Ann. Anim. Sci., Vol. 19, No. 4 (2019) 905–935 DOI: 10.2478/aoas-2019-0043

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HERBAL MEDICINE ADDITIVES AS POWERFUL AGENTS TO CONTROL AND PREVENT AVIAN INFLUENZA VIRUS IN POULTRY – A REVIEW

Ahmed R. Gado[1♦], Hany F. Ellakany[1], Ahmed R. Elbestawy[1], Mohamed E. Abd El-Hack[2♦], Asmaa F. Khafaga[3], Ayman E. Taha[4], Muhammad Arif[5], Samir A. Mahgoub[6]

[1]Department of Poultry and Fish Diseases, Faculty of Veterinary Medicine, Damanhour University, Egypt
[2]Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt
[3]Department of Pathology, Faculty of Veterinary Medicine, Alexandria University, Edfina 22758, Egypt
[4]Department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Alexandria University, Edfina 22578, Egypt
[5]Department of Animal Sciences, College of Agriculture, University of Sargodha, 40100, Pakistan
[6]Department of Microbiology, Faculty of Agriculture, Zagazig University, Zagazig 44111, Egypt
♦Corresponding authors: m.ezzat@zu.edu.eg; dr.mohamed.e.abdalhaq@gmail.com (Mohamed E. Abd El-Hack); ahmed.gado@vetmed.dmu.edu.eg (Ahmed R. Gado)

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 hemagglutinin 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, Astragalus, *Cochinchina 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 purpurea*, Ginseng stem-and-leaf saponins (GSLS), Astragalus polysaccharides (APS), *Myrtus communis* oil, Garlic powder, Turmeric, Thyme and Curcumin. This review aimed to evaluate 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). Avian influenza A (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 AIA 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). HPAI virus strains of subtype H5N1 caused several outbreaks in various countries of southeast Asia, such as Cambodia, China, Indonesia, India, Bangladesh, Thailand, Vietnam, 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, 2012). In fact, the use of antiviral drug families such as neuraminidase and hemagglutinin 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 a, b), ectoparasites (Abbas et al., 2018), reproductive and renal toxicity (Khafaga and Bayad, 2016 a, b), heat stress (Khafaga et al., 2019 a, b), 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., 2015, 2016, 2018, 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 was usually followed by failure due to antigenic drift of AIV (Lee et al., 2004; Busani et al., 2009; Capua et al., 2009).

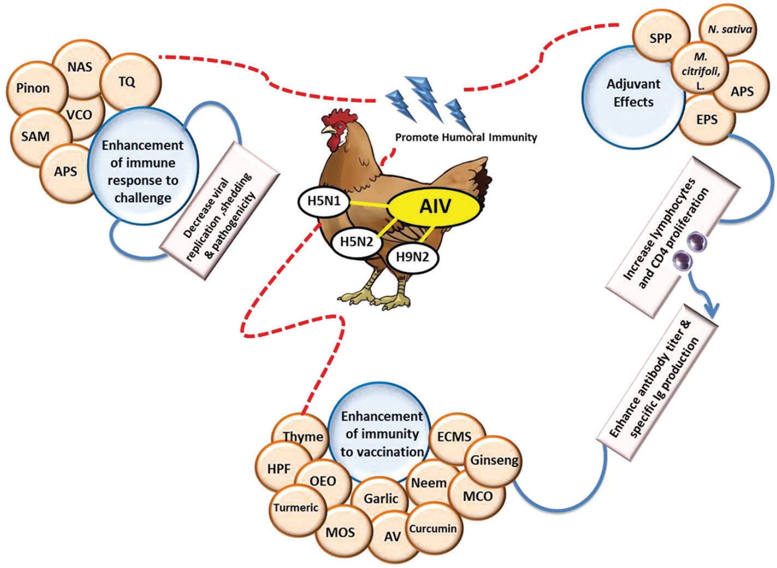
There are several factors that contribute to the inadequacy of vaccination as a standalone tool for AI prevention including: (1) inactivated vaccines are specific to only one subtype of AI, poor vaccine application (Suarez and Schultz-Cherry, 2000); (2) vaccine interference with maternally-derived antibodies 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., 2011 a, b; Park et al., 2011; Lee et al., 2012; Lee and Song, 2013); (6) variable host response to vaccination among different species and breeds of poultry (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

A great number of herbal extracts and botanicals has been investigated for their antiviral property. Generally, herbal preparations have potential strengthening impact on immune system, which resulted in fighting ability against invading viral infectious (Ganjhu et al., 2015; Sun et al., 2018; Alagawany et al., 2018, 2019). 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 stimulates release of IFN-γ, enhances Th-1 responses, and promotes the activity of CD^{+8} cytotoxic T cell, thereby playing a pivotal role in controlling viral infection (Trinchieri, 2003). In addition, various studies reported an 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 a pivotal role in phagocytic promotion, which is essential to attenuating viral infection, replication and spread (Qureshi et al., 2017). Type I interferons (IFN-α and IFN-β) are cytokines eliciting antiviral, antiproliferative, and immunomodulatory effects (Cheng et al., 2007). Concerning anti-influenza plants and their mechanism of antiviral action, 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 for 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 for avian influenza viruses is presented in Table 1.



PSP: *Pinus monophylla* shell polysaccharide; **TQ**: Thymoquinone; **VCO**: Virgin coconut oil; **SAM**: *Sambucus nigra*; **APS**: Astragalus 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**: Mannanoligosaccharides; **AV**: Aloe vera; **CRM**: Curcumin; **MCO**: *Myrtus communis* oil; **GSLS**: Ginseng stem-and-leaf saponins; **ECMS**: *Cochinchina momordica*

Figure 1. Different types of herbal plants and their role in improvement of chicken humoral immunity

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 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 CD^{+4} content and T lymphocyte multiplication were enhanced in all treated groups.

Table 1. Summary for the major findings related to uses of botanicals for avian influenza viruses

	Herbal	Dose level(s)	Species/strain	Main findings	Ref.
1	2	3	4	5	6
Adjuvant effects	*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 membranaceus 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)
Enhancement of immunity to vaccination	*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	Yuniwarti et al. (2012)

Table 1 – contd.

1	2	3	4	5	6
Enhancement of immunity to vaccination	*Echinacea purpurea*	0.1% aqueous Echinacea	One-day-old male broiler chickens (Ross 308)	• Treatment did not 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)
		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 to AI vaccination	Landy et al. (2011 b)
		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%	Najafzadeh et al. (2011)
		E. purpurea root powder in diet (0.1% and 0.5%)	Broiler chickens	• Marked alteration in feed conversion ratio, number of lymphocytes and heterophils, total counts of white blood cells (WBCs), and antibody titers against NDV and AIV	Dehkordi et al. (2011)
	Rosmarinus officinalis L. (rosemary)	Rosemary powder (0, 0.5, or 1.0%) and Vitamin E (0, 100, or 200 mg/kg)	Broiler male chicks	• 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)
	Eucalyptus globulus	Essential oils of eucalyptus and peppermint	Broiler chicks	• 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 (GSLS)	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)
Cochinchina momordica (ECMS)	5, 10, 20, 40 and 80 µg/dose	Two-week-old broiler chicks	• 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 chicks	• There is no significant modulation of antibody titers against AI as measured at 1, 35, and 42 days of age	Hosseinzadeh et al. (2014)
Myrtus 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)
Allium sativum (garlic)	1 and 3% fresh garlic powder	Broiler chicks	• Neither treatments nor removal of garlic had any effect on antibody titers to H9N2 vaccine	Jafari et al. (2009)
	2 and 4 g/kg garlic powder	Broiler chickens	• Non-significant enhancement in the humoral antibodies against AIV and NDV	Toghyani et al. (2011)
	100, 150, or 200 g/ton	Broiler chickens	• Significant impact on NDV and AIV antibodies titers • Diet supplemented with 200 g/ton of garlic powder had better positive effect.	Eid and Iraqi (2014)
Mannanoligosaccharides (MOS) and humate (HU)	0.1, 0.2 and 0.3%	Broiler chickens	• MOS supplementation led to marked increment in AIV antibody titers • MOS and 0.3% HU stimulating the humoral immunity against AIV vaccine	Tohid et al. (2010)

Table 1 – contd.

1	2	3	4	5	6
	Curcuma domestica Val. (turmeric) powder	3.3, 6.6 and 10 g/kg	Broiler chickens	• No significant impact on NDV and AIV antibody titer. • No significant enhancement of growth performance parameters except for feed efficiency.	Nouzarian et al. (2011)
		50% ethanol extract of turmeric	Layer chicken	• Significant increase of total heterophils after vaccination • Treatment and vaccine did not significantly affect the total basophils number	Widhowati et al. (2018)
	Herbal formula (viranur, turmeric and *Phyllanthus*)	Herbal solution (KB) (5 g turmeric and 25 g *Phyllanthus*), herbal solution (KC) (36 g herbals formula viranur and 25 g *Phyllanthus*)	Broiler chickens	• The weight index of thymus, spleen, and Fabricius bursa had no significant alteration • Non-significant difference in the spleen pathology • Lymphocyte increase in lymphoid follicles of the bursa of Fabricius in KC group and more widening in the cortex than medulla of the thymus in both group	Hartati et al. (2015)
	Turmeric and *Thymus vulgaris*	5 g/kg turmeric + thyme powder 2.5 g/kg turmeric powder + 2.5 g/kg thyme powder	Broiler chicks Ross 308	• Addition of different concentrations of turmeric and thyme powders increased body weight and antibody titers against NDV and AIV	Fallah and Mirzaei (2016)
	Curcuma longa and lutein	CRM or LTN (200 mg/kg diet)	Broiler chicks	• Significant elevation in NDV and AIV antibody titers in CRM-supplemented chicks over chicks in other groups • CRM-supplementation stimulates proliferation of B and T lymphocyte in both LPS- and non-LPS-induced chicks.	Rajput et al. (2013)
	Thymus vulgaris	5 and 10 g/kg	Broiler chicks	• Dietary inclusion of 5 g/kg improve growth performance without any deleterious effect on immune status and serum biochemical parameters	Toghyani et al. (2010)

	Thyme extract (0.1%, 0.15% and 0.2%) in DW	Broiler chicks	• 0.2% thyme extract induce significant increase in specific antibody response against H9N2 vaccine 14 days after vaccination	Talazadeh et al. (2015)
	0.1% and 0.2% Antibiofin® (containing *Thymus vulgaris*) in DW	Broiler chicks	• Elevation in specific antibody titer against H9N2 vaccine 14 and 28 days after vaccination	Talazadeh et al. (2016)
Sweet orange (*Citrus sinensis*) peel extract (SOPE)	1000 and 1250 ppm of in DW	Broiler chicks	• Dose-dependent significant elevation in serum antibody titers responses to all vaccines	Zohreh et al. (2014)
	0.25%, 0.5%, 0.75% and 1% of DCSP	Broiler chicks	• Total sheep red blood cells were significantly changed • Specific antibodies for AIV and NDV had no significant alteration • The lowest AI titer was obtained at 0 and 0.25%, while the	Ebrahimi et al. (2015)
Nettle (*Urtica dioica*) and ginger	2 g/kg nettle powder; 4 g/kg ginger powder and 2 g/kg ginger + 2 g/kg nettle powder	One-day-old broilers (Ross 308)	• Non-significant difference in antibody titers against NDV, AIV and SRBC • Serum antioxidant capacity was significantly elevated by nettle or ginger	Toghyani et al. (2015)
Oregano essential oil (OEO)	0.005 and 0.01%	Broiler chickens	• Higher dose improved parameters of growth performance in young birds • Improved specific antibody titers against NDV and AIV-HI	Galal et al. (2016)
Oil extracted propolis (OEP)	OEP (0, 40, 70, 100, 400, 700 and 1000 mg/kg)	Broiler chickens	• Significant increase in AI, ND and IBD antibody titer • No effect on IB titers • High concentration of OEP induces negative impact on broiler humoral immunity	Taheri et al. (2005)
	OEP (50, 100, 200, and 300 mg/kg)	One-day-old broiler chicks (Ross 308)	• Non-significant alteration in growth performance parameters • Non-significant effected on humoral immune function	Gheisari et al. (2017)

Table 1 – contd.

1	2	3	4	5	6
		OEP (1000 and 5000 mg/kg)	Mixed-sex quail chicks	• Significant difference in specific serum AIV antibody titers and sheep red blood cells, difference heterophils to lymphocytes ratio	Babaei et al. (2016)
	Hypericum perforatum L. (HPE)	250, 500, and 1,000 mg/kg	Broiler chickens	• 500 mg/kg concentration enhanced H5 antibody titer by 9.82% after the first vaccination and by 30.63% after the second one	Jiang et al. (2012)
	Mentha spicata extract	0.2%, 0.4% and 0.6%	Broiler chickens	• Non-significant difference in relative weights of immune organs and serum antibody titers to AIV and NDV	Nanekarani et al. (2012)
	Tribulus terrestris L.	1 or 5 g/kg		• Increased serum antibody titers against NDV • 1 g/kg vine showed the highest antibody titer against AIV and sheep RBCs	Yazdi et al. (2014)
	Satureja hortensis L.	5 and 10 g/kg	Broiler chicks (Ross 308)	• 5 g/kg savory increased antibody titers against SRBC • No remarkable effect on specific antibody titers against NDV and AIV at 42 days of age	Ghalamkari et al. (2011)
	Neem (*Azadirachta indica*)	7 and 12 g of neem fruit powder/kg	One-day-old broiler chicks	• Induce desirable impact on broilers immune responses without any reported negative effect on growth performance • Treatment with 7 g neem/kg increased antibody titers against SRBC	Landy et al. (2011)
	Sumac (*Rhus coriaria* L.) fruit powder	3 and 7 g/kg sumac powder		• No marked effect on antibody titers against SRBC, NDV and AIV • Heterophile to lymphocyte ratio were improved in response to 7 g/kg dose and AGP	Toghyani and Faghan (2017)
	Radix astragali, *R. glycyrrizae*, *R. codonopis*, and *Herba epimedii* aqueous extracts	–	Broiler chicks	• Did not affect antibody titers against NDV and AIV-H5	Liu et al. (2009)

Enhancement of immune response to challenge	Virgin coconut oil (VCO)	10 ml VCO/kg	Broiler chickens	• Significant increase in specific antibody titers against H5N1, lymphocyte count, CD4 count and number of CD8 after challenge with AI virus • An increase in chicken viability and significant reduction of mortality	Yuniwarti et al. (2015)
	Echinacea purpurea (EF) and *Sambucus nigra*	Extracts of EF and SAM in DW	Broiler chickens	• EF induce reduction of the fecal viral shedding in all days post-challenge • SAM resulted in significant reduction in the number of tracheal positive samples as compared to untreated and EF-treated groups	Karimi et al. (2014)
	Peppermint and eucalyptus essential oils	Mentofin® (containing eucalyptus and peppermint essential oils)	Broilers challenged intratracheally by *Mycoplasma gallisepticum*	• Significant reduction in tracheal deciliation, mucosal hypertrophy, goblet cell degeneration and heterophil infiltration	Barbour et al. (2010)
	Astragalus polysaccharide (APS)	Dietary supplementation of APS	Broilers chickens	• APS reduce replication of H9N2 and enhance early humoral immunity in young chicks	Kallon et al. (2013)
	NAS preparation	0.2 or 0.1 g/kg/d	Broilers chickens	• Prevented the shedding of H9N2 virus at the 7th day PI	Shang et al. (2010)
	Polyphenolic compounds of green tea	Dietary inclusion of 10 g green tea by-products/kg	Broilers chickens	• Significant antiviral effect in chickens • Strong anti-influenza activity • Dietary supplementation of catechins 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)	–	Broilers chickens	• 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	Setiyono and Bermawie (2014)

Table 1 – contd.

1	2	3	4	5	6
	Nigella sativa seeds	1% and 3% levels of *Nigella sativa seeds*	Unvaccinated turkey to H9N2 infection	• Significant decreased in clinical signs and viral shedding • Significant increase in serum antibody titers against H9N2 and increased IFN-γ mRNA expression • Reduction of pathogenicity of the virus in birds receiving 3% level	Umar et al. (2015)
	Thymoquinone (TQ) and curcumin (Cur)	–	Unvaccinated turkeys	• 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	Umar et al. (2016)
	Pinus monophylla (Pinon) shell polysaccharide (PSP)	–	Young chicks	• Enhanced expression of IL-6 with elevated antibody titers • PSP administration decreases the replication of H9N2 and enhances early humoral immunity	Xie et al. (2012)
	Olea europaea (olive) leaf extracts (OLE)	–	Broilers chickens	• Both OLE treatment 3 days pre- and post-infection or only post infection resulted in 70 % protection of birds • Pre-treatment dela	Saif (2015)

Astragalus membranaceus 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 effects of APS–sEPS on NDV and AIV vaccines, at a 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 b-globin/IgG chimeric intron and neomycine pCIneo 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 damnacanthal 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®, 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 investigated 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 results showed an increment in number of lymphocytes and Th-CD4 in AI vaccinated birds that received 10 mL per kg diet compared to unvaccinated chicks that received the same level of VCO. However, a decrease in these numbers was reported in chicks given 15 ml VCO/kg diet; this increase may be attributed to increased proliferation of lymphocytes through formation of phospholipids 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 lymphocytes in chicks given 15 ml VCO/kg diet may be attributed 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 did not affect antibody titers against AI, ND, total anti-SRBC (at 21 days of age) and IgM (at 21 and 42 days of age) (Miran et al., 2010). Similarly, Landy et al. (2011 a, b) 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 increased production of antibodies in chicks given *E. purpurea* continuously at the level of 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 controlling 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 conversion 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 and Fallah, 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 days. A total number of 270 one-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 can enhance broilers' humoral immunity, but it is 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 illustrated the effect of GSLS and Astragalus 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 induced 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. The obtained results showed that the pretreatment of CEF could enhance the anti-viral activity of GPS; also, that GPS is able to improve the early humoral immunity in young chicks.

Cochinchina momordica (Bitter melon, Bitter apple, Bitter gourd, Bitter squash or 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-week-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 authors attributed this nonsignificant effect to strict biosecurity measures observed throughout the experimental period (Hosseinzadeh et al., 2014).

Myrtus communis oil (MCO) (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 significantly 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 improved the antibody titers against AIV and NDV.

Aloe vera (AV) (Indian aloe, Chinese aloe, Barbados aloe, True aloe or 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 were supplemented with Aloe vera (3% in drinking water), garlic powder (3% in diet), and a 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 at 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 the 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 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., 2010).

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 of 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 did not 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 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 7 days of age followed by AI vaccine at 14 days. 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 of the herbal 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 stimulated 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 induced better pigmentation efficiency, however curcumin-treated chicks showed enhanced immune status (Rajput et al., 2013).

Thymus vulgaris (German thyme, Common thyme, Garden thyme or 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 induced 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® (containing *Thymus vulgaris*) in drinking water were tested for their impact on immune response of broiler chicks against AIV subtype H9N2 vaccine. The obtained results revealed that Antibiofin® at dose level of 0.1% and 0.2% elevated the specific antibody titer against H9N2 vaccine 14 and 28 days after vaccination compared to the control group (Talazadeh et al., 2016).

Citrus sinensis peel extract (SOPE) (Sweet orange)

Various concentrations 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% concentrations, while the highest one was obtained at 0.5 concentration (Ebrahimi et al., 2015).

Urtica dioica (Nettle) and Zingiber officinale (Ginger)

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 were vaccinated with NDV and AI and supplemented with OEO. It could be concluded that dietary inclusion of OEO is 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. Chickens received 7 different levels of OEP (0, 40, 70, 100, 400, 700 and 1000 mg/kg), and serum samples were collected twice at days 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 induced 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) 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 differences in heterophils to lymphocytes ratio were observed among treatment and control groups (P<0.01) (Babaei et al., 2016).

Hypericum perforatum L. (HPE) (Common St John's Wort, Perforate St John's-Wort, or 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 20th 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 or Mackerel mint) extract

The drinking water application of 0.2%, 0.4% and 0.6% concentrations of *Mentha spicata* extract caused 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 promoter. 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 a, b).

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 did not induce a marked effect on antibody titers against SRBC, NDV and AIV (P>0.05) but heterophile to lymphocyte ratio was 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 or Licorice)

Liu et al. (2009) compared the immunomodulatory effect of *Radix astragali*, *Radix glycyrrizae*, *Radix codonopis*, and *Herba epimedii* aqueous extracts in clinically 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 were not 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 started from 7 days 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 elder, European elder, Elderberry, European black elderberry, or 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 is able to reduce shedding of H9N2 virus from trachea and feces (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 to control groups (Barbour et al., 2010).

Astragalus polysaccharide (APS) (Astragalus)

Li et al. (2011) mentioned that APS is a common immune adjuvant which is 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 first 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 0.1 g/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 contrast, 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 formulas 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., 2016 a).

Thymoquinone (TQ) and curcumin (Cur)

Umar et al. (2016 b) 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 treatments 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 cornerstones 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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Received: 27 III 2019
Accepted: 8 VII 2019