimedium polysaccharides (EPS)*  
and sulfated APS (sAPS) | 150, 100 and 50 mg/kg | Broiler chicken | • Enhancement of antibody titers against AIV and NDV in Sulfated EPS- treated group | Guo *et al.* (2012) |
| *Nigella sativa* | - | SPF chickens | • The induced potent cell mediated immunity reached up to 86% phagocytic percent and 0.5 lymphocyte proliferation at 14 days post vaccination. | Mady *et al.* (2013) |
| *Morinda citrifolia, L.* | 29 mg, 58 mg, and 116 mg/chicken/day | 28-week-old Lohmann laying chickens | • Dose level of 58 mg/chicken/day had a better capability to enhance serum production of specific immunoglobulin (IgY) and IgG against H5N2 vaccine. | Sasmito (2012) |
| *Pleurotus ostreatus* (Oyster mushroom) | 10, and 20 g/kg | Ross 308 male broiler chicks | • Non- significant change was reported in ND and AI antibody responses of chicks at any level of supplements | Toghyani *et al.* (2012) |
|  | 1% and 2% | Chicken | • Slight increase in influenza antibody titers | Fard *et al.* (2014) |
| Virgin coconut oil | 0, 5, 10, 15 mL/kg feed | Vaccinated and unvaccinated chicken | • An increment in number of lymphocytes and Th-CD4 in AI vaccinated birds received 10 mL per kg diet  
• Decrease in these numbers was reported in chicks given 15 mL VCO/kg diet | Yuniwart *et al.* (2012) |
| 0.1% aqueous Echinacea | One-day old male broiler chickens (Ross 308) | | • Treatment didn’t affect antibody titers against AI, ND, total anti-SRBC (at 21 day of age) and IgM (at 21 and 42 days of age) | Miran *et al.* (2010) |
| *Echinacea purpurea* | Dried aerial part powder of E. purpurea (5 g and 10 g/kg diet),  
Ethanolic extract (0.25 g / kg diet) | One-day old broiler chicks (Ross 308) | • Chicks exhibited non-significant response against AI vaccination, | Landy *et al.* (2011b) |
|  | 0.1% and 0.5% Echinacea in the diet | Broiler chickens | • Significant difference in the antibody titer against influenza vaccine  
• Better impact was obtained in dose level of 0.5% than 0.1%. | Najafzad *eh et al.* (2011) |
<table>
<thead>
<tr>
<th>Supplement</th>
<th>Treatment</th>
<th>Chicken Type</th>
<th>Effects</th>
<th>Reference</th>
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<tr>
<td><em>E. purpurea</em> root powder</td>
<td>0.1% and 0.5% in diet</td>
<td>Broiler chickens</td>
<td>• Marked alteration in feed conversation ratio, number of lymphocytes and heterophils, total counts of white blood cells (WBCs), and antibody titers against NDV and AIV</td>
<td>Dehkordi <em>et al.</em> (2011)</td>
</tr>
</tbody>
</table>
| *R. officinalis* L. (Rosemary)  | 0.0, 0.5, or 1.0% and Vitamine E (0, 100, or 200 mg/kg) | Broiler male chickens           | • Both RP and/or VE had no significant impact on antibody titers and lymphoid tissues weight.  
• Significant effect on level of plasma globulin was obtained with supplementation of RP and VE combination | Rostami *et al.* (2018)              |
| *E. globulus* Essential oils of |           | Broiler chickens               | • Significant increase in HI titers against ND and AI vaccines  
• Significant improvement of macrophages phagocytic activity was reported at 14, 28 and 42 days. | Awaad *et al.* (2010)               |
| *Ginseng stem-and-leaf saponins*| 5 mg/kg of BW | Broiler chickens               | • Recovery of IgA+ cells, intestinal intraepithelial lymphocytes and solenocyte proliferation  
• The specific antibody response against ND-AI vaccine was enhanced in Cy-immunosuppressed chickens | Zhai *et al.* (2011)                |
| *Ginseng*                        | 5, 10, and 20 mg/kg | Broiler chickens               | • Pretreatment with CEF could enhance the anti-viral activity of GPS  
• GPS able to improve the early humoral immunity in young chicks | Kallon, and Abdullahi (2015)         |
| *C. Momordica* (ECMS)           | 5, 10, 20, 40 and 80 μg/dose | Two weeks old Broiler chickens | • Numerical increase in the levels of antibody  
• Chicks supplemented with 10 and 20 μg/dose exhibited significant improvement of total IgG on day 28 | Rajput *et al.* (2007)              |
| *Coriander*                      | 2% from broiler diets | Broiler chickens               | • There is no significant modulation of antibody titers against AI as measured at 1, 35, and 42 days of age | Hosseinzadeh *et al.* (2014)         |
| *M. Communis* (MCO)             | 100, 200, 300 mg/Kg | Ross 308 broiler chickens      | • Significant increase in the antibody titers against AIV and NDV 200 mg/Kg concentration of MCO had significant better effect | Mahmoodi *et al.* (2014)             |
| *Aloe vera*                      | 0.5%, 0.75% and 1% in drinking water | Broilers chicken               | • Inclusion of AV at level 1% induced greater antibody titers against SRBC  
• All treatments had no effect on antibody titer against AIV | Shokraneh *et al.*., (2016)          |
|                                 | 3% in DW, garlic 3% in diet, aloe vera (1.5% in DW) + garlic (1.5% in diet) | one-day-old, Ross chicks         | • No significant variation was reported between control and treated chicks in NDV and AIV antibody titers  
• High expression of both viruses’ antibody titers was noticed on 18 and 28 days in combined AV-garlic group | Fallah (2014)                       |
<p>| <em>A. sativum</em> (Garlic)            | 1 and 3% fresh garlic powder | Broiler chickens               | • Neither treatments nor removal of garlic had any effect on antibody titers to H9N2 vaccine | Jafari <em>et al.</em> (2009)              |
|                                 | 2 and 4 g/kg garlic powder | Broiler chickens               | • Non-significant enhancement in the humoral antibodies against AIV and NDV | Toghyani <em>et al.</em> (2011)            |</p>
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Treatment</th>
<th>鸡品种</th>
<th>Impact on NDV and AIV antibody titers</th>
<th>Notes</th>
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<tbody>
<tr>
<td>Eid, and Iraqi (2014)</td>
<td>100, 150, or 200 g/ton</td>
<td>瓦腊鸡肉</td>
<td>• Significant impact on NDV and AIV antibodies titers</td>
<td>饲料补充200 g/ton的大蒜粉有更好的正面效果。</td>
</tr>
<tr>
<td>Tohid et al. (2010)</td>
<td>0.1, 0.2 and 0.3%</td>
<td>瓦腊鸡肉</td>
<td>• MOS supplementation led to marked increment in AIV antibody titers</td>
<td>MOS和0.3%HU刺激的体液免疫对人体抵抗AIV疫苗有显著影响。</td>
</tr>
<tr>
<td>Nouzarian et al. (2011)</td>
<td>3.3, 6.6 and 10 g/kg</td>
<td>瓦腊鸡肉</td>
<td>• No significant impact on NDV and AIV antibody titer.</td>
<td>MOS和0.3%HU刺激的人体的体液免疫对AIV疫苗有显著的影响。</td>
</tr>
<tr>
<td>Widioha et al. (2018)</td>
<td>50% ethanol extract of turmeric</td>
<td>粉状鸡</td>
<td>• Significant increase of total heterophils after vaccination</td>
<td>处理和疫苗未显著影响总单核细胞数量。</td>
</tr>
<tr>
<td>Hartati et al. (2015)</td>
<td>5 g/kg turmeric &amp; thyme powder 2.5 g/kg turmeric powder + 2.5 g/kg thyme powder</td>
<td>瓦腊鸡肉</td>
<td>• Addition of different concentrations of turmeric and thyme powders increased body weight and antibody titers against NDV and AIV</td>
<td>苏木和香茅的添加显著增重量和抗体对NDV和AIV。</td>
</tr>
<tr>
<td>Rajput et al. (2013)</td>
<td>CRM or LTN (200 mg/kg diet)</td>
<td>瓦腊鸡肉</td>
<td>• Significant elevation in NDV and AIV antibody titers in CRM-supplemented chicks over chicks in other groups</td>
<td>CRM- supplement stimulates proliferation of B and T lymphocytes in both LPS- and non-LPS-induced chicks。</td>
</tr>
<tr>
<td>Toghyani et al. (2010)</td>
<td>5 and 10 g/kg</td>
<td>瓦腊鸡肉</td>
<td>• Dietary inclusion of 3 kg improve growth performance without any deleterious effect on immune status and serum biochemical parameters</td>
<td>未显示有显著差异。</td>
</tr>
<tr>
<td>Saki et al. (2014)</td>
<td>Thyme Essence (Zero, 0.10, 0.15 and 0.20 mL/L)</td>
<td>瓦腊鸡肉</td>
<td>• The serum antibody titers did not show significant differences between different treatments</td>
<td>Thyme extract (0.1%, 0.15% and 0.2%) in DW</td>
</tr>
<tr>
<td>Talazadeh et al. (2015)</td>
<td>0.2% thyme extract induce significant increase in specific antibody response against H9N2 vaccine 14 days after vaccination</td>
<td>瓦腊鸡肉</td>
<td>• Elevation in specific antibody titer against H9N2 vaccine 14 and 28 days after vaccination</td>
<td>Thyme extract (0.1%, 0.15% and 0.2%) in DW</td>
</tr>
</tbody>
</table>

**Herbal Formula**

**Turmeric**

**Thyme**

**Curcuma longa** and *lutein*

**Thymus vulgaris**

**Mannanoligosaccharides (MOS) and Humate (HU)**

**Curcuma domestica Val. (Turmeric) powder**
<table>
<thead>
<tr>
<th>Source</th>
<th>Treatment/Extract</th>
<th>Organism/Study Design/Condition</th>
<th>Findings</th>
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<td>Zohreh et al. (2014)</td>
<td>Thymus vulgaris in DW</td>
<td>Broiler chicks</td>
<td>Dose-dependent significant elevation in serum antibody titers responses to all vaccines</td>
</tr>
<tr>
<td>Ebrahimi et al. (2015)</td>
<td>Sweet orange (Citrus sinensis) peel extract (SOPE)</td>
<td>Broiler chicks</td>
<td>Total sheep red blood cells were significantly changed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Specific antibodies for AIV and NDV had no significant alteration.</td>
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<tr>
<td></td>
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<td></td>
<td>The lowest AI titer was obtained at 0 and 0.25%, while the highest one was obtained at 0.5 concentration</td>
</tr>
<tr>
<td>Toghyani et al. (2015)</td>
<td>Nettle (Urtica dioica) and ginger</td>
<td>One-day-old broilers (Ross 308)</td>
<td>Non-significant difference in antibody titers against NDV, AIV and SRBC.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serum antioxidant capacity was significantly elevated by nettle or ginger</td>
</tr>
<tr>
<td>Galal et al. (2016)</td>
<td>Oregano essential oil (OEO)</td>
<td>Broiler chicks</td>
<td>Higher dose improved parameters of growth performance in young birds</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Improved specific antibody titers against NDV and AIV-HI</td>
</tr>
<tr>
<td>Taheri et al. (2005)</td>
<td>Oil extracted propolis (OEP)</td>
<td>Broiler chickens</td>
<td>Significant increase in AI, ND and IBD antibody titer</td>
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<td></td>
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<td></td>
<td>No effect on IB titers</td>
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<td></td>
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<td>High concentration of OEP induces negative impact on broiler humoral immunity</td>
</tr>
<tr>
<td>Gheisari et al. (2017)</td>
<td>Oregano essential oil (OEO)</td>
<td>Mixed-sex quail chicks</td>
<td>Significant difference in specific serum AIV antibody titers and sheep red blood cells,</td>
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<td></td>
<td></td>
<td></td>
<td>difference heterophils to lymphocytes ratio</td>
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<tr>
<td>Babaee et al. (2016)</td>
<td>Oregano essential oil (OEO)</td>
<td>Broiler chickens</td>
<td>500 mg/kg concentration enhanced H5 antibody titer by 9.82% after the first vaccination and by 30.63% after the second one.</td>
</tr>
<tr>
<td>Jiang et al. (2012)</td>
<td>Hypericum perforatum L. (HPE)</td>
<td>Broiler chickens</td>
<td>Significant difference in relative weights of immune organs and serum antibody titers against NDV and AIV</td>
</tr>
<tr>
<td>Nanekaran et al. (2012)</td>
<td>Mentha spicata extract</td>
<td>Broiler chickens</td>
<td>Increased serum antibody titers against NDV</td>
</tr>
<tr>
<td>Yazdi et al. (2014)</td>
<td>Tribulus terrestris L.</td>
<td>Broiler chickens</td>
<td>1 kg vine showed the highest antibody titer against AIV and sheep RBCs</td>
</tr>
<tr>
<td>Ghalamkari et al. (2011)</td>
<td>Satureja hortensis L.</td>
<td>Broiler chicks (Ross 308)</td>
<td>5 g/kg savory increased antibody titers against SRBC</td>
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<td></td>
<td></td>
<td></td>
<td>No remarkable effect on specific antibody titers against NDV and AIV at 42 days of age.</td>
</tr>
<tr>
<td>Landy et al. (2011)</td>
<td>Neem (Azadirachta indica)</td>
<td>One-day-old broiler chicks (Ross 308)</td>
<td>Induce desirable impact on broilers immune responses without any reported negative effect on growth performance.</td>
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<td>Treatment with 7 g neem/ kg increased antibody titers against SRBC</td>
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<tr>
<td>Toghyani, and</td>
<td>Sumac (Rhus coriaria L.) fruit powder</td>
<td>One-day-old broiler chicks</td>
<td>No marked effect on antibody titers against SRBC, NDV and AIV</td>
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<td></td>
<td>Heterophile to lymphocyte ratio were improved in response to 7 g/kg dose and AGP</td>
</tr>
<tr>
<td>Enhancement of immune response to challenge</td>
<td>Radix astragali, R. glycyrrizae, R. codonopis, and Herba epimedi aqueous extracts</td>
<td>-</td>
<td>Broiler chicks</td>
</tr>
<tr>
<td>Virgin coconut oil (VCO)</td>
<td>10 ml VCO/kg</td>
<td>Broiler chickens</td>
<td>Significant increase in specific antibody titers against H5N1, lymphocyte count, CD4 count and number of CD8 after challenge with AI virus.</td>
</tr>
<tr>
<td>Echinacea purpurea (EF) and Sambucus nigra</td>
<td>Extracts of EF and SAM in DW</td>
<td>Broiler chickens</td>
<td>EF induce reduction of the fecal viral shedding in all days post-challenge</td>
</tr>
<tr>
<td>Peppermint and eucalyptus essential oils</td>
<td>Mentofin®, (containing eucalyptus and peppermint essential oils)</td>
<td>Broilers challenged intratracheally by <em>Mycoplasma gallisepticum</em></td>
<td>Significant reduction in tracheal deciliation, mucosal hypertrophy, goblet cell degeneration and heterophil infiltration</td>
</tr>
<tr>
<td>Astragalus polysaccharide (APS)</td>
<td>Dietary supplementation of APS</td>
<td>Broilers chickens</td>
<td>APS reduce replication of H9N2 and enhance early humoral immunity in young chicks</td>
</tr>
<tr>
<td>NAS preparation</td>
<td>0.2 or 0.1g/kg/d</td>
<td>Broilers chickens</td>
<td>Prevented the shedding of H9N2 virus at the 7th day PI.</td>
</tr>
<tr>
<td>Polyphenolic compounds of green tea</td>
<td>Dietary inclusion of 10 g green tea by-products/kg</td>
<td>Broilers chickens</td>
<td>Significant antiviral effect in chickens.</td>
</tr>
<tr>
<td>Herbal I (sambiloto, temu ireng, adas bintang, sirih merah) and herbal II (sambiloto, adas bintang, sirih merah)</td>
<td>-</td>
<td>Broilers chickens</td>
<td>Congestion and edema of respiratory tract epithelium, lymphoid depletion of spleen and bursa of Fabricius with spread of virus particles in tissue of respiratory and lymphoid system</td>
</tr>
<tr>
<td>Nigella sativa seeds</td>
<td>1% and 3% levels of <em>Nigella sativa</em> seeds</td>
<td>Unvaccinated turkey to H9N2 infection</td>
<td>Significant decreased in clinical signs and viral shedding</td>
</tr>
<tr>
<td>Treatment</td>
<td>Group</td>
<td>Observations</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
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<tr>
<td>Thymoquinone (TQ) and curcumin (Cur)</td>
<td>Unvaccinated turkeys</td>
<td>- Improvement in production of specific antibodies, viral shedding, cytokine expression and suppression of viral pathogenicity. Groups treated with combination of TQ and Cur showed the best result.</td>
<td>Umar et al. (2016)</td>
</tr>
<tr>
<td>Pinus monophylla (Pinon) shell polysaccharide (PSP)</td>
<td>Young chicks</td>
<td>- Enhanced expression of IL-6 with elevated antibody titers. PSP administration decreases the replication of H9N2 and enhances early humoral immunity.</td>
<td>Xie et al. (2012)</td>
</tr>
<tr>
<td><em>Olea europaea</em> (Olive) leaf extracts (OLE)</td>
<td>Broilers chickens</td>
<td>- Both OLE treatment 3 days pre- and post-infection or only post infection resulted in 70% protection of birds. Pre-treatment delayed the beginning of mortalities.</td>
<td>Saif (2015)</td>
</tr>
</tbody>
</table>