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Review article

The Use of Medicinal Plants in Avian Colibacillosis Management: A Review

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Abstract

Avian colibacillosis is regarded as one of the most severe bacterial diseases in the poultry industry worldwide. Due to the emerging antimicrobial resistance issue in isolated *E. coli* from poultry sources, the application of medicinal plants to treat colibacillosis is widely being studied due to the presence of the respective bioactive compounds. This article reviewed studies between the year 2016 to 2022 that used medicinal plants as avian colibacillosis treatment in *in-vitro* and *in-vivo* studies. The review provided insights to the readers regarding the type of plants used, the form of treatments and outcomes of previous experiments. A better understanding of the information addressed in this review article helps poultry researchers and industry to explore the potential of various medicinal plants in managing, reducing, and eliminating avian colibacillosis from poultry flocks.

Keywords: Avian colibacillosis, Herbal medicine, Natural products, Phytotherapy

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INTRODUCTION

Escherichia coli is a gram-negative rod-shaped bacteria under the Enterobacteriaceae family (Spickler, 2016; Nolan, 2019). The bacteria are ubiquitous, normally found in the intestine as commensals, and can be found in the environment as well in foods (Spickler, 2016; Nolan, 2019). Being a large and diverse group of bacteria, possession of virulence factor is being used to determine the pathogenic strains, which will further be classified into pathotypes (Spickler, 2016; Nolan, 2019). Colibacillosis refers to any infection or disease caused by *E. coli* (Merriam-Webster, 2023). Meanwhile, colisepticaemia is a systemic disease caused by coliform bacteria via circulatory blood invasion (Grünberg, 2020). The disease is recognised in many species of animals, including avian (American Association of Avian Pathologists, 2013; Nolan, 2019; Grünberg, 2020).

Avian colibacillosis is a major endemic disease in poultry that causes huge economic losses through mortality and low productivity from diseased chickens (AAAP, 2013; Nolan, 2019). In Indonesia, the estimated economic direct and indirect losses due to avian colibacillosis were discussed by Wibisono et al. (2018) that reached 13.10% of total poultry assets in Indonesia. The direct losses were calculation of harvest weight loss, total mortality and decreased egg production while the indirect losses included expenditure costs such as cleaning, disinfection and labour compensation (Wibisono et al., 2018). A retrospective study of 380 diagnosed poultry cases in a Kenyan laboratory revealed bacterial disease is the major cause of poultry mortality and morbidity at 45.2% with colibacillosis as the leading bacterial condition compared to other pathogens (n=303, 79.7%) (Katu et al., 2020).

The use of various plants as medicine to treat colibacillosis is widely being studied due to the presence of the respective bioactive compounds, mainly secondary metabolites that possess desired effects such as antimicrobial, antioxidant and anti-inflammatory traits (Teoh, 2015). Secondary metabolites are small molecular compounds naturally produced via secondary metabolism to ensure their survival in the environment (Teoh, 2015). Each medicinal plant contains different secondary metabolites that depend on the cultivated soil conditions, plant age, types and doses of fertilisers and environmental stressors (Sugiharto, 2021). Conditions of bioactive compounds in terms of concentration and activity are also determined by the form of supplementation or administration (powder or meal, extract, encapsulated, filtrate or decocted product) or method of extraction by using different types and concentrations of solvents (Sugiharto, 2021).

In regards to utilising medicinal plants as colibacillosis treatment, Aminzare et al. (2017) reported that 44 traditional plants were studied at their potential as phytotherapy against isolates of *E. coli* conducted in different countries. Numerous and diverse research has been conducted using multiple medicinal plants to study the therapeutic effects on colibacillosis-infected chickens throughout the years. However, reviews on application of medicinal plants to specifically treat avian colibacillosis in *in-vitro* and *in-vivo* studies have never been done prior to this publication. The knowledge would be remarkably useful in guiding future researchers in determining the type of plants to be used, the methodology and expected outcomes when designing their experiments. Hence, this review aims to elaborate on the use of medicinal plants in treating avian colibacillosis.

E. coli INFECTION IN POULTRY INDUSTRY

E. coli is a pathogen causes avian disease that is called avian colibacillosis as a primary or secondary infection. The aetiological agent commonly causes major outbreaks is *E. coli* from serotypes O1, O2, O35, O36, and O78 (American Association of Avian Pathologists, 2013). Commonly, the agent for avian colibacillosis is called avian pathogenic *E. coli* (APEC). The pathotyping defining feature is a number of virulence genes clustered in the plasmid of the bacteria (Nolan, 2019; Kathayat et al., 2021). However, Collingwood et al. (2014) disagreed that all *E. coli* in avian colibacillosis cases can be classified as APEC. Although certain pathogenic *E. coli* can be classified as APEC, the disease is not caused by a specific single pathotype of *E. coli* as the strains are diverse, and typically colibacillosis in poultry is caused by opportunistic infections that rise from commensal genotypes that carry APEC virulence factors.

Predisposing factors usually are related to poor hygiene in the poultry house environment and secondary to immunosuppression or respiratory diseases (AAAP, 2013; Nolan, 2019). The natural route of infection originates from a high bacterial load in the faeces in the environment or initial exposure through contaminated eggs that are being transmitted via the respiratory tract, damaged skin barrier or intestinal mucosa, cloaca and navel (Nolan, 2019). Common diseases that play a significant role in being primary causes are mycoplasmosis, infectious bronchitis, Newcastle disease, haemorrhagic enteritis and turkey bordetellosis (Nolan, 2019). Another predisposing condition is immunosuppression due to environmental stress from poor housing conditions (AAAP, 2013).

There are non-specific clinical symptoms vary with age, organs, and concurrent disease (Nolan, 2019). Infected birds can develop air sacculitis, pericarditis, enteritis, cellulitis, omphalitis, peritonitis, salpingitis, synovitis, osteoarthritis, and systemic infections like septicaemia and coligranuloma (AAAP, 2013). The disease infects all types of poultry, including chickens, ducks, and turkeys, regardless of breed or age group (AAAP, 2013). Acute septicaemia infecting young birds has few lesions, including hyperemic liver, spleen, and body cavity fluids (Nolan, 2019). If the birds survive the septicaemic phase, histopathological and post-mortem lesions may include subacute fibrinopurulent lesions in various organs and lymphocyte depletion in lymphoid organs (Nolan, 2019).

The typical diagnostic procedure involves the isolation of *E. coli* from samples including heart blood, liver or other organs with lesions (Nolan, 2019). Nonetheless, the proper diagnosis of the disease is not simply by depending on isolating *E. coli* (AAAP, 2013). To diagnose the disease as primary colibacillosis, the isolated coliform usually undergoes serotyping to detect the major pathogenic serotypes, plus other primary infectious diseases that need to be ruled out prior (AAAP, 2013). Secondary colibacillosis is diagnosed when the bacteria are isolated secondary or due to other primary infections (AAAP, 2013). Another method to detect the pathogenicity of the isolates is by polymerase chain reaction (PCR) to detect the plasmid-borne virulence genes or by inoculating embryonated eggs via allantoic sac at 12 days of age followed lesions observation (Nolan, 2019).

Avian colibacillosis prevention primarily focuses on farm management to minimise bacteria exposure (AAAP, 2013; Nolan, 2019). Poor housing conditions cause stress and predispose to infections, which can lead to secondary colibacillosis (Nolan, 2019). Vaccination is not widely used in colibacillosis control because APEC strains are diverse and less effective in protecting against heterologous strains (Kathayat et al., 2021).

Antibiotics have long been the first-line treatment for avian colibacillosis. Tetracycline, neomycin, streptomycin, sulfa-group drugs, and others were among the common antibiotics used in farms (AAAP, 2013; Nolan, 2019). As a result of the widespread use of antibiotics, treatments with the aforementioned antibiotics were no longer effective because most of the isolates were resistant to them (AAAP, 2013; Nolan, 2019). Antimicrobial resistance in poultry is becoming a major global issue. According to one study, the prevalence of resistant APEC isolates against commonly used antibiotics such as ampicillin, amoxicillin, and tetracycline was reported to be over 80%, indicating concerns about higher levels of antimicrobial resistance (Nhung et al., 2017). In Malaysia, 81.6% of *E. coli* isolated from chickens were multiple drug resistant with most isolates were resistant against erythromycin, tetracycline, spectinomycin, trimethoprim and flumequin (Ramlan et al., 2016). Ramlan also reported that 19.2% of 125 isolates tested were resistant against more than eight antibiotics. Another study in east coast Malaysia poultry farms revealed that 100% of *E. coli* isolated from 384 cloacal swabs were multiple drug resistant (MDR) with the highest top five prevalence of resistance occurred against erythromycin, chloramphenicol, tetracycline, ampicillin and sulfamethoxazole-trimethoprim (Ibrahim et al., 2021). The findings were in line with other study that isolated high percentage of multiple drug resistant *E. coli* from chickens, humans, rodents and soils in Tanzania (Sonola et al., 2021), and also a nationwide level study in Lebanon poultry farms which concluded that their farms are reservoir for extended spectrum β-lactamases (ESBL) and AmpC gram negative bacilli that included *E. coli* (Dandachi, 2018).

The threat of antimicrobial resistance does not only concern the poultry industry, but is also regarded as a major public health issue as chickens are one of the major sources of protein. Based on the Department of Statistics Malaysia in 2020, the per capita consumption of poultry meat was at 47.4kg/year which was the highest in the country compared to eggs, pork, milk, mutton and beef. Due to this fact, it is extremely alarming when Devan et al., (2022) detected colistin-resistant *E. coli* isolated from broilers in Kelantan, Malaysia. A total of 23.08% of the isolates from 91 cloacal swabs were positive with colistin-resistant encoding genes *mcr*-1 (Devan et al., 2022). The finding was significant as colistin was described as the last resort in treating infection by multiple antimicrobial resistant bacteria such as carbapenem-resistant Enterobacteriaceae (Arcilla et al., 2016). In addition, Study by Rafiq et al. in 2022 observed that the prevalence of isolated foodborne pathogen was higher in poultry-based food compared to the livestock itself. High percentage of multiple drug resistant *E. coli* was reported in raw broiler and layer chicken meat from poultry shop in Bangladesh (75.06%, n=381) with resistance towards ampicillin (98.95%), erythromycin (89.5%) and tetracycline (85.3%) (Rahman et al., 2020). The findings suggested that it is high time for poultry industry players to search for other alternatives beside antibiotics.

Antibiotic alternatives are becoming more popular and are being encouraged in research to overcome antimicrobial treatment failures and resistance (El-Saadony et al., 2022). Extensive research is currently being conducted using natural plant extracts to investigate the potential of antibiotic alternatives as avian bacterial disease treatment, as well as studies in nanotechnology and beneficial bacterial strains (Aminzare et al., 2017; El-Saadony et al., 2022).

THE USE OF MEDICINAL PLANTS IN TREATING COLIBACILLOSIS

In-vitro Study

Sessou et al. (2018), revealed that essential oils extracted from *Aeollanthus pubescens* showed antibacterial effects using agar diffusion and microdilution methods against *E. coli* isolated from poultry. The isolate was susceptible to the whole plant oil, flower oil and leaf stem oil. Nevertheless, when compared with antibiotics, tetracolivit showed better activity, while oxytetracycline was inferior to the extracts. No activity was observed for extracts of *Euphorbia hirta*, *Psidium guajava* and *Clausena anisata.*

Ethanol extract of *Piper betle* leaves study by Kulnanan et al. (2022) revealed the antibacterial effect of the plant against APEC clinical isolates via disc diffusion assay, MIC and MBC determination (0.5-1.0 mg/mL), and time kill assay. Bacterial cells challenged with the extract were disrupted, in the breakdown, and longer without septum compared to the control. The extract was also observed to inhibit APEC from forming biofilm and eradicate established biofilm. Through scanning electron microscope (SEM), the bacterial adhesion to the surfaces was reduced with the extract administration against APEC.
Positive *in-vitro* inhibitory effect of garlic (*Allium sativum*) against multidrug-resistant (MDR) *E. coli* isolates from diseased chickens and their virulence genes expression were observed by Gharib et al. (2017). A total of 14 MDR isolates were susceptible to aqueous garlic extract. The gene expression of intimin, which is necessary for attachment (*eaeA*) and verotoxin producing genes (*vt2e*) was inhibited and downregulated with the treatment of garlic subinhibitory concentration at 1%.

Essential oils from several plants were examined to have an antibacterial effect against a colibacillosis poultry isolate via disc diffusion and MIC determination. The essential oils were from cinnamon *(Cinnamomum zeylanicum* at 2.52 mg/mL), lemon grass (*Cymbopogon citratus* at 1.118 mg/mL), litsea (*Litsea cubeba* at 1.106 mg/mL), peppermint (*Mentha piperita* at 1.14 mg/mL) and clove (*Syzygium aromaticum* at 1.318 mg/mL) (Ebani et al., 2018).

An *in-vitro* study by Chenniappan et al. (2020) showed the methanolic *Cissus quadrangularis* L. stem extract was effective against *E. coli* isolated from samples of diseased poultry. The antibacterial activity efficacy levels were followed by ethanol, petroleum ether and aqueous extracts. The study also found multivarious active compounds from the leaves extracts, which acted by different mechanisms in the antimicrobial properties.

The effects of allicin oil from *A. sativum,* and cinnamon oil from *C. zeylanicum* were tested against MDR *E. coli* isolated from chickens with respiratory and/or gastrointestinal problems. Both allicin and cinnamon oils exhibited antibacterial effects on the isolate at 250 and 500 ppm, and 125 and 63 ppm, respectively. The downregulation of multiple tested virulence genes was also observed after treatment with both oils (Hassan et al., 2022).

Table 1 *In-vitro* studies on the effects of selected medicinal plants in extract form against *E. coli*

Extracts				
Plant	**Study Design**	**Concentration**	**Outcome**	**Reference**
Family: Alliaceae Genus: *Allium* Species: *sativum*	Antimicrobial against MDR *E. coli* diseased chicken isolates, expression of virulence genes after treatment	Aqueous extract (100%, 50% and 25%) for diffusion assay, increment of 0.1 mg/µl each from 0.1 to 1 mg/µl for MIC, SIC	14 MDR isolates susceptible, inhibited and downregulated eaeA and vt2e genes with treatment at 1% SIC	Gharib et al. (2017)
Family: Passifloraceae Genus: *Cucumis* Species: *quadrangularis*	Antibacterial against *E. coli* diseased chicken isolates	Discs with 1000 µg-4000 µg stem extracts (methanol, ethanol, aqueous and petroleum ether)	Best antibacterial activity with methanol followed by ethanol, petroleum ether and aqueous extracts.	Chenniappan et al. (2020)
Family: Piperaceae Genus: *Piper* Species: *betle*	Antibacterial, antibiofilm, and anti-adhesion activities against APEC isolates	12.5 µL (200 mg/mL) of plant extract on the disc to produce 2.5 mg/disc	Bactericidal activity, disruption and breakdown of bacterial cells, longer cells without a septum, inhibited and eradicated biofilm, reduction of bacterial adhesion to surfaces	Kulnanan et al. (2022)

Table 2 *In-vitro* studies on the effects of selected medicinal plants in essential oils form against *E. coli*

Essential Oils				
Plant	**Study Design**	**Concentration**	**Outcome**	**Reference**
Family: Lamiacea Genus: *Aeollanthus* Species: *pubescens*	Antibacterial effect against *E. coli* isolated from poultry	Sterile discs impregnated with 1, 2, 3, 4, 5 and 10 µL of essential oil	Susceptible against whole plant oil, flower oil and leaf stem oil, better activity than oxytetracycline	Sessou et al. (2018)
Family: Alliaceae Genus: *Allium* Species: *sativum* Family: Lauraceae Genus: *Cinnamomum* Species: *zeylanicum*	Antimicrobial activities against MDR *E. coli* isolates of diseased chickens	Essential oils were prepared as 10% (10,000 ppm)in DMSO as the original solution	Exhibited antibacterial effects, downregulation of multiple tested virulence genes	Hassan et al. (2022)
Family: Lauraceae Genus: *Cinnamomum* Species: *zeylanicum* Family: Poaceae Genus: *Cymbopogon* Species: *citratus* Family: Lauraceae Genus: *Litsea* Species: *cubeba* Family: Lamiaceae Genus: *Mentha* Species: *piperita* Family: Myrtaceae Genus: *Syzygium* Species: *aromaticum*	Antibacterial against poultry colibacillosis isolate	Essential oils at 1:10 dilution in dimethyl sulfoxide for diffusion assay, starting dilution at 10% (v/v) for MIC	Susceptible to all listed essential oils with the best result at the mixtures of *Cinnamomum zeylanicum* & *Syzygium aromaticum*	Ebani et al. (2018)

In-vivo Study

Supplementation of 20% or 20 ml/L *Withania somnifera* root extract in the water of experimental colibacillosis infected chicken managed to lower the magnitude of the haematological parameter changes. Supplemented chickens had lower values of increased serum alanine transaminase, aspartate transaminase, lactate dehydrogenase, and creatine phosphokinase and decreased total protein and albumin compared to the control. Other positive impacts include a significantly higher activity of oxidative blood parameters such as superoxide dismutase, catalase, glutathione reductase, and glutathione-S-transferase enzymes. The *E. coli* specific antibody titre and lymphocyte proliferation response was higher in supplemented chickens suggesting better humoral and cellular immune response. Clinically, *W. somnifera* roots supplement reduced the infection's severity, mortality and recovery period. The severity of the gross and histopathological lesions was mild, with 31.48% and 34.38% supplementation protection percentage, respectively (Kumari et al., 2020).

A study on the haematobiochemical parameters of infected chickens supplemented with *Andrographis paniculata* in feed resulted in increased haemoglobin, packed cell volume (PCV), total erythrocyte count, absolute lymphocyte count, total serum protein and albumin values while at the same time decreased absolute heterophil count, and aspartate aminotransferase (AST) and alanine transaminase (ALT) values compared to the unsupplemented group. The study concluded that colibacillosis hampered haematobiochemical profile in infected chickens, but *A. paniculata* supplementation successfully improved the values toward normal values of healthy chickens (Sonwane et al., 2017).

The use of *Azadirachta indica* leaf extract showed that water supplemented with the plant reduced the severity of the gross and histopathological lesions of the visceral organs such as the liver, heart, lung, kidney and intestine in colibacillosis infected chickens (Sharma et al., 2016).

Another *in-vivo* study showed that the magnitude of serum biochemistry parameter changes related to colibacillosis, such as AST, ALT, LDH increase, and creatine phosphokinase decrease, was significantly lower in chickens that received *Emblica officinalis* extract as feed supplement compared to the unsupplemented chickens. The severity and recovery period of the infection was reduced with *E. officinalis* supplement, as evidenced by the serum biochemistry results (Rath et al., 2017).

Kilany et al. (2018) found that the supplement of *Ocimum basilicum* (sweet basil) and *Moringa oleifera* in feed improved immunostimulant and antioxidant activities in colibacillosis infected chicken. The supplementation significantly increased total body weight, decreased IL-6, IgG and IgM levels, decreased MDA levels and increased superoxide dismutase and glutathione levels. In addition, the histopathological lesions at the intestine, liver, kidney, bursa, thymus and spleen were improved in the supplemented group.

According to Hasan and Sadeq (2020), on the effect of *Mentha piperita* on colibacillosis infected chickens, the study showed positive impacts on feed intake, feed conversion ratio and body weight gain significantly. On the jejunum morphology, supplementation of *M. piperita* significantly affected the muscle thickness and villus to -to-crypt ratio; however, there was no significant effect on villus height and crypt depth. The result of the serum biochemistry was varied and non-significant.

A Traditional Chinese Medicine (TCM) that comprised of *Taraxacum* and *Astragalus membranaceus* combined with probiotic *Bacillus subtilis* and *Lactobacillus* had significant synergistic antibacterial activity on *E. coli* when compared to TCM and probiotics used separately. Other synergistic effects include increased body weight gain, decreased diarrhoea rate and mortality, alleviated intestinal and hepatic pathological changes, increased IL-2 and IL-10 mRNA expression and inhibited TLR-4 mRNA expression. The optimal proportion formula of *Taraxacum* extracts: total flavonoids of *Astragalus*: polysaccharides of Astragalus: probiotics was 5: 2: 2: 2 (Liang et al., 2021).

The effects of *A. sativum* (garlic) and *Zingiber officinale* extracts showed that *A. sativum* extract possessed a broader antimicrobial spectrum against MDR *E. coli* O78 and *Staphylococcus aureus* isolates as compared to *Z. officinale*. *In-vivo* study on similar extracts exhibited immunomodulatory effects on colibacillosis infected chickens through phagocytosis augmentation, bactericidal activity enhancement, nitric oxide (NO) production reduction, plus triggering IL-1β, IL-6 and IFN-γ cytokines expression levels. Besides, the drastic reduction in the *E. coli* O78 colony forming units (CFU) isolated from the vital organs further supported the protective mechanisms of the extracts (Elmowalid et al., 2019). Similar findings by Damanik et al. (2018) on the use of *A. sativum* and *Z. officinale*. The study showed that the extracts' administration showed that the extracts' administration would decrease the CFU count of E. coli in the faeces of colibacillosis-challenged chickens. The combination of both extracts was better in result compared to separate administration. The garlic extract reduced the bacterial count to 67.12%, the ginger extract to 58%, and the combined extracts to 76.05%.

The study of *Nigella sativa* (black cumin) seed powder and *Saccharomyces cerevisiae* treatment revealed better growth performance in infected chickens. The weight gain was higher when treated with *N. sativa* despite lower feed intake, while the feed conversion ratio was better in treated chickens compared to the negative control. Plus, the faecal microbial load was lower in both treatments. No significant results were observed on comparing both treatments in production performance and faecal microbial loads, and blood counts and haemoglobin concentrations among all groups (Haq et al., 2020).

A study on the ameliorative effect of *Murraya koenigii* (curry leaves) powder and levofloxacin showed positive results on haematological and biochemical parameters of colibacillosis chickens. Compared to non-treated chickens, curry leaves powder treatment showed improved values of total protein, albumin, globulin, creatinine, AST and ALT. In combination with levofloxacin, the plant treatment generated better haematological and biochemical parameters, which were as good as the healthy control group (Tandale et al., 2019).

In Hashem et al. (2020), they discovered the impact of supplementing *Echinacea purpurea* in broilers with colibacillosis. Less clinical symptoms were observed in supplemented broilers, with 15% mortality. In the leukogram, non-significant changes were found in total leukocyte counts, with significantly decreased heterophils, monocytes and increased lymphocytes. The AST, ALT and GGT enzymes were significantly decreased. Additionally, improved protein values were noted, with a significant increase in the total and alpha globulins and a significant decrease in the albumin globulin ratio.

The increased lysozyme level in the supplemented group was lower than the non-supplemented which exhibited improved immune response. Mild lesions were observed in the liver, kidney and intestine, while the spleen was normal, confirming the plant's benefits.

Administration of *A. sativum* extract to infected chicken showed that the treatment had less severe clinical signs, lower mortality at 6.6% and higher body weight than the untreated groups (Elamary et al., 2018).

Treatment of *M. oleifera* on colibacillosis chicken showed significant improvement in the feed intake but decreased when the enrichment went beyond 100 mL level. Similarly, in the FCR, where it was significantly increased, but no improvement after the enrichment level was at 100 mL and beyond. At 100 mL/L and 150 mL/L extracts, the treatment increased final body weight, crude protein, gross energy digestibility and carcass yield while decreasing heterophils: lymphocytes ratio. To conclude, the extract improved growth performance, carcass quality, welfare issues and nutrient digestibility (Ullah et al., 2022).

Tinospora cordifolia stem extract possessed significant protective effects in chickens challenged with *E. coli* O78. The clinical signs appeared later than the non-supplemented groups, with similar clinical signs but less severe in shorter periods. The mean body weight of infected and supplemented chickens at day 25 post-infection was comparable to that of the healthy, unsupplemented control. Faster bacterial clearance of the group with extract enrichment was observed, with zero mean viable bacterial cell count in the liver compared to untreated at 0.004±0.001 viable bacterial count (Mean±SE) (Mamta and Jakhar, 2017).

Ocimum gratissimum extract was used to control colibacillosis in chickens. The administered extract managed to improve the weight gain in the infected chickens. For the total bacterial counts in the caecum, the *E. coli* count was reduced, while concurrently, the lactic acid bacteria count was higher. Caecal histopathology results revealed vacuolations which were interpreted as mucosal lipidosis, where the presence of short chain fatty acids produced by prebiotic fermentation would supposedly involve repairing damaged tissues via immune modulation (Ikele et al., 2020).

The study of *Piper auritum* and *Ocimum basilicum* as phytobiotics in treating colibacillosis produced positive results. The administration of 30 mL extract at 10% via oral route to infected chickens promoted the feed intake, leading to significant carcass weight differences. The total bacterial count in lungs, hearts, livers and spleens was lower in both extracts-supplemented chickens and the values were even lower than in the commercial antibiotic treatment group (Aguilar-Urquizo et al., 2020).

In-vivo colibacillosis study in chickens found that administering neem *Azadirachta indica* (neem) leaf powder at 2 g/kg in feed showed reduced severity in clinical signs. The gross lesions of heart, liver, lung, air sacs, kidney and intestine were less severe in supplemented groups. Similar observations were made in the histopathological lesions. The mortality rate of the supplemented group was lower than the unsupplemented by 12% (Patil et al., 2018).

Andrographis paniculata extract was used on layers infected with APEC and the results showed improvement on hen day production at 30% treatment. Treated groups also showed lower feed conversion rates and as the treated concentration increased (Hidanah et al., 2020).

Similar effects of *A. paniculata* on colibacillosis chickens observed by Sonwane et al. (2019). The infected groups manifested no clinical signs with the supplementation of the plant. Milder gross lesions at post-mortem were examined with the provision of the plant in the feed of the treated groups. The histopathological lesions of the lung, liver, heart, kidney, spleen and duodenum were present but milder than the severe lesions in the untreated group.

In Tidiane et al. (2017), study utilised *Thonningia sanguinea* aqueous extract on systemic chicken colibacillosis. Administration of the extract to infected chickens showed better clinical index, higher mean weight gain, reduced morbidity and mortality, milder organ lesions, and total elimination in hepatic bacterial load. Besides moisture content, other parameters were more excellent in the extract-treated group compared to colistin treatment.

Table 3 *In-vivo* studies on the effect of selected medicinal plants in extract form used in management of colibacillosis experimentally infected chickens

Extracts			
Plant	**Method of Administration**	**Outcome**	**Reference**
Family: Meliaceae Genus: *Azadirachta* Species: *indica*	10% neem leaf extract in water	Reduced severity of gross and histopathological lesions of visceral organs such as the liver, heart, lung, kidney and intestine	Sharma et al. (2016)
Family: Acanthaceae Genus: *Andrographis* Species: *paniculata*	10%, 20%, 30% ethanolic extract	Antibacterial, 30% extract increased hen day production	Hidanah et al. (2020)
Family: Alliaceae Genus: *Allium* Species: *sativum*	Approximately 1 mL of 80 mg/mL extract orally daily for five days 24 hours after infection	Minimum inhibitory concentration is 15 to 30 mg/mL, while the minimum bactericidal concentration 20 to 80 mg/mL, active substances decrease the ability of *E. coli* to form a biofilm, less severe clinical signs, lower mortality at 6.6%, higher body weight	Elamary et al. (2018)
Family: Alliaceae Genus: *Allium* Species: *sativum* Family: Zingiberaceae Genus: *Zingiber* Species: *officinale*	Aqueous garlic extract at 10-15%, ginger extract at 15 g/kg diet	Broad antimicrobial effects, immunomodulatory effects (phagocytosis augmentation, bactericidal activity enhancement, nitric oxide (NO) production reduction, plus triggering IL-1β, IL-6 and IFN-γ cytokines expression levels), reduced bacterial CFU from vital organs	Elmowalid et al. (2019)
Family: Alliaceae Genus: *Allium* Species: *sativum* Family: Zingiberaceae Genus: *Zingiber* Species: *officinale*	1% ethanolic extract	Decreased number of bacteria in faeces, garlic extract at 67.12%, ginger extract at 58%, combined extracts at 76.05%.	Damanik et al. (2018)
Family: Euphorbiaceae Genus: *Emblica* Species: *officinalis*	Grinded dry fruit extract of at 10 g/kg feed	Serum biochemistry parameter changes such as AST, ALT, LDH increase, and creatine phosphokinase decrease were significantly lower in magnitude	Rath et al. (2017)
Family: Asteraceae Genus: *Echinacea* Species: *purpurea*	Powdered extract 5 mg/kg feed	Less clinical symptoms and mortality improves haematological and serum biochemical adverse effects, mild lesions in histopathology of liver, kidneys, intestines, and spleen	Hashem et al. (2020)

Table 3 *In-vivo* studies on the effect of selected medicinal plants in extract form used in management of colibacillosis experimentally infected chickens (Cont.)

Extracts			
Plant	**Method of Administration**	**Outcome**	**Reference**
Family: Moringaceae Genus: *Moringa* Species: *oleifera*	Aqueous leaves extract at 50 mL/L, 100 mL/L, 150 mL/L	Significantly antibacterial at 12% aqueous extract, improved feed intake and FCR at 100mL, improved final body weight gain, carcass yield, heterophils: lymphocytes decreased, improved crude protein and gross energy digestibility	Ullah et al. (2022)
Family: Lamiaceae Genus: *Ocimum* Species: *gratissimum*	Ethanolic leaves extract at 40 g/L	Improved weight gain, reduced caecal total *E. coli* count, increased caecal total lactic acid bacteria count, mucosal lipidosis in the caecum, immunomodulation	Ikele et al. (2020)
Family: Piperaceae Genus: *Piper* Species: *auritum* Family: Lamiaceae Genus: *Ocimum* Species: *basilicum*	30 mL of alcoholic leaf extracts at 10% per oral	Antibacterial, promoted feed intake, significant body weight differences, lower total bacterial counts in lungs, hearts, livers and spleens	Aguilar-Urquizo et al. (2020)
Family: Menispermaceae Genus: *Tinospora* Species: *cordifolia*	Semisolid powder stem extract at 1 g/kg feed	Less severe clinical signs at shorter periods, low mortality rates and ameliorative effect on body weight gain and reduced bacterial load	Mamta and Jakhar (2017)
Family: Balanophoraceae Genus: *Thonningia* Species: *sanguinea*	Inflorescences aqueous extract at 50 mL at 10 mg/mL, or about 500 mg in water after thirsting from 18 hours to 7 hours in the morning daily for one week	Better clinical index, higher mean weight gain, reduced morbidity and mortality, milder organ lesions, and total elimination in hepatic bacterial load. Parameters better than colistin treatment	Tidiane et al. (2017)
Family: Asteraceae Genus: *Taraxacum* Species: Not mentioned Family: Leguminosae *Genus: Astragalus* Species: *membranaceus*	9 g/kg BW in water (*Taraxacum* extracts + total flavonoids of *Astragalus* + polysaccharides of *Astragalus*)	Better synergistic effects with probiotics on antibacterial activity, improved diarrhoea indicators, immune organ index, hepatic and intestinal histopathological changes, and regulation of IL-2, IL-10, and TLR-4 mRNA expression.	Liang et al. (2021)
Family: Solanaceae Genus: *Withania* Species: *somnifera*	20% or 20 ml/L root extract in the water	Lower magnitude haematological parameters changes, higher oxidative blood parameters, reduction in severity, mortality, and recovery period, enhanced humoral and cellular immune responses	Kumari et al. (2020)

Table 4 *In-vivo* studies on the effect of selected medicinal plants in non-extract powder form used in management of colibacillosis experimentally infected chickens

Non-extracts			
Plant	**Method of Administration**	**Outcome**	**Reference**
Family: Meliaceae Genus: *Azadirachta* Species: *indica*	Leaf powder at 2 g per kg of feed	Less severity of clinical symptoms and mortality, mild heart, liver, lung, air sacs, kidney and intestine pathological lesions,	Patil et al. (2018)
Family: Acanthaceae Genus: *Andrographis* Species: *paniculata*	Dried leaves powder or 5 gm/kg of feed	Increased Hb, PCV, TEC, decreased absolute heterophil count, increased absolute lymphocyte count, increased serum total protein and albumin, comparable AST, ALT values with healthy control	Sonwane et al. (2017)
Family: Acanthaceae Genus: *Andrographis* Species: *paniculata*	Dried leaf powder at 5 g/kg of feed	No clinical signs, milder gross and histopathological lesions at the lung, liver, heart, kidney, spleen and duodenum	Sonwane et al. (2019)
Family: Rutaceae Genus: *Murraya* Species: *koenigii*	Leaves powder at 1% of feed	Partial improvement in total protein, albumin, globulin, creatinine, AST and ALT. Combined with levofloxacin generated better haematological and biochemical parameters	Tandale et al. (2019)
Family: Lamiaceae Genus: *Mentha* Species: *piperita*	Powder at 0.5% in feed (5 g/kg), 1% in feed (10g/kg), 0.5% in water, 1% in water	Positive impacts on the feed intake, feed conversion ratio and body weight gain, jejunum muscle thickness and jejunum villus to crypt ratio	Hasan and Sadeq (2020)
Family: Ranunculaceae Genus: *Nigella* Species: *sativa*	2% seed powder in feed	Better growth performance in weight gain and FCR, low faecal microbial load	Haq et al. (2020)
Family: Lamiaceae Genus: *Ocimum* Species: *basilicum* Family: Moringaceae Genus: *Moringa* Species: *oleifera*	Powdered leaves in feed at 0.5% *O. basilicum*, 5% *M. oleifera*	Improved growth performance, immune response, antioxidant action, and histopathological lesions	Kilany et al. (2018)

FUTURE RECOMMENDATIONS

This review article included a broad range of studies to provide a narrative summary of medicinal plants that had been used as treatment of avian colibacillosis. Moving forward, it is highly recommended that systematic reviews on studies that tested medicinal plants as avian colibacillosis treatment be conducted as the review would be more objective and comprehensive. Systematic reviews would produce minimal bias and reproducible results, as the methodology of selecting and evaluating literatures involves multiple databases and more rigorous. Meta-analysis would also greatly improve the quality of the systematic review to statistically assess the strength of all published experimental evidences on medicinal plants in managing avian colibacillosis.

CONCLUSIONS

Research on medicinal plants as therapy against avian colibacillosis had been conducted using multiple species of plants in various forms that include essential oils, extracts or grounded powder. Evidences from both in-vitro and in-vivo conducted experiments resulted in positive outcomes that lead to the promising potential of phytotherapy in managing avian colibacillosis in poultry.

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AUTHOR CONTRIBUTIONS

Muhammad Ali Imran Mohamed Kamil: Prepared the first draft of the manuscript, collected data from previous journal articles

Luqman Abu-Bakar: Prepared the abstract and conclusion section, decided the direction of the manuscript writing, reviewed and corrected the whole draft before submission, supervised data collection

Mohd Farhan Hanif Reduan: Main supervisor for the whole draft preparation, reviewed, proof read and corrected the draft before submission, facilitated the submission process

Intan Noor Aina Kamaruzaman: Provided resources and funded the research, insights on the antimicrobial resistance in avian colibacillosis treatment in the manuscript

Siti Nor Azizah Mahamud: The main supervisor and contributor for poultry medicine and treatment aspect in the manuscript

Amirul Faiz Mohd Azmi: Provided insights on the feed supplements and nutritional aspects of poultry industry in the manuscript

Mohammad Sabri Abdul Rahman: Contributed on the epidemiological aspect in understanding avian colibacillosis in the manuscript

Muhammad Luqman Nordin: Provided insights on the pharmacological aspect of various ethnomedicine in the manuscript

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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