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Review article

Unveiling the phytobiotic potentials of clove *(Syzygium aromaticum)* for poultry immunology: The need for more molecular research

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Abstract

Poultry production faces numerous challenges, including infectious diseases, stress, and the need for sustainable alternatives to antibiotics. Herbal additives and probiotics have gained attention as potential immunomodulatory agents to enhance the immune response and overall health of poultry. This review highlights the current understanding of the immunomodulatory effects of dietary Clove, *Syzygium aromaticum* on poultry, with a particular emphasis on the need for more molecular research to unravel the underlying mechanisms. This knowledge will facilitate the development of targeted approaches to optimize poultry health, welfare, and productivity in the face of emerging climate change and other challenges.

Keywords: Antibody, Climate change, Herbal additives, Immunostimulants, Infectious diseases

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INTRODUCTION

The demand for poultry products is on the increase (Kleyn and Ciacciariello, 2021), necessitating the development of sustainable and safe production practices. The use of prebiotics, probiotics, symbiotics, enzymes, organic acids, immunostimulants, essential oils and phytogenic/phytobiotic herbs to enhance birds' natural immunity is on the increase while antibiotics are being discouraged (Reda et al., 2020; Sheiha et al., 2020; Abd El-Ghany et al., 2021; Mohamed, 2022). Being growth, production, immune status and meat quality enhancers without leaving residues as well as being environmentally friendly, herb spices and their bioactive constituents are increasingly becoming significant in poultry production (El-Hack and Alagawany, 2015; El-Hack et al., 2015; Abd El-Hack et al., 2016; Gadde et al., 2017; Chowdhury et al., 2018; Kunnumakkara et al., 2018; Al-Mufarrej et al., 2019; Hussein et al., 2019). Herbal additives are fast becoming increasingly preferred to antibiotics (Muaz et al., 2018; Hussein et al., 2020), as they offer immunomodulatory effects that can enhance the innate and adaptive immune responses in poultry.

Various herbal additives, such as garlic, turmeric and ginseng, have been investigated for their immunomodulatory properties in poultry (Hartady et al., 2021; Abd El-Hack et al., 2022). These additives have shown promising effects on immune system improvement, production of antibodies and secretion of cytokines as well as phagocytic activities (Mazziotta et al., 2023; Seidavi et al., 2023). However, the specific molecular pathways and cellular receptors involved in mediating these immunomodulatory effects has not been largely reported.

Due to its healing properties, *clove* (*Syzygium aromaticum*) has been regarded as a very important medicinal plant (Bellakhdar, 1997). Eugenol, eugenol acetate and caryophyllene have been revealed as the major compounds in clove essential oil (EO); which are responsible for varieties of biological activities attributable to *S. aromaticum*, including antioxidant, anti-inflammatory and antibacterial effects, among others (Ali et.al., 2012; Mittal et al., 2014; El Faqer et al., 2022). Figures 1 and 2 show the physical appearance of clove and chemical structures of some of the compounds in it respectively.

Figure 1 Physical appearance of clove (Syzygium aromaticum) (Yameen et al., 2023)

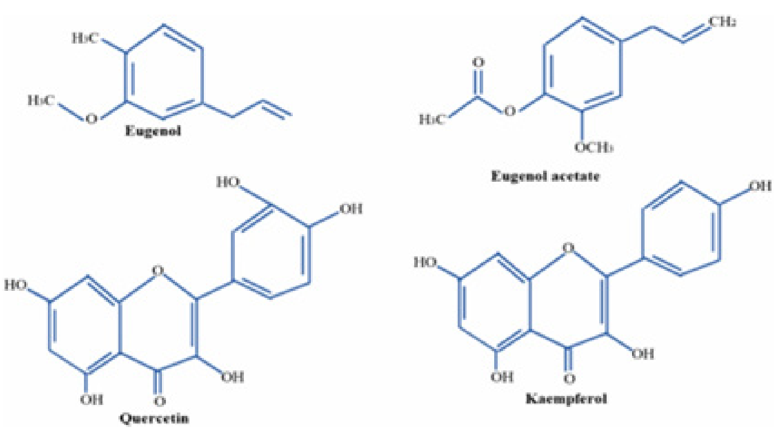

Figure 2 Chemical structures of some of the compounds in cloves (Idowu et al., 2021)

It has been reported from several studies that dietary spices containing essential oils or their bioactive compounds can improve digestion (Murugesan et al., 2015; Mustafa, 2016; Mandey et al., 2019; Suliman et al., 2021). They promote secretions of bile salt hydrolase and other digestive enzymes from the intestinal mucosa and pancreas (Jang et al., 2007). It has been insinuated that clove has capability for the modulation of intestinal microbiota population, enhancement of nutrient digestibility and modification of the intestinal mucosa structure (Suliman et al., 2021).

Cloves, among many other medicinal herbs and spices, have shown potentials to reduce the massive economic losses attributable to coccidiosis in the poultry industry (AL-Fifi, 2007; De Pablos et al., 2010; Zaman et al., 2012; Rakhmani et al., 2014; Molan et al., 2015; Srisanyong et al., 2021). Coccidiosis has been described as a devastating and economically damaging parasitic poultry disease which causes poor growth, reduced feed conversion ratio, high morbidity and mortality (Mund et al., 2017; Kadykalo et al., 2018; Qaid et al., 2020).

Although several studies have been conducted to evaluate the effect of broiler chicken diets supplemented with cloves on performance, carcass characteristics, and production traits of broilers (Boyraz and Özcan, 2006; Mukhtar, 2011; Agostini et al., 2012; Salman and Ibrahim, 2012; Azadegan et al., 2014; Mohammadi et al., 2014), a comprehensive understanding of the molecular mechanisms underlying these effects is still lacking.

MAIN TEXT

Phytobiotics

Phytobiotics have been reported by researchers to have pharmacological effects, which can be attributed to their phytoconstituents, including steroids, saponins, flavonoids, glycosides, tannins, alkaloids, terpenes, etc. (Agidew, 2022; Sharma et al., 2023). They are very useful to humans in both traditional and alternative medicine as well as in nutrition as flavours and food preservatives (Grashorn, 2010; Agidew, 2022). It has been confirmed via many in vitro and in vivo studies that phytobiotics have many indications in animal nutrition, such as feed intake stimulation, stimulation of digestive enzyme secretion, digestibility enhancement and gut microflora manipulation as well as nutrigenomics, antioxidant, antimicrobial, coccidiostatic, antistress, cholesterol-lowering, anthelmintic and immunomodulatory activities (Panda et al., 2006; Grashorn, 2010; Prabakar et al., 2016; Batiha et al., 2020; Ghanima et al., 2020; Arif et al., 2022). In poultry, feed palatability and sensory quality; growth promotion in improved weight gain and feed conversion ratio as well as reduced mortality; gut function and nutrient digestibility for improved growth performance; gut microflora control for less diseases of the GIT, improved growth and reduced mortality; immune function enhancement for improved health; and carcass meat safety in reduced microbial load and improved sensory quality are confirmed phytogenic effects (Mountzouris et al., 2009; Grashorn, 2010; Prabakar et al., 2016).

Chemical composition of clove extracts

Batiha et al. (2020) listed eugenyl acetate, eugenol and β-caryophyllene as the most important phytochemical constituents of clove oil. According to Golmakani et al. (2017), eugenol is the major compound found in cloves essential oil, accounting for at least 50 %, while about 40 % consist of eugenyl acetate, β-caryophyllene, and α-humulene. Also, there are minor or trace components like diethyl phthalate, caryophyllene oxide, cadinene, α-copaene, 4-(2-propenyl)-phenol, chavicol and α-cubebene, which account for less than 10 %. The screening of clove extracts by El Faqer et al. (2022) revealed the presence of phenols, flavonoids, flavones aglycones, coumarins and tannins. *S. aromaticum* has also been shown to be rich in phytochemicals such as sesquiterpenes, monoterpenes, hydrocarbon and phenolic compounds (Batiha et al., 2020).

Bioactivities and pharmacological effects of clove extracts

Several authors have mentioned that cloves essential oil has antiseptic (Khalilzadeh et al., 2016; Wei et al., 2016; Shahbazi, 2019), natural stimulant (Shahbazi, 2019), carminative (Guan et al., 2007; Din et al., 2015; Wei et al., 2016; Shahbazi, 2019), anticoagulant (Khalil et al., 2017; Uchôa Lopes et al., 2020), anthelminthic (Shahbazi, 2019), antiemetic (Guan et al., 2007), antidiarrheal (Wei et al., 2016; Khalil et al., 2017; Uchôa Lopes et al., 2020), antispasmodic (Guan et al., 2007; Wei et al., 2016), hepatoprotective (Din et al., 2015), spasmolytic (Din et al., 2015), antimutagenic (Kennouche et al., 2015; Khalil et al., 2017, Thapa et al., 2019), anticonvulsant (Kennouche et al., 2015), antidepressant (Liu et al., 2015), renal reinforcement (Guan et al., 2007; Khalil et al., 2017), antipyretic (Uchôa Lopes et al., 2020), neuroprotective (Khalil et al., 2017), antistress (Salman and Ibrahim, 2012; Suliman et al., 2021), antiallergic (Guan et al., 2007; Khalilzadeh et al., 2016; Wei et al., 2016), antidiabetic (Khalil et al., 2017; Behbahani et al., 2019), and hypocholesterolemic (Khalil et al., 2017) as well as analgesic, anticancer, anesthetic, insecticidal, mosquito repellant and aphrodisiac effects (Batiha et al., 2020; Vigad et al., 2021).

Essential oils possess phytogenic and pharmacological characteristics and their supplementation has been discovered to enable modulation of intestinal microbiota in poultry, inhibition of growth of specific bacterial pathogens, improvement of immunity, and enhancement of meat production via better utilization of nutrients and energy (Seidavi et al., 2022). Milićević et al. (2022) came up with a rather uncommon use for essential oils as alternative environmental biopesticide. They presented that cloves essential oil (CEO) can be packaged for preservation of stored products or fresh fruits.

Also, pharmacological effects of clove, which is phytobiotic, against pathogenic parasites and microorganisms, including pathogenic bacteria, *Plasmodium, Babesia, Theileria parasites, Herpes simplex*, and hepatitis C viruses have been checked (Batiha et al., 2020). However, these effects have not been completely studied, and may be explored as new research ideas for clove essential oil.

Potentials of clove as Poultry feed additive

Growth Performance

Utilization of clove and its essential oil as feed additives and growth promoters in poultry has resulted in improved growth performance, control of some intestinal pathogens, digestion stimulation and antiseptic effects. It has also been shown that clove and its essential oil possess strong antimicrobial, antifungal, antiparasitic, anti-inflammatory, anesthetic, anti-carcinogenic and antioxidant abilities (Najafi and Torki, 2010). Clove essential oil as a dietary supplementation has also been tipped for improvement of growth performance, including weight gain and feed conversion, as well as partial fixing of coccidial problems in gut integrity and meat quality of broiler chickens (Zhang et al., 2023).

However, inclusion level must be taken into consideration when using cloves in poultry production. Greater growth performance has been found in birds fed diets with inclusion levels of 100 and 200 mg/kg clove in a study (Agostini et al., 2012) in which different levels of clove oil inclusion (0, 100, 200, 1000 and 2500 mg/kg) were used. In another study (Mohammadi et al., 2014), lower performances were observed in broiler chickens fed 500 mg/kg than those fed 100 and 300 mg/kg clove oil. Meanwhile, it has been reported that excessive dosage can be toxic to animals and/or result in abnormal functions based on the rate of absorption and assimilation (Jouany and Moravi, 2007). Al-Mufarrej et al. (2019) in their publication concluded that clove supplementation at levels greater than 2 % can negatively affect broiler chickens' performance; without affecting liver health and immune-response. According to them however, there is still need for further research to explain the underlying mechanism.

Also, Jouany and Moravi (2007) had reported significantly lowered alanine absorption from rat intestine when fed diet with inclusion of eugenol and cinnamaldehyde of clove with concentrations 850 – 1000 mg/kg. Meat with low cholesterol can be produced when cloves powder and oil are combined due to the presence of phytochemicals (Adu et al., 2020).

According to Mukhtar (2011), reduced mortality rate as well as decreased total cholesterol levels were observed along with enhanced live body weight, feed intake, feed conversion ratio and carcass yield. Cloves also consist of an anti-stress agent (Salman and Ibrahim, 2012). Furthermore, Salman and Ibrahim (2012) reported that the performance of heat-stressed broiler chickens was enhanced by feed diets with 0.8 % each inclusion of clove powder and clove flower oil combination and drinking water with 0.4 % aqueous clove extract inclusion (Suliman et al., 2021). Elbaz et al. (2022) concluded from their research that clove essential oil is a potential alternative to antibiotics; its supplementation can improve growth performance as well as enhance immunity and gut health of broiler chickens.

Serum biochemistry

Reduced blood glucose levels have been reported in clove essential oil-containing treatments compared to control treatment (Mohammadi et al., 2014) which agreed with the report by Baker et al. (2008) and Yedjou et al. (2023) on the use of medicinal herbs to stimulate insulin secretion thereby reducing blood glucose levels. Clove essential oil has also been found to possess hypocholesterolemic ability, which may result from an inhibition of the hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase activity, a significant regulatory enzyme in the synthesis of cholesterol (Hong et al., 2012; Mohammadi et al., 2014). Meanwhile, cholesterolemic effects of essential oils has been known to be strain-specific/breed-specific and gender-specific as well as dependent on age and feed composition (Lee et al., 2003; Hussein et al., 2023).

Al-Mufarrej et al. (2019) reported that high clove supplementation of 4 % to 6 % gave rise to reduced total serum protein and albumin as well as the greatest aspartate aminotransferase activity in broiler chickens at 35 days.

Wati et al. (2015) reported significant effect of phytogenic feed additives (clove, peppermint, fennel, oak, melissa balm, thyme and anise) supplementation in increase in the lymphocyte percentage as well as decrease in the heterophil percentage and the heterophyl/lymphocyte (H/L) ratio; attributable to the antioxidant and anti-inflammatory properties of clove buds. The oxidative stress in the immune cells reduced while the lymphocytes count increased due to the strong antioxidant effect of clove buds (Gandomani et al., 2014; Ben Naser et al., 2023). Clove as an aromatic antioxidant herb lowers the H/L ratio in poultry blood, thereby enhancing their immunity (Ojala et al., 2000; Ben Naser et al., 2023).

Low abdominal fat is of significant economic importance to poultry farmers as it gives rise to better carcass yield, which in turn increases total productivity (Elbaz et al., 2023). According to Mahrous *et al.* (2017), serum total cholesterol was significantly reduced in broiler chickens with inclusion of clove powder in their diet. Also, Venkadeswaran *et al.* (2014) found that total cholesterol, LDL-cholesterol, and triglyceride levels were reduced with inclusion of eugenol, which is a major constituent of cloves. It can be explained that clove improves the health status of broilers via hypolipidemic activity, by preventing or minimizing the production of free radicals in their bodies (Hussein et al., 2019; Sultana et al., 2023). Probiotics supplementation has been found to reduce lipogenesis or fatty acids synthesis by reducing the acetyl-CoA carboxylase enzyme, mitigating its catalytic role in the process (Homma and Shinohara, 2004; Axling, 2012). Elbaz et al. (2023) reported that probiotic or clove essential oil supplementation significantly reduced serum triglycerides, cholesterol, LDL and ALT during thermal stress.

Gut Health

Clove is a popularly known phytobiotic plant that can improve poultry gut health, whether used alone or combined with other plants (Batiha et al., 2020; Arif et al., 2022; Islam et al., 2023). It enhances the gastrointestinal ecosystem by hindering growth of pathogenic microorganisms and regulating the gut morphologic and morphometric characteristics (Agostini et al., 2012; Wati et al., 2015; Chowdhury et al., 2018; Chakma et al., 2020; Islam et al., 2023).

The morphologic and morphometric characteristics of broilers' intestines are indications of their health status, nutrient absorbability and immunocompetence (Nicholson et al., 2012). Intestinal digestibility, nutrient absorbability and gut health have been reported to improve with increase in absorptive villus surface area, enhancing broilers' growth performance (Laudadio et al., 2012; Mahdavi et al., 2014; Mohamed et al., 2014; Islam et al., 2023). Mohammadi et al. (2014) observed that the histomorphological changes in the intestine of the broiler chickens in their study indicated that clove essential oil treatments resulted in higher villus length in the duodenum and jejunum compared to the control treatment ($P < 0.01$). Al-Mufarrej et al. (2019) also reported an increase in intestinal length resulting from clove dietary supplementation at 10 g/kg inclusion rate but reduction at 20 g/kg or more, which is in alignment with the observation of Islam et al. (2023). Othman et al. (2022) from their findings reported that clove bud powder supplementation in broiler chicks' feed improved villus length and villus height to crypt depth ratio of the chicks fed 1 % inclusion compared to the control. The chicks were raised for 6 weeks. Diarrhea, for which *Escherichia coli* is greatly responsible, is one of the most frequently occurring deadly diseases in poultry (Wang et al., 2017; Liang et al., 2021). It has been reported that clove essential oil inclusion treatment increased *Lactobacillus* counts and decreased *E. coli* counts compared to the control, thereby improving cecal microbial status of broiler chickens in the treatment groups significantly (Mohammadi et al., 2014; Islam et al., 2023). In another study where dietary inclusion levels of clove were tested, feed to gain ratio was observed to have been modified with the inclusion of clove (Agostini et al., 2012). It was further revealed via evaluation of microbiology and intestinal morphology of ileal content and tissue, that though the enterobacteriaceae counts were unaffected at 21 days, *Lactobacillus* was increased by clove supplementation compared to the result from the birds fed the control diet.

Coccidiosis has been described as a devastating and economically damaging parasitic poultry disease which causes poor growth, reduced feed conversion ratio, high morbidity and mortality (Mund et al., 2017; Kadykalo et al., 2018; Qaid et al., 2020). The disease is caused by the apicomplexan parasite of the genus Eimeria which has seven known species (Fatoba and Adeleke, 2018), which attacks the intestinal epithelium and the connective tissue of the mucosa. The use of natural products such as probiotics, plant extracts and fungal extracts as prevention and treatment of avian coccidiosis has been widely reported (Allen et al., 1998; Dalloul et al., 2005; Lee et al., 2007; Abbas et al., 2010; Kim et al., 2013; Al-Shera ji et al., 2013; Almeida et al., 2014). Dietary supplementation of clove and other essential oils have been studied for their potential to prevent and ameliorate coccidial damage in avian gut integrity (Zhang et al., 2023).

The Need for More Molecular Research

The biological and biochemical activities of dietary clove supplementation in poultry have been the crux of recent researches (Al-Mufarrej et al., 2019; Suliman et al., 2021). Despite the growing body of evidence supporting the immunomodulatory effects of clove as an herbal additive in poultry, there is a significant gap in our understanding of the underlying molecular mechanisms. The limited availability of molecular studies hampers our ability to fully exploit the potential of cloves for optimizing poultry health and production. Besides the generally known antioxidant and anti-inflammatory properties of essential oils, the characterization of the signaling pathways involved in the modulation of intestinal immune activities of poultry through the use of dietary cloves should be carried out in vivo.

Such study was carried out to evaluate the effect of the Oregano essential oil on chicken interferon–alpha signalling pathway components post NDV vaccination using quantitative real-time PCR (qRT-PCR) (Galal et al., 2016). The molecular underpinnings of the immunomodulatory effects of cloves on poultry should be further studied using gene expression analyses of intestinal cytokines and other immune genes in quantitative real-time polymerase chain reactions as it has been done for other phytobiotics and essential oils (Kim et al, 2013; Qin, 2016; Shimao et al. 2019; Kikusato et al, 2020, Kikusato, 2021). Well-designed studies should be conducted to ascertain optimum dietary levels that maximizes phytobiotic benefits without cytotoxic effects. Similar studies have been carried out to study the effect of probiotics, specifically Bacillus-based (*B. subtilis and B. amyloliquefaciens*) diet supplementation in broiler chickens where tight junction protein gene expression correlated with intestinal immune activity and gut barrier integrity (Kim and Lillehoj, 2019). Adhikari et al. (2020) also studied the effect of probiotics on the expression of immune genes during Salmonella enteritidis infection.

Molecular Research Approaches

To bridge this knowledge gap, future research should focus on molecular approaches such as transcriptomics, proteomics, and metabolomics. These techniques can provide valuable insights into the gene expression patterns, protein profiles, and metabolic pathways associated with the immunomodulatory effects of herbal additives and probiotics. Additionally, the use of advanced molecular tools, including next-generation sequencing and bioinformatics, will enable a deeper understanding of the complex host-microbe interactions and signaling pathways involved.

Chicken Specific Kinome Array analysis can be carried out to study the immunometabolic changes that occur in chickens when fed with dietary cloves. Also, rRNA-based Microbiota analysis can be carried out on pre-infected and post-infected chickens fed with supplemental cloves to quantify its immunomodulatory effect. In addition, interactive effect of genotype and dietary supplementation with cloves can be studied to identify metabolic biochemical differences which may be strain-specific. The expression of genes related to innate immunity such as TLR3, TLR5, and NF-κB, and cytokines like IL-1β, IL-6, IL-15, and IFN-γ could be compared in clove-fed and control birds.

There is a revived scientific interest in drug discovery from natural sources (Najmi et al., 2022). An example is the bioactivity-guided fractionation technique which has been the focus of recent researches (Iqbal et al., 2015; Nothias et al., 2018; Tu et al., 2019; Abdallah et al., 2021). Evaluation of antiplasmodial and antimicrobial activities of cloves can be conducted by subjecting it to bioassay-guided isolation of phytoconstituents using flash chromatography followed by characterization using NMR and HR-ESI-MS techniques as carried out on *Terminalia albida* by Baldé et al. (2021).

Other modern approaches such as molecular modeling, virtual screening, natural product library, and database mining can be explored to establish clove (*Syzygium aromaticum)* as a veritable alternative to antibiotic growth promoter for poultry.

However, potential challenges may arise to this advancement due to variability in herbal additive compositions, high cost of conducting high through-put molecular research, newly developing expertise in emerging research methodologies, on-going research discoveries, limited facilities and equipment, as well as technical difficulties in translating in vitro findings to in vivo applications, especially in developing countries.

CONCLUSIONS

Clove (*S. aromaticum*) as an herbal additive offers promising immunomodulatory effects in poultry, enhancing their immune response and overall health. However, there is a critical need for more molecular research to unravel the underlying mechanisms and identify key molecular targets. By employing advanced molecular techniques, researchers can gain a deeper understanding of the complex interactions between this additive and the host immune system. This knowledge will facilitate the development of targeted approaches to optimize poultry health, welfare, and productivity in the face of emerging climate change and other challenges.

AUTHOR CONTRIBUTIONS

Itunuola Anne Folarin conceived, structured and wrote the most part of this article. Silifat Adewunmi Olanloye wrote some parts while Gbolahan Muyiwa Folarin also contributed parts, edited and formatted the article. All the authors contributed to the content, as well as read, edited, and fine-tuned the manuscript. All authors approve the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interest, financial or otherwise.

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