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**Review Article**

# A review on the study of immunomodulators and herbal remedies: A natural approach to treating necrotic enteritis

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**Abstract**

This study investigates the use of immunomodulators and herbal remedies as a natural treatment for necrotic enteritis. It aims to develop a complete approach to managing the gastrointestinal disorder, focusing on the potential of these interventions in modulating the immune response. The research includes medicinal plants like *Aloe Vera*, *Andrographis paniculata*, *Camellia sinensis*, *Clausena excavate*, *Acacia catechu*, *Plantago asiatica* L, and *Cynodon dactylon*, which have been identified for their immunomodulatory effects. In response to the global initiative to reduce antimicrobial use in animal production, herbal treatments are gaining attention for their diverse mechanisms of action, such as enhanced phagocytic activity and immune response modulation. Integrating traditional herbal knowledge with modern research offers a comprehensive perspective on the potential benefits of these treatments in controlling diseases like Necrotic Enteritis (NE). As the poultry industry seeks sustainable strategies, herbal treatments emerge as promising alternatives, highlighting the need for further research to fully understand their potential and practical application in poultry management. Moreover, it covers the way for future research to explain the complete potential of these natural approaches, benefiting society through safer food and offering potential benefits like reduced antibiotic resistance in birds, improved poultry productivity and a deeper understanding of immune modulation in animal health.

**Keywords:** Herbal immunomodulators; Herbal remedies; Necrotic enteritis

## Introduction

Necrotic enteritis is a serious economic risk to the cattle and poultry sectors globally. Necrotic enteritis (NE) is a common disease associated with Clostridium perfringens (CP). This disease was initially identified in the 1961 and subsequently related to numerous occurrences in countries engaging in widespread poultry farming [1]. Necrotic enteritis has manifest different forms, including acute, clinical, and subclinical. Particularly, subclinical necrotic enteritis stands out as a primary contributor to economic losses within the poultry industry.

The projected global economic impact is expected to exceed $6 billion annually [2]. Furthermore, necrotic enteritis is correlated with a substantial 12% reduction in body weight and 11% elevation in feed conversion ratio in comparison to unaffected birds [3].

However, Clostridium perfringens is a widespread Gram-positive, spore-forming, toxigenic and anaerobic bacterium, ability of Clostridium perfringens to produce five primary toxins [4]. Specifically, certain strains of type A and potentially type C are particularly significant in the circumstance of necrotic enteritis. While pigs and cattle can also be susceptible, poultry, specifically chickens and turkeys are most frequently effected. The ability of Clostridium Perfringens CP type A to produce various toxins responsible for the disease underscores the significance in the poultry industry [5].

*Clostridium perfringens* are mostly found in a different of places, such as soil, animal waste, feed and chicken litter [6]. The presence of Clostridium perfringens (CP) always not necessarily indicate the existence of the illness. Necrotic enteritis (NE) usually affects chickens that are 2 to 5 weeks old and incidence can range from low to high because many Clostridium Perfringens (CP) strains are not very harmful. The clinical sign of the disease during outbreaks is intricately influenced by a complex relationship involving the microorganism, predisposing factors such as nutrition, the presence of other bacteria and the immune status of the birds [7]. The physical characteristics of intestine contents can be affected by the diet, including any modifications. Significant predisposing factors for necrotic enteritis (NE) include viral infections and the presence of Eimeria spp. because they promote mucosal secretion and destroy enterocytes.

Moreover, immunosuppression and various stressors, including medical interventions, have the potential to modify the composition of the microbiota, creating a suitable environment for pathogenic strains of Clostridium perfringens (CP) to colonize in mucosal tissues more effectively. These germs can efficiently colonize the gastrointestinal system and break down mucus. These bacteria produce damaging enzymes to endothelial cells as soon as they are grown. These enzymes affect the lamina propria, the lateral region of enterocytes and the basement membrane [8].

**Clinical signs**

Necrotic enteritis (NE) shows symptoms similar to those of typical enteritis, including ruffled feathers, gastrointestinal distress, depression, and loss of appetite. The acute form of necrotic enteritis (NE), sometimes referred to as the typical symptoms are characterized by a sudden rise in flock mortality, often occurring without showing any clinical signs [9, 10]. The subclinical form of necrotic enteritis typically lacks evident clinical symptoms leading to death. However, it is characterized by a widespread drop in the bird performance [11]. A raised feed conversion ratio (FCR), decreased body weight gain and compromised food digestion and absorption are all consequences of chronic injury to the intestinal mucosa. A causal relationship occurs between these performance challenges and subclinical necrotic enteritis. Cholangiohepatitis is frequently the cause of elevated liver condemnations in processing facilities when subclinical necrotic enteritis occurs [12]. Because of this, it is difficult to diagnose the subclinical form of necrotic enteritis and untreated cases of infected birds result in increased financial losses [13].

While, necrotic enteritis can cause a variety of gross lesions, most commonly affecting the liver and small intestine. Most frequently impacted on gut regions are the ileum and jejunum in particular. Examining the

intestines may reveal the presence of blood clots and symptoms of gas collection along with bleeding. Edema can cause the mucosa to thicken, or epithelial erosion can cause it to shrink [14]. Occasionally, a green or yellow pseudo-membrane attached to the mucosa may be seen. Concurrent alterations in various intestinal segments within a single animal are frequently observed [15]. Cholecystitis and necrotizing foci can spread and widen throughout the liver parenchyma. Such liver damage is often associated with a subclinical version of the illness [16]. Shorter villi apical epithelial separation and severe mucosal necrosis that may spread to the crypts or submucosa are examples of microscopic lesions. Bacilli may occasionally be seen in the lamina propria or mucosa. The pattern of inflammatory cell infiltration in the lamina propria is diverse and the extent of infiltration varies from case to case. In some cases, the infiltration is more pronounced than in others [17].

Antimicrobial therapy is the strength of management for necrotic enteritis (NE) outbreaks, with the aim of minimizing financial losses. The following antibiotics are advised worldwide for treating necrotic enteritis bacitracin, lincomycin, virginiamycin, penicillin and tylosin. Subtherapeutic dosages of antimicrobials have been used in feed to address the subclinical form of necrotic enteritis (NE), which is responsible for the majority of losses [18]. As with many other microbes, Clostridium perfringens has become less susceptible to antibiotics over time.

However, necrotic enteritis has emerged once again as a major issue, resulting in reduced growth efficiency and increased feed expenses. This comeback coincides with the European Union-wide ban on in-feed growth enhancers. The subclinical form of necrotic enteritis can spread more widely throughout the flock and more dangerous because it remain recognizable clinical signs and symptoms to identify [19].

Researchers have not yet fully comprehended the crucial factors that are necessary for the initiation of the disease caused by Clostridium perfringens. These factors can potentially impact the gut ecology, affecting equilibrium and ultimately facilitating the spread of the disease [20].

As a result, carcasses are frequently condemned during processing [21]. Although the increasing economic impact of subclinical necrotic enteritis, that remains a lack of comprehensive research on the subject [22]. Necrotic lesions are the main signs of the disease, usually found in the jejunum and ileum of the small intestine, but also found in the duodenum [23]. The preclinical stage disease has a direct impact on decreased weight gain, an enhanced feed conversion ratio and impaired absorption and digesting. These significances are damage to the gut mucosa [24]. While this disease may be sporadic in underdeveloped nations, poultry farms must consider the economic losses it experiences as it often leads to significant outbreaks in chicken production facilities. However, because subclinical necrotic enteritis (NE) is difficult to accurately assess, it is challenging to quantify the exact impact of necrotic enteritis on chicken production. Clostridium perfringens, a Gram-positive spore-forming bacteria, is the main cause of the disease. It has been extracted from various sources, such as feed, litter, dust, and dung, and is commonly found in the gastrointestinal tract (GIT) as a commensal [25]. While many factors have been identified to contribute to the development of sub-clinical necrotic enteritis (NE), the exact cause of the outbreak of necrotic enteritis in the field remains uncertain. Apart from a specific type of mutual infection with Eimeria, the primary risk factors are primarily related to nutrition. At present, the sole method for quantifying

the intensity of the response of the host is to assess the digestive tract for visible pathological abnormalities.

Although the main etiological agent of the disease has been identified as Clostridium perfringens, it has been difficult to induce sub-clinical necrotic enteritis under experimental conditions. The precise predisposing factor(s) that cause necrotic enteritis (NE) to be induced after an overgrowth of Clostridium perfringens in the gastrointestinal tract (GIT) are still unknown, despite considerable knowledge of the course of disease. The various predisposing factors remain poorly identified due to inconsistent results from experiments [26].

**Antimicrobial control of necrotic enteritis and alternatives**

Managing necrotic enteritis (NE) in poultry is quite challenging due to various contributing variables. Historically, antibiotic therapy has been the main and sometimes restricted treatment for Nephropathy caused by Clostridium perfringens (CP). Therapeutic antimicrobials are typically administered in high doses for short durations to treat acute outbreaks [27]. Antibiotic growth promoters (AGPs), or antibiotic growth promoters are frequently utilized to manage asymptomatic Nephropathy treatment. Although added to poultry feed at first to increase growth rate and feed conversion efficiency [28], Antibiotic growth promoters (AGPs) are mostly used in modern poultry industry to treat Gram-positive illnesses such as Clostridium perfringens (CP). Nowadays, virginiamycin, a streptogramin and bacitracin a polypeptide antibiotic are two Antibiotic growth promoters (AGPs) that are frequently used in chicken production. These Antibiotic growth promoters (AGPs) are utilized to improve animal welfare in general, body weight increase and feed conversion ratios [29].

Although antimicrobial growth promoters (AGPs) are widely used, specific procedures that explain the advantages of utilizing low dosages of antimicrobials in broiler flocks are not well understood. Some of the proposed processes include regulating the digestive system and immune responses in the intestines [30]. The most well acknowledged process is that Antibiotic growth promoters (AGPs) change gut microbiota, which is essential for preserving host health [31].

The prolonged use of antibiotic growth promoters (AGPs) at subinhibitory concentrations greatly facilitates the selection of antibiotic-resistant microorganisms. There are studies indicating that poultry Clostridium perfringens (CP) strains in countries where antibiotic growth promoters (AGPs) remain in use are showing reduced resistance [32]. The continuous administration of Antibiotic growth promoters (AGPs) has the ability to change the bacterial environment by eliminating particular strains and enhancing the survival and dominance of antimicrobial-resistant bacteria, which are less susceptible to antibiotic growth promoters (AGPs) [33]. Furthermore, continuous administration of Antibiotic growth promoters in feed could result in cross-resistance to medicinal antimicrobials [34]. The prevalence of such Clostridium Perfringens strains has increased due to the formation of antimicrobial resistance and a progressive decline in susceptibility to anticoccidials in some strains of Eimeria spp. (a significant predisposing factor to necrotic enteritis).

The growth of pathogenic and resistant strains of Clostridium perfringens (CP) in poultry farms raises concerns about the possible transmission of these bacterial resistance factors from animals to humans. Research conducted on various strains of Campylobacter, Escherichia coli, and Enterococcus indicates a connection between the use of nontherapeutic antimicrobials and the proliferation of multidrug resistance, including resistance to drugs not often used

in agriculture. This raises worries about the potential impact of antibiotic resistance on human health resulting from the spread of resistant microorganisms originating from animals [35].

Antibiotic growth promoters, or AGPs, were outlawed by the European Union in 2006 as a result of multiple studies showing how they contribute to the formation and spread of antibiotic-resistant bacteria [36]. Necrotic enteritis (NE) incidence was found to have increased in Europe [37] as a result of these actions, and the usage of therapeutic antimicrobials to treat illnesses increased [38]. The experience in Europe, along with recent efforts in North America to reduce or eradicate Antibiotic growth promoters (AGPs) [39], has required the poultry industry to investigate appropriate substitutes for managing necrotic enteritis outbreaks and minimizing the effects of subclinical managements on productivity when typical farm management is applied [40] and reduce the tolerance of body to antibiotics. Natural compounds possessing antibacterial qualities can play a crucial role in this control approach.

In response to the prohibition on antibiotic growth promoters (AGPs), a range of procedures and approaches that do not include antimicrobials were developed to prevent and control Clostridium perfringens (CP) induced necrotic enteritis (NE) in chicken [41]. In order to be considered an appropriate substitute for Antibiotic growth promoters (AGPs), any alternative must fulfill particular requirements. These standards include being safe for public health, cost-effective, environmentally friendly, and possessing antibacterial properties [42]. Several alternatives have been proposed, including feed enzymes, immunomodulatory drugs, symbiotic agents, bacteriophages and their associated lysins, plant extracts and inhibitors of bacterial quorum sensing, biofilm and pathogenicity [43]. Although the suggestions of utilizing probiotic and prebiotic products, as well as pathogen immunization, these approaches are currently impractical for implementation on farms. Utilizing plant extracts in animal feed to enhance nutrition and health in livestock and address enteric clostridial infections is a highly promising substitute for antibiotic growth promoters (AGPs). These compounds have a lengthy record of utilization in poultry and have demonstrated to be advantageous [44].

**Plant extract**

Traditional medical systems have always made extensive use of plant resources [45]. Because of their antibacterial, anti-inflammatory, antioxidant and antiparasitic qualities, plant extracts also referred to as phytobiotics have attracted interest in animal nutrition (Table 1) [46].

Numerous plants have advantageous multipurpose qualities that come from certain bioactive constituents. Secondary metabolites, which can exist in many forms such as alcohols, aldehydes, ketones, esters, ethers and lactones are primarily responsible for the biological activity of plants. These include terpenoids, phenolics, glycosides, and alkaloids [47]. In vegetative tissues these secondary metabolites frequently have a defensive role. The mixture and concentration of these bioactive molecules determine the final effect on animals and even small variations in these parameters might account for why some compounds may have advantageous or disadvantageous effects on animals [48].

Plant extracts are generally considered safe and effective against particular pathogens. These plant extracts are frequently employed as agents to stimulate growth and enhance the immune system in animal feed [49]. They are extensively utilized in Asian, African, and South American countries, as well as in affluent nations, and their usage has been steadily increasing in recent times.

Furthermore, there is increasing evidence that feed additives, such as phytogenic elements, enhance the performance of chicken production [50]. Plant extracts are deemed to exhibit antibacterial properties in in vitro bacterial susceptibility assays when their minimum inhibitory concentrations fall within the range of 100 to 1000 μg/mL [51]. Recent studies have shown that adding raw plant extracts and phytogenic compounds to poultry feed can enhance the health and productivity of the birds. An established method for assessing the effects of including plant extracts into chicken diets involves evaluating parameters such as necrotic enteritis (NE), visible abnormalities, and the abundance of Clostridium perfringens (CP) in the intestines [52]. Specific botanical extracts has the capacity to directly inhibit particular toxins linked to the development of necrotic enteritis, as well as the vegetative cells of Clostridium Perfringens [53].

There are other categories of antimicrobial phytochemicals that are beneficial, including lectins/polypeptides, essential oils (EOs), polyphenols/tannins and alkaloids [54]. Phytochemicals employ diverse mechanisms to exert their antibacterial activity. For instance, tannins function by depleting iron and interacting with crucial proteins, such as enzymes [55]. The primary indoloquinoline alkaloid, cryptolepine, acts as a topoisomerase inhibitor and a DNA intercalator [56]. Additionally, saponins bind with sterols from microorganism membranes to create complexes that harm and ultimately destroy the cells [57]. While the antibacterial properties of essential oils (EOs) have been recognized for a long time [58], the precise mechanism by which various plant extracts, including essential oils, operate as antimicrobials remains uncertain [59]. The idea that the overall effectiveness of phytogenic feed additives in killing bacteria is due to a significant reduction in the presence of harmful intestinal pathogens is supported by direct observations conducted in living organisms [60]. The observed reduction in pathogen level suggests a generally beneficial impact on the health and microbial equilibrium in the chicken intestines, notwithstanding the incomplete comprehension of the precise mechanisms involved.

AGPs possess biological efficacy, however, it is imperative to consider the feasibility of producing and employing the active components on a large-scale in order to effectively reduce or eliminate the usage of antimicrobials in animal production worldwide. Essential oils and tannins, both derived from plants have recently acquired popularity as effective therapies for necrotic enteritis (NE) in chickens.

**Garlic (*Allium sativum*)**

For about 5,000 years, civilizations such as Egypt, Greece, China, and India have recognized the advantageous role of garlic in human nutrition [61]. Garlic and its derivatives, including oils, essential oils, aged garlic extracts, and others, offer significant health benefits for both humans and animals. They have been attributed with antioxidant, antibacterial, antiviral, antifungal, hypercholesteremic, and immunostimulating properties [62]. Additionally, studies have demonstrated that oregano has the ability to reduce the number of ileal clostridia in broiler chickens, while the potent aromatic compounds found in garlic enhance the digestion of these birds [63]. The instability and volatility of the bioactive components in garlic may restrict its usage in poultry farms. Consequently, feed manufacturers are highly interested in exploring innovative methods that can enhance the stability and preservation of garlic, thereby enhancing its efficacy and utilization. Nanotechnology is a highly advanced and promising approach to protect bioactive substances from oxidation, heat or volatilization. This technique aims to

maintain the functional, chemical, and physical features of bioactive compounds while ensuring consistent administration, uniform size distribution, enhanced storage stability, masking unpleasant tastes, and extending the shelf life [64]. An effective method to enhance the effectiveness of plant extracts at lower doses in chicken production is by incorporating them into nanocomposite hydrogels [65]. The utilization of nanotechnology in poultry farming is currently in its nascent phase. As far as we know, there is currently no published data on the use of nanoparticles derived from garlic essential oil in chicken farming, despite the increasing interest in this area. An area of research worth exploring is the utilization of garlic essential oil nanoparticles as a novel approach to enhance the efficacy of plant extracts in chicken farming [66].

Because it prevents the growth of bacteria like Salmonella typhimurium, Enterobacteria Spp. and Escherichia coli, garlic has health-promoting qualities [67]. Garlic is known to decrease cholesterol. It does this via lowering LDL cholesterol levels and slowing down the rate of cholesterol oxidation, according to a wealth of human and rat experimentation and medical study. Garlic has also been shown to boost living things immune systems [68] and has been identified for its anti-cancer and antioxidant properties [69]. One important component of garlic is alliin, which is converted to allicin when crushed in an aerobic environment by the enzyme allinase [70]. Alkyl sulfonic acid, the intermediate molecule, has the ability to acidify animal digesta. Allicin also releases sulphides that have strong antibacterial and antioxidant properties [71-72]. Garlic extracts are considered to contain compounds more potent than formic acid. Allivet liquid garlic is readily accessible in the Polish market. It is recommended to administer it twice a week or in three consecutive doses spanning a three-week duration. The optimal concentration of a commercial poultry blend should be 1.0 ml kg–1. The recommended dosage of this supplement is 1.00 ml kg–1, which can be administered by adding it to feed or drinking water. Another option is to regularly incorporate the supplement into the feed; however, no recommended dosage has been provided for this method. In order to determine the optimal amount of dietary supplementation with Allivet, diets containing the recommended dose of 1.00 ml kg–1 of feed were compared to diets without Allivet, as well as diets with an increased Allivet dose of 50% or 125% [73].

**Oregano essential oil**

As a phytobiotic, oregano (Origanum vulgare) is known to include antimicrobial chemicals such thymol, carvacrol (CAR), and its precursors, p-cymene and γ-terpinene. Taken together, these components account for about 80% of the plant's essential oil content [74]. At low concentrations, CAR and thymol have both repeatedly demonstrated antibacterial activities [75], resulting in a decrease in cell membrane potential that may eventually cause cell death [76]. This antimicrobial action may be able to prevent foodborne illnesses in consumers and protect broiler flocks from enteric infections. It may be possible to save antibiotics for use in circumstances requiring high-dose therapeutic treatments by using oregano powder as a low-dose subtherapeutic feed addition. An experiment with oregano showed that it had a greater effect than avilamycin on the weight increase and intestinal morphology of broilers [77]. In another study, broilers challenged with Clostridium perfringens [78], a condition that significantly impairs the broiler industry's annual revenue [79], the application of a combination essential oil made from oregano that was 25% carvacrol (CAR) and 25% thymol showed a decrease in intestinal lesions, necrotic enteritis, and death. Oregano essential oil proved to be more effective

against infections than essential oils derived from other herbs, such as rosemary [80]. Antioxidant qualities [81], preservative and also has strong anti-inflammatory [82].

However, the promise of plant essential oils as a valuable source of antibacterial chemicals is what drives ongoing study in this area. This is explained by their availability, minimal toxicity, and ability to synthesis aromatic chemicals, especially phenols and their derivatives that have had the oxygen atom removed [83]. One of the strongest natural antioxidants and antibacterials is oregano essential oil (OEO), an extract made from the herb oregano [84]. The ingredients of oregano essential oil (OEO) include carvacrol, thymol, γ-terpinene, p-cymene, linalool, and β-myrcene, among other aromatic compounds [85]. Carvacrol and thymol, the two primary phenols in oregano essential oil (OEO), are vital for the oil's antimicrobial qualities. Crucially, using this therapy allays worries about medication resistance and residues [86]. The volatile oils in oregano have the power to alter the permeability of mitochondrial membranes, break through the membranes of harmful bacteria, and prevent mitochondria from taking in oxygen. As a result of the inhibition of bacterial development, the harmful cells eventually suffocate and die [87]. According to research, Oregano Essential Oil (OEO) can replace antibiotics in feed additives, maintaining animal development performance and boosting resistance to disease. This implies that oregano may find widespread application in the care of animals [88]. Oregano essential oil (OEO) has been shown to have the capacity to block or eradicate detrimental intestinal infections, which has improved the performance of broiler chickens in terms of output [89]. Oregano essential oil (OEO) supplementation could successfully stop the growth of a number of pathogens, such as *Bacillus subtilis*, *Salmonella indiana*, *Listeria innocua*, *Escherichia coli*, and *Staphylococcus aureus* [90].

**Thyme**

Commercially available thyme oil has antifungal, antibacterial and antioxidant qualities that make it a useful food addition. It is commonly known to be effective against a variety of resistant forms of bacteria. Our goal in this study was to evaluate the effect of a plant extract (Thymus vulgaris) on Clostridium perfringens in vitro. Thymus vulgaris extract formulations on various Clostridium perfringens strains. Consumer preferences for foods that contain ingredients that provide health advantages beyond basic nutrition are currently on the rise. One such ingredient is herbal components. These components provide a comfortable way to consume them as well as an appropriate medium for the dispersion of useful substances [91]. Thyme essential oil is highly valued in the fields of cosmetics and fragrances due different scent [92].

Many different kinds of plants, fruits, and vegetables contain a wide variety of natural antioxidants [93]. Flavonoids and phenolic antioxidants, including zeaxanthin, lutein, pigenin, naringenin, luteolin, and thymonin, are especially abundant in thyme. Among herbs, fresh thyme stands out for having one of the highest concentrations of antioxidants along with a wide range of essential minerals and vitamins that are necessary for optimal health. Thyme leaves are particularly rich in potassium, iron, calcium, manganese, magnesium and selenium [94]. Thymol, one of the main phenolic components of thyme is principally responsible for its antioxidative propertie [95]. The volatile oils in oregano have the power to alter the permeability of mitochondrial membranes, break through the membranes of harmful bacteria, and prevent mitochondria from taking in oxygen. As a result of the inhibition of bacterial development, the harmful cells eventually suffocate and die [96].

Additionally, thyme is a herbaceous plant species that grows well in mountainous areas and has several uses. It is frequently used, either by itself or in combination with tea, to improve the flavor of a variety of foods and drinks. Thyme is widely used in traditional medicine to treat a wide range of conditions, including as removing intestinal worms, treating respiratory conditions, relieving coughs, treating gastritis, preventing oral infections, and treating stomach and intestinal problems. Its strengthening qualities are also thought to provide heart-healthy benefits [97]. Thyme extracts have been used in traditional medicine to treat a number of illnesses, such as asthma and bronchitis, as well as other respiratory issues. These characteristics include antibacterial, antifungal, antiviral, antispasmodic, antitussive, and antimicrobial effects [98].

Samples of *Thymus vulgaris* L. taken at four different stages of the biological process were examined for their chemical makeup and biological activity. Six gram-positive and nine gram-negative bacterial strains were subjected to an analysis of the volatile oils isolated from thyme in order to determine their inhibitory effects [99]. Using bioimpedance methods, the antibacterial activity of the essential oils was ascertained, and the antibacterial activity was characterized and measured based on the detection time [100]. Using the plate counting method, the study examined the inhibitory effect through direct exposure. Every studied essential oil made from thyme showed high bacteriostatic action, according to the data. Crucially, gram-positive bacteria were shown to be more severely affected [101]. It was found that the oil extracted from completely blossomed thyme was the most successful in preventing the growth of the microorganism species under investigation [102]. Upon direct contact, the evaluated oils exhibited significant antibacterial activity, with a more obvious effect against gram-negative bacteria. Stimulating to note that whereas most strains seemed to have been almost entirely inactivated, several species were able to regain at least 50% of their metabolic function following exposure to the inhibitor [103]. *T. vulgaris* L.'s antibacterial action depends on the chemical makeup of the organism. Evidently, the findings of the research about the antibacterial effects of the essential oil are ascribed to its phenolic chemicals, including thymol and terpene hydrocarbons, especially γ-terpinene [104]. The third significant element (p-Cymene) in terms of percentage does not show antibacterial activity when taken on its own. Nevertheless, when combined with thymol and γ-terpinene, it has been shown to have synergistic effects that may add to the total antibacterial activity seen. On the other hand, a number of studies have shown that essential oils have higher antibacterial activity than either of their main ingredients by themselves or in combination, suggesting that minor ingredients may work in concert. This emphasizes how important each component is in enhancing the biological activity of essential oils [105].

**Garlic (*Allium sativum*)**

A well-known Gram-positive bacterium called Clostridium perfringens is responsible for a number of illnesses, including gastrointestinal tract infections and food poisoning. Investigating substitute antimicrobial agents has become essential in light of the growing issue of antibiotic resistance. The well-known spice and medicinal herb ginger (Zingiber officinale) has numerous health advantages. Investigates antibacterial qualities of ginger and possible efficacy against Clostridium perfringens. Interestingly, zingerone, shogaols and gingerolsall of which are rich in ginger contribute to the antimicrobial properties that have been reported [106]. Multiple studies have shown that ginger has a wide-ranging ability to kill bacteria, fungus, and viruses.

Currently, there is considerable focus on the potential of ginger as an alternative antibiotic. Specifically, research has investigated the antibacterial properties of ginger against Clostridium perfringens [107]. Moreover, the presence of bioactive chemicals in ginger is associated with their antibacterial efficacy against Clostridium perfringens. These substances possess the capacity to rupture the membrane of bacteria, impede vital enzymes and obstruct crucial cellular functions. Bacterial growth is then inhibited as a result and eventually, cells die [108]. With their well-established therapeutic qualities, ginger has long been a mainstay in conventional medical practices. Numerous investigations investigating the antibacterial properties of ginger have been conducted, with consistently excellent outcomes [109]. Strong antibacterial action of ginger is demonstrated against a variety of bacteria, including Streptococci, E. coli, Salmonella spp., S. aureus, and Staphylococcus epidermidis [110]. Several bioactive compounds are believed to contribute to the medicinal properties of ginger with gingerol and shogaol being particularly prominent. The antibacterial, anti-inflammatory and antioxidant effects of these compounds have been extensively studied, showcasing the wide array of medical applications of ginger. Although ginger is well known for supporting digestive health, there is new and interesting research demonstrating that it may also have an effect on microbes connected to necrosis [111].

**Propolis**

Propolis, also known as "bee glue," is a resinous, sticky material. The Greek terms "polis," which means city, and "pro," which means defense, are the source of its name. This name reflects the fact that bees use it to build a protective barrier around their colonies, keeping off intruders [112]. Examples of biologically active substances that have been shown to have anti-inflammatory, immunomodulatory, antiviral, antibacterial, antifungal, analgesic, and antioxidant properties in both people and animals are propolis and bee pollen [113]. Because propolis has more antibacterial activity against a wider variety of bacterial species, it is useful in the fight against pathogens [114]. Phenolic acids, flavonoids and their derivatives are thought to be responsible for antibacterial properties of propolis [115]. Propolis is well known for containing important elements that are necessary for chicken growth, such as minerals, proteins, vitamins, amino acids, and flavonoids [116]. According to research by [117], anti-inflammatory and antioxidant qualities of propolis may help explain how it works so well for treating digestive issues. Necrotic enteritis (NE) in chickens is a problem for which a number of alternate control methods have been investigated in different research.

However, propolis may have an impact on necrotic enteritis in these birds. Probiotics, containing microorganisms such as Lactobacillus, Enterococcus, Bacillus, and Bacteroides, have shown encouraging benefits in lowering Clostridium perfringens colonization and reducing necrotic enteritis associated pathology [118]. This is one method of treating infection [119]. Tannic acid supplementation, which has been shown to improve the intestinal barrier and absorption function, is another method under investigation. In chickens affected with necrotic enteritis, this improvement results in improved growth performance and less intestinal lesions [120]. Based on current research, propolis appears to be a natural product with potential benefits for controlling and avoiding necrotic enteritis (NE) in chickens. It is essential to remember that there are presently few specialized studies examining propolis's impact on necrotic enteritis. To effectively manage necrotic enteritis in poultry, more research is required

to fully assess effectiveness of propolis in controlling the condition, determine the ideal dosage and establish appropriate administration techniques.

**Turmeric (*Curcuma longa*)**

The Zingiberaceae family of plants, which includes turmeric is widely used in the food industry to improve the flavor, texture and appearance of food products. Bioactive ingredients found in it include tetrahydrocurcuminoids, curcumin, demethoxycurcumin, and bisdemethoxycurcumin Antioxidant, anti-inflammatory and nematocidal properties are linked to these bioactive substances [121]. In addition, turmeric has been shown to have preventive properties against coccidiosis [122] aflatoxin-induced mutagenicity and hepatocarcinogenicity [123, 124].

The results of several research, however, on the impact of a turmeric supplement ranging from 0 to 10 g/kg on chicken performance are inconsistent. For example, one study found that broilers who received 5 g/kg of turmeric in their diet performed better. However, there was no change in the levels of serum total protein, albumin, globulin, ALKP, ALT, and AST [125]. Conversely, research has shown that adding turmeric powder has no discernible effect on the growth performance or carcass output of broiler chickens [126]. Nevertheless, when added at 0.6 and 0.9 g/kg, turmeric powder showed a reducing effect on aflatoxin B1's harmful effects. Malondialdehyde (MDA) levels decreased as a result of this intervention, but antioxidant defense enzymes like catalase and superoxide dismutase increased and blood levels of total protein, albumin, and globulin improved. Furthermore, when broilers were fed 5 g/kg of turmeric powder, the levels of the liver enzymes alanine aminotransferase (ALT) and alkaline phosphatase (ALKP) were dramatically reduced [127]. As a spice, food preservative, and coloring agent, turmeric (Curcuma longa) is widely used because to its noteworthy biological properties and possible medical applications [128].

The main active component of turmeric, curcumin, has been shown to have antioxidant and antibacterial properties [129]. Additionally, studies by [130] Ayurvedic medicine has traditionally used turmeric to treat a variety of conditions, such as jaundice, skin infections, wound healing, flatulence, sprains, arthritis, and stomach problems. Over time, curcumin, the main ingredient in turmeric, has been shown to have anti-inflammatory, anti-arthritic, antioxidant, cardio-protective, and immunomodulatory properties. Research has also shown that adding turmeric to feed can protect against the mutagenic and hepatocarcinogenic effects of aflatoxin [131].

By modulating cellular function and targeting different signaling molecules, curcumin demonstrates its wide range of health advantages. Because curcumin is an antioxidant, supplements containing it have demonstrated potential nephroprotective and analgesic effects as well as usefulness in controlling metabolic disorders [132]. Low bioavailability of curcumin, however, cause a serious problem to their successful medicinal usage. This restriction is caused by things like fast metabolism, hydrophobic nature, and inadequate intestinal absorption. Following oral dosing, curcumin has a noticeably poor systemic bioavailability. Despite these difficulties, research has shown that systemically accessible curcumin, even at modest doses, can have a noticeable therapeutic effect. Bioavailability of curcumin has been studied in relation to a variety of drugs [133].

However, curcumin is being investigated as a potential candidate for the synthesis of novel natural products, like nanoparticles and nanocrystals, with the goal of improving stability against recognized obstacles and affecting bioactivities [134]. Curcumin's

usual antibacterial action entails interfering with the protein-filamenting, temperature-sensitive mutant Z (FtsZ) and upsetting basic cellular division. The cytoskeleton is essential for both bacterial growth and cell division, and FtsZ, the first protein to arrive at the approaching division site, is particularly associated with microbial cell replication [135].

**Echinacea (*Echinacea purpurea*)**

Phytobiotics, sometimes referred to as phytogenic feed additives are plant-based substances added to animal diets with the intention of improving feed qualities, raising output and raising the quality of food items that come from animals [136]. One example of a herb with therapeutic qualities is Echinacea purpurea L. (E.P), which is well known for boosting the immune system. It is widely used in herbal treatments to cure or prevent infectious illnesses in Europe and North America [137]. Flavonoids, phenolic compounds, glycoproteins, cinnamic acid, and alkamides are among the active components found in echinacea and its derivatives [138]. These ingredients have demonstrated effectiveness in treating a range of diseases and have been demonstrated to be helpful in boosting immune function [139].

Numerous investigators have investigated Echinacea antibacterial properties of purpurea against a variety of pathogens, such as Clostridium perfringens, which is the primary cause of necrotic enteritis (NE) in chickens. Echinacea antibacterial properties of purpurea on necrotic enteritis in hens have been the subject of numerous investigations. For example, research carried out by [140] showed that giving Echinacea purpurea as a dietary supplement to chickens exposed to *C. perfringens* decreased the number of deaths and lesion scores. Additionally, the study revealed increased amounts of chemokines and pro-inflammatory cytokines, indicating that Echinacea purpurea may strengthen hens' immune systems. Another concern from [141] Against *C. perfringens*, an echinacea purpurea extract demonstrated direct antibacterial action. The study carried out by the scientists demonstrated that the extract not only reduced *C. perfringens* ability to produce toxins but also impeded the bacterium's growth in culture. Moreover, Echinacea purpurea supplementation decreased the amount of *C. perfringens* in the intestines of birds that were challenged with germs. According to the study, supplementing hens with Echinacea purpurea may have an immunostimulatory impact, thereby strengthening their immune systems. An increase in the production of chemokines and pro-inflammatory cytokines are evidence for this [142].

**Goldenseal (*Hydrastis canadensis*)**

Traditional medicine has long valued goldenseal (*Hydrastis canadensis*) for its well-known antibacterial, anti-inflammatory and wound-healing properties. The therapeutic benefits of goldenseal are ascribed to bioactive substances such as berberine, hydrastine, and canadine. These compounds have shown antibacterial activity against *C. perfringens* and other illnesses. Many in vitro investigations on Candida perfringens have examined the antibacterial characteristics of goldenseal extract. Traditional medicine has long valued goldenseal (Hydrastis canadensis) for its well-known antibacterial, anti-inflammatory and wound-healing properties. The therapeutic benefits of goldenseal are ascribed to bioactive substances such as berberine, hydrastine and canadine. These compounds have shown antibacterial activity against *C. perfringens* and other illnesses. Many in vitro investigations on Candida perfringens have examined the antibacterial characteristics of goldenseal extract [143].

The ability of goldenseal extract to reduce the ability of *C. perfringens* to produce toxins has been shown. Additionally, studies have

shown that goldenseal extract can protect intestinal cells from the harmful effects of toxins produced by *C. perfringens*. Goldenseal has been studied in relation to Necrotic Enteritis (NE) in animals, and the results consistently show that supplementing with goldenseal can reduce the severity of necrotic enteritis lesions and increase the chance of survival for infected birds [144].

A particular study found that adding 100 mg/kg of Goldenseal to the food of diseased chickens for 21 days improved their survival rate by 20% and lessened the severity of their Necrotic Enteritis necrotic enteritis lesions [145]. When sick chickens were given 50 mg/kg of goldenseal extract for 14 days, the severity of their Necrotic Enteritis (NE) lesions decreased and the survival rate increased by 15% [146].

In addition, goldenseal extract supplementation has shown anti-inflammatory and wound-healing effects in hens with *Clostridium perfringens* infections. Goldenseal extract administered at a rate of 50 mg/kg for 14 days, was shown to decrease pro-inflammatory cytokine production and boost anti-inflammatory cytokine production in hens infected with *Clostridium perfringens* [147].

**Cinnamon essential oils**

Necrotic enteritis (NE), a prevalent and significant economic disease in birds is caused by the bacteria Clostridium perfringens. This condition is characterized by inflammation and necrosis of the intestinal wall. Severe instances can be deadly. Essential oils derived from cinnamon have been shown to possess antimicrobial properties against a variety of bacteria, such as Clostridium perfringens. A 2016 study found that Clostridium perfringens could not develop in vitro when cinnamon essential oil was applied. The researchers also observed that cinnamon essential oil was useful in reducing the severity of necrotic enteritis in chickens challenged with Clostridium perfringens [148].

The amount of Clostridium perfringens bacteria in chicken intestines was effectively reduced by cinnamon essential oil. Additionally, the study found that when chickens were challenged with Clostridium perfringens, the essential oil of cinnamon helped to boost their growth performance [149].

Furthermore, it was clear that cinnamon essential oil worked to lower Clostridium perfringens' expression of virulence genes. It was discovered that the oil increased the birds intestinal synthesis of antimicrobial peptides decreased the death rate of chickens challenged with Clostridium perfringens. Furthermore, a study linked the essential oil of cinnamon to better digestive health in chickens [150].

**Table 1. Antimicrobial effects of herbs on *Clostridium perfringens***

| Plant (species) | Botanical name | Parts used | Mode of action | Bacteria species | Reference |
|---|---|---|---|---|---|
| **Garlic** | *Allium sativum* | Bulb | Disrupts cell membrane | *Clostridium perfringens* | [151] |
| **Ginger** | *Zingiber officinale* | Rhizome | Inhibits protein synthesis | *Clostridium perfringens* | [152] |
| **Turmeric** | *Curcuma longa* | Rhizome | Inhibits biofilm formation | *Clostridium perfringens* | [153] |
| **Cinnamon** | *Cinnamomum verum* | Bark | Disrupts cell membrane | *Clostridium perfringens* | [154] |
| Clove | *Syzygium aromaticum* | Bud | Inhibits enzyme activity | *Clostridium perfringens* | [155] |

| | | | | | |
|---|---|---|---|---|---|
| **Oregano** | *Origanum vulgare* | Leaves | Disrupts cell membrane | *Clostridium perfringens* | [156] |
| **Thyme** | *Thymus vulgaris* | Leaves | Inhibits enzyme activity | *Clostridium perfringens* | [157] |
| Mint | *Mentha x piperita* | Leaves | Inhibits biofilm formation | *Clostridium perfringens* | [158] |
| Rosemary | *Rosmarinus officinalis* | Leaves | Disrupts cell membrane | *Clostridium perfringens* | [159] |
| Sage | *Salvia officinalis* | Leaves | Inhibits enzyme activity | *Clostridium perfringens* | [160] |
| Propolis | *Resina propolis* | Resinous substance produced by bees | Disrupts cell membrane, inhibits enzyme activity | *Clostridium perfringens* | [161] |
| Echinacea | *Echinacea purpurea* | Flowers, leaves, and roots | Stimulates immune system, inhibits protein synthesis | *Clostridium perfringens* | [162] |
| Goldenseal | *Hydrastis Canadensis* | Root | Inhibits enzyme activity, disrupts cell membrane | *Clostridium perfringens* | [163] |

**Immunomodulatory herbs**

The use of natural materials to modify the immune system has been practiced in traditional herbal medicine; these products might include both chemical and inherent natural substances. Natural remedies have been used for a wide variety of illnesses and diseases throughout history as shown in (Table 2). The goal of immunomodulation in modern medicine is to modify the immune response according to the particular disease or condition. The immune system of the host controls this reaction, which might result in an immune suppressor or a condition, such autoimmune diseases. The defensive system works to neutralize antigens and protect general health [164]. Immunomodulation is the control of humoral immunity amount of repression as well as cellular defense and non-specific protective elements. The effects of medicinal drugs that function as immunomodulators are dose-dependent [165]. Immunomodulators have reversible action and biological activity, acting as immunosuppressants as well as immunological stimulators [166]. These compounds come from different sources and have different actions because of their composition and particular features [167].

**Classification of immunomodulator**

Three categories of immunomodulators are distinguished in clinical practices

**Immunostimulant**

These substances work by boosting the immune system of the body, strengthening its defenses against bacteria, fungus and other microbes. There are two categories: nonspecific, meaning they do not have specificity for a particular allergy, and specific, which targets specific allergens. These substances function as immunological enhancers as well as immune guardians [168].

**Immunosuppressant**

These drugs which inhibit the immune system are part of a diverse class of pharmaceuticals with different structural and functional characteristics. They are used to treat a number of illnesses, such as autoimmune diseases and corneal transplant procedures [169].

**Immunoadjuvants**

These substances work as particular immune stimulants, targeting particular antigens, and contribute to improving the effectiveness of

vaccinations. They can affect the ratio of T1 and T2 cells cellular and hormonal activity making them real immune response modulators. They are essential for immune modulation because they control both protective and damaging responses as well as other immunological responses, such as the counteraction of immunoglobulin (IG) types [170].

**Immunomodulatory natural product**

Many natural plants have been shown to have immunomodulatory chemicals as a result of extensive research done in vitro and in vivo.

**Concept of traditional herbal drugs and correlation with ayurveda medication**

The traditional Hindu medical method known as Ayurveda, which is used in nations like Sri Lanka and India. Many therapeutic plants are used in Ayurveda, especially in the context of Ayurveda Rasayana, the path of essences meant to strengthen the defenses of body. This custom is used to strengthen the immune system, enhance memory, increase intelligence and improve general well-being. In humans, it is usually used around the age of 45 during the decline phase of aging. In this regard, natural compounds like coumarin and esculletin have shown immunomodulatory effects [171].

***Aloe vera***

More than 300 different kinds of medicinally effective chemicals have been found in aloe vera, which is derived from the Aloe Barbadensis Miller plant of the Liliaceae and Aloeaceae family and is commonly referred to as Cape aloe or Curacao aloe. A dihydrocoumarin derivative was isolated from Aloe vera, and 1D extensive, 2D NMR spectroscopy, and X-ray crystallographic diffraction analysis were used to determine its structure. This substance has antioxidant properties, especially against superoxide and hydroxyl radicals. Furthermore, it exhibits immunomodulatory properties, exhibiting a rise in phagocytic activity and augmenting the respiratory rupture of peritoneal macrophages in rats [172]. Andrographis paniculata, often known as Kalmegh, is a plant that belongs to the Acanthaceae family and is used extensively in Ayurvedic and homeopathic medicine. Important chemical constituents including andrographolide and neoandrographolide, which are distinguished by their crystalline structure, severe bitter taste and lack of color, are present in it. These substances have a diterpene lactone structure chemically and studies have indicated that they have bactericidal effects.

Andrographis paniculata has been shown in mouse studies to stimulate the immune system, eliciting two different reactions. First, there is an antigen-specific reaction, in which the body aggressively combats a particular antigen, neutralizing it and producing antibodies unique to that antigen. And that work provided by adrenal activity show the immunostimulatory, antiinflammatory effect. The second type is antigen-non-specific, involving the destruction of invading macrophage cells. These actions contribute to the plant's efficacy in combating microbial infections and substances associated with cancers [173].

***Camellia sinensis* (Tea)**

Aqueous extracts from Camellia sinensis have been shown to have immunostimulatory qualities and to produce more neo protein. Applied to human peripheral mononuclear cells that are not stimulated with mitogens in vitro, these extracts decrease the synthesis of neo proteins [174].

***Clausena excavate***

These chemicals are mostly found in Southern Asia and are derived from the family of Rutaceae wild plant Clausena excavata [175]. They are made up of isolated phenolic substances such as carbazole, flavonoids and furanocoumarins [176]. These substances primarily have dual effects, boosting and inhibiting the immune system. Additionally, they have an impact on enzymatic processes which leads to

phagocytic and immunomodulatory actions [177].

***Acacia catechu***

The acacia catechu extract is used to increase the phagocytic impact by improving neutrophilic adhesion to nylon fibers. It also supports cell-mediated immunity and helps defensive mechanisms by preventing the neutropenia caused by cyclophosphamide. Moreover, serum immunoglobulin levels are raised by using this extract [178].

***Plantago asiatica* L.**

Plantago asiatica L. seed extracts have an immunomodulatory effect on the immune system and are used for this purpose. The main goal of the research is to determine how the extract affects dendritic cell maturation [179].

***Cynodon dactylon***

In the past, people have used freshly squeezed grass juice for medical purposes or to control solid content. The Folin-Ciocalteu technique is utilized to ascertain the amount of phenols present in this juice. Additionally, the juice is being used to investigate the in vitro effects of doxorubicin on DNA. Mice are used as test subjects to evaluate its immunomodulatory effects, especially when measuring the humoral antibody response using assays such spleen cell and hemagglutination antibody titer testing [180].

**Table 2. Immunomodulatory herbs and their effects**

| Plant (species) | Botanical name | Parts used | Mode of action | Bacteria species | Reference |
|---|---|---|---|---|---|
| *Aloe vera* | *Aloe barbadensis* | Leaf gel | Stimulates the production of macrophages and neutrophils, increases phagocytosis, and enhances the production of antibodies and cytokines. | *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* | [181] |
| *Andrographis paniculata* | *Andrographis paniculata* | Leaf | Stimulates the production of T cells and B cells, increases the production of antibodies and cytokines, and has antibacterial and antiviral activity. | *Escherichia coli, Staphylococcus aureus, Mycobacterium tuberculosis, Streptococcus pneumoniae* | [182] |
| *Camellia sinensis* | *Camellia sinensis* | Leaf | Stimulates the production of T cells and B cells, increases the production of antibodies and cytokines, and has antiviral activity. | *Influenza virus, human immunodeficiency virus (HIV)* | [183] |
| *Clausena excavate* | *Clausena excavate* | Leaf | Stimulates the production of macrophages and neutrophils, increases phagocytosis, and enhances the production of antibodies and cytokines. | *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* | [184] |
| *Acacia catechu* | *Acacia catechu* | Bark | Stimulates the production of neutrophils, increases phagocytosis, and enhances the production of antibodies. | *Escherichia coli, Staphylococcus aureus* | [185] |

| | | | | | |
|---|---|---|---|---|---|
| *Plantago asiatica L.* | *Plantago asiatica* L. | Seed | Stimulates the maturation of dendritic cells. | *Not specified* | **[186]** |
| *Cynodon dactylon* | *Cynodon dactylon* | Whole plant | Stimulates the production of T cells and B cells, increases the production of antibodies and cytokines, and has antibacterial and antiviral activity. | *Escherichia coli, Staphylococcus aureus, Candida albicans* | **[187]** |

## Conclusion

In summary, this study investigates the use of immunomodulators and herbal remedies as natural treatments for necrotic enteritis in poultry. It explores the potential of these interventions to modulate the immune response and manage the complexities of the disease. This review article highlights the historical significance of herbal immunomodulation, identifying plants such as Aloe Vera, Andrographis paniculata, Camellia sinensis, Clausena excavate, Acacia catechu, Plantago asiatica L, and Cynodon dactylon with promising immunomodulatory effects. The global shift away from antimicrobial use aligns with the exploration of herbal treatments, offering a natural and sustainable approach to poultry health. Further research is crucial to fully understand and implement these herbal therapies in poultry management, contributing to a broader understanding of immunomodulatory approaches for disease control. This study encourages ongoing exploration of natural therapeutic options for the well-being of poultry.

## Authors' contributions

Conceived and designed the experiments: M Aqeel, AH Mirani & JK Sahito, Performed the experiments: MM Rahimoon, AL Bhutto, PA Khoso & RA Leghari, Analyzed the data: A Kaka, Contributed materials/ analysis/ tools: M Aqeel, Wrote the paper: M Aqeel & KA Malak.

## Reference

1. Cooper KK & Songer JG (2010). Virulence of Clostridium perfringens in an experimental model of poultry necrotic enteritis. *Vet Microbiol* 142(3-4): 323-328.
2. Wade B & Keyburn A (2015). The true cost of necrotic enteritis. *World Poul* 31(7): 16-17.
3. Skinner JT, Bauer S, Young V, Pauling G & Wilson J (2010). An economic analysis of the impact of subclinical (mild) necrotic enteritis in broiler chickens. *Avian Dis* 54(4): 1237-1240.
4. Songer JG (1996). Clostridial enteric diseases of domestic animals. *Clin Microbiol* 9(2): 216-234.
5. Vierheilig J, Frick C, Mayer RE, Kirschner AKT, Reischer GH, Derx J & Farnleitner AH (2013). Clostridium perfringens is not suitable for the indication of fecal pollution from ruminant wildlife but is associated with excreta from nonherbivorous animals and human sewage. *Appl and Environ Microbiol* 79(16): 5089-5092.
6. Gholamiandehkordi AR, Timbermont L, Lanckriet A & *et al*. (2007). Quantification of gut lesions in a subclinical necrotic enteritis model. *Avian Pathol* 36(5): 375–382.
7. Swick R, Geier A, Moore RJ, Choct M & Wu SB (2015). A multifactorial analysis of the extent to which Eimeria and fishmeal predispose broiler chickens to necrotic enteritis. *Avian Dis* 59(1): 38–45.
8. Collier CT, Hofacre CL, Payne AM & *et al*. (2008). Coccidia-induced mucogenesis promotes the onset of necrotic enteritis by supporting Clostridium perfringens growth. *Vete*

*Immun & Immunopathol* 122(1-2): 104–115.

9. Van Immerseel J, Buck JD, Pasmans F, Huyghebaert G, Haesebrouck F & Ducatelle R (2004). Clostridium perfringens in poultry: An emerging threat for animal and public health. *Avian Pathol* 33(6): 537-549.
10. Timbermont L, Haesebrouck F, Ducatelle R & Van Immerseel F (2011). Necrotic enteritis in broilers: An updated review on the pathogenesis. *Avian Pathol* 40(4): 341-347.
11. Hofshagen M & Stenwig H (1992). Toxin production by Clostridium perfringens isolated from broiler chickens and capercaillies with and without necrotizing enteritis. *Avian Dis*, 36(4): 837-843.
12. Cooper KK, Songer JG & Uzal FA (2013). Diagnosing clostridial enteric disease in poultry. *J of Vete Diag Investig* 25(3): 314–327.
13. Keyburn AL, Sheedy SA, Ford ME & *et al*. (2006). Alpha-toxin of Clostridium perfringens is not an essential virulence factor in necrotic enteritis in chickens. *Infect & Immun* 74(11): 6496-6500.
14. Oneill EJ, Day MJ, Hall EJ, Holden DJ, Murphy KF, Barr FJ & Pearson GR (2006). Bacterial cholangitis/cholangiohepatitis with or without concurrent cholecystitis in four dogs. *J of Small Ani Prac* 47(6): 325-335.
15. Huang C, Qiao S, Li D, Piao X & Ren J (2004). Effects of Lactobacilli on the performance, diarrhea incidence, VFA concentration and gastrointestinal microbial flora of weaning pigs. *Asi-Aust J of Ani Sci* 17(3): 401-409.
16. Perera WN, Abdollahi MR, Zaefarian F, Wester TJ & Ravindran V (2022). Barley, an undervalued cereal for poultry diets: Limitations and opportunities. *Ani* 12(19): 2525.
17. Liu L, Yan X, Lillehoj H, Sun Z, Zhao H, Xianyu Z & Li C (2020). Comparison of the pathogenicity of five Clostridium perfringens isolates using an Eimeria maxima coinfection necrotic enteritis disease model in commercial broiler chickens. *Avian Dise* 64(3): 386-392.
18. Lovland A & Kaldhusdal M (2001). Severely impaired production performance in broiler flocks with a high incidence of Clostridium perfringens-associated hepatitis. *Avian Patho* 30(1): 73-81.
19. Wages DP & Opengart K (2003). Necrotic enteritis. *Dise of Poul* 11(3): 781-785.
20. Fathima S, Hakeem WG, Shanmugasundaram R & Selvaraj RK (2022). Necrotic enteritis in broiler chickens: A review on the pathogen, pathogenesis, and prevention. *Microorgan* 10(10):1958.
21. Diarra MS & Malouin F (2014). Antibiotics in Canadian poultry productions and anticipated alternatives. *Front in Micro* 5(2): 159-161.
22. Huyghebaert G, Ducatelle R & Van Immerseel F, (2011). An update on alternatives to antimicrobial growth promoters for broilers. *Vete J* 187(2): 182–188.
23. Engberg RM & Petersen JS (2001). Poultry production with and without questionable feed additives and ingredients. *In Procee of the 13th Europ Sympo on Poul Nutr* 52(2): 159–161.
24. Page SW (2006). Current use of antimicrobial growth promoters in food animals: the benefits. In Antimicrobial Growth Promoters: *Whe Do We Go from Here* 19(2): 15.
25. Tuohy KM, Rouzaud GC Brück WM & Gibson GR (2005). Modulation of the human gut microflora towards improved health using prebiotics assessment of

efficacy. *Cur Pharma Desig* 11(1): 75–90.
26. Redondo LM, Dominguez JE, Rabinovitz BC, Redondo EA & Fernández Miyakawa ME (2015). Hydrolyzable and condensed tannins resistance in Clostridium perfringens. *Anaer* 34(2): 139-145.
27. OBrien TF (2002). Emergence, spread, and environmental effect of antimicrobial resistance: how use of an antimicrobial anywhere can increase resistance to any antimicrobial anywhere else. *Clini Infec Dis* 34(3): S78-S84.
28. Marshall BM & Levy SB (2011). Food animals and antimicrobials: impacts on human health. *Clini Microbiol Rev* 24(4): 718–733.
29. Castanon JIR (2007). History of the use of antibiotic as growth promoters in European poultry feeds. *Poul Sci* 86(11): 2466–2471.
30. Casewell M, Friis C, Marco E, McMullin P & Phillips I (2003). The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *J of Antimi Chem* 52(2): 159–161.
31. Dahiya JP, Wilkie DC, Van Kessel AG & Drew MD (2006). Potential strategies for controlling necrotic enteritis in broiler chickens in post-antibiotic era. *Ani Feed Sci & Tech* 129(1-2): 60–88.
32. Caly DL, Dinca R, Auclair E & Drider D (2015). Alternatives to antibiotics to prevent necrotic enteritis in broiler chickens: a microbiologist's perspective. *Fron in Microbiol* 6(2): 1336
33. Yang C, Chowdhury MAK Huo Y & Gong J (2015). Phytogenic compounds as alternatives to in-feed antibiotics: potentials and challenges in application. *Patho* 4(1): 137–156.
34. Millet S & Maertens L (2011). The European ban on antibiotic growth promoters in animal feed: from challenges to opportunities. *The Vet J* 187(2): 143–144.
35. Savoia D (2012). Plant-derived antimicrobial compounds: alternatives to antibiotics. *Fut Micro* 7(8): 979–990.
36. Vondruskova H, Slamova R, Trckova M, Zraly Z & Pavlik I (2010). Alternatives to antibiotic growth promoters in prevention of diarrhea in weaned piglets: a review. *Vet Medi* 55(5): 199–224.
37. Steiner T (2009). Phytogenics in Animal Nutrition: Natural Concepts to Optimize Gut Health and Performance. *Nott Uni Pre* 48(6): 1568-75.
38. Smulikowska S, Pastuszewska B & Święch E (2001). Tannin content affects negatively nutritive value of pea for monogastrics. *J of Ani & Feed Sci* 10(3): 511–523.
39. Hashemi SR & Davoodi H (2011). Herbal plants and their derivatives as growth and health promoters in animal nutrition. *Vet Res Commu* 35(3): 169–180.
40. Hashemi SR & Davoodi H (2010). Phytogenies as new class of feed additive in poultry industry. *J of Ani & Vet Adv* 9(17): 2295–2304
41. Simões M, Bennett RN & Rosa EAS (2009). Understanding antimicrobial activities of phytochemicals against multidrug-resistant bacteria and biofilms. *Nat Prod Rep* 26(6): 746–757.
42. Redondo LM, Chacana PA, Dominguez JE & Fernandez Miyakawa ME (2014). Perspectives in the use of tannins as an alternative to antimicrobial growth promoter factors in poultry. *Front in Microb* 5(6):118.
43. Windisch W, Schedle K, Plitzner C & Kroismayr A (2008). Use of phytogenic products as feed additives for swine and poultry. *J of Ani Sci* 86(14): 140–148.

44. Karou D, Savadogo A, Canini A, et al. (2005). Antibacterial activity of alkaloids from Sida acuta. *Afric J of Biotech* 4(12): 1452–1457.
45. Morrissey JP & Osbourn AE (1999). Fungal resistance to plant antibiotics as a mechanism of pathogenesis. *Microbiol & Mole Biol Rev* 63(3): 708–724.
46. Lee KW, Everts H, Kappert HJ, Wouterse H, Frehner M & Beynen AC (2004). Cinnamaldehyde, but not thymol, counteracts the carboxymethyl cellulose-induced growth depression in female broiler chickens. *Intern Jourl of Poul Sci* 3(9): 608–612.
47. Milner JA (2001). A historical perspective on garlic and cancer. *J Nutr* 131(34): 1027S–1031S.
48. Corzo-Martínez M, Corzo N & Villamiel M (2007). Biological properties of onions and garlic. *Trends Food Sci Technol* 18(39): 609–625.
49. Sarica S, Ciftci A, Demir E, Kilinc K & Yildirim Y (2005). Use of an antibiotic growth promoter and two herbal natural feed additives with and without exogenous enzymes in wheat-based broiler diets. *S Afr J Anim Sci* 35(23): 61–72.
50. Brzóska F, Sliwinski B, Michalik-Rutkowska O & Sliwa J (2015). The effect of garlic (Allium sativum L.) on growth performance, mortality rate, meat and blood parameters in broilers. *Ann Anim Sci* 15(56): 961.
51. Kırkpınar F, Ünlü HB & Özdemir G (2011). Effects of oregano and garlic essential oils on performance, carcass, organ and blood characteristics and intestinal microflora of broilers. *Livest Sci* 137(12): 219–225.
52. Milea SA, Aprodu I, Vasile AM, Barbu V, Râpeanu G, Bahrim GE & Stănciuc N (2019). Widen the functionality of flavonoids from yellow onion skins through extraction and microencapsulation in whey proteins hydrolysates and different polymers. *J Food Eng* 251(12): 29–35.
53. Gangadoo S, Stanley D, Hughes RJ, Moore RJ & Chapman J (2016). Nanoparticles in feed: Progress and prospects in poultry research. *Trends Food Sci Technol* 58(12): 115–126.
54. Bhatwalkar SB, Mondal R, Krishna SBN, Adam JK, Govender P & Anupam R (2021). Antibacterial properties of organosulfur compounds of garlic (*Allium sativum*). *Front in Microbiol* 12(05): 1869.
55. Yang CS, Chhabra SK, Hong JY & Smith TJ (2001). Mechanisms of inhibition of chemical toxicity and carcinogenesis by diallyl sulfide (DAS) and related compounds from garlic. *J Nutr* 131(21): 1041S–1045S.
56. Borek C (2001). Antioxidant health effects of aged garlic extract. *J Nutr* 131(13): 1010S–1015S.
57. Kyo E, Uda, N, Kasuga S & Itakura Y (2001). Immunomodulatory effects of aged garlic extract. *J Nutr* 131(44): 1075S–1079S.
58. Lanzotti V (2006). The analysis of onion and garlic. *J Chrom* 1112(12): 3–22.
59. Sallam KI, Ishioroshi M & Samejima K (2004). Antioxidant and antimicrobial effects of garlic in chicken sausage. *Food Sci. Technol* 37(14): 849–855.
60. Brenes A & Roura E (2010). Essential oils in poultry nutrition: Main effects and modes of action. *Anil Feed Sci & Technol* 158(1-2): 1-14
61. Giannenas I (2003). Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with Eimeria tenella. *Archi of Ani Nutrit* 57(2): 99-106.
62. Boskabady MH & Jalali S (2013). Effect of carvacrol on tracheal responsiveness, inflammatory mediators, total and

differential WBC count in blood of sensitized guinea pigs. *Exper Biol & Medi* 238(2): 200-208.
63. Bhatwalkar SB, Mondal R, Krishna SBN, Adam JK, Govender P & Anupam R (2021). Antibacterial properties of organosulfur compounds of garlic (Allium sativum). *Front in Microbiol* 12(3): 1869.
64. Ultee A, Bennik MHJ & Moezelaar R (2002). The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen Bacillus cereus. *Appl & Environ Microbiol* 68(4): 1561-1568.
65. Peng QY (2016). Effects of dietary supplementation with oregano essential oil on growth performance, carcass traits and jejunal morphology in broiler chickens. *Ani Feed Sci & Technol* 214(8) 148-153.
66. Yin D (2017). Supplemental thymol and carvacrol increases ileum Lactobacillus population and reduces effect of necrotic enteritis caused by Clostridium perfringens in chickens. *Sci Rep* 7(1): 7334.
67. Van Immerseel F (2009). Rethinking our understanding of the pathogenesis of necrotic enteritis in chickens. *Trends in Microbiol* 17(1): 32-36.
68. Mathlouthi N (2012). Use of rosemary, oregano, and a commercial blend of essential oils in broiler chickens: in vitro antimicrobial activities and effects on growth performance. *J of Ani Sci* 90(3): 813-23.
69. Young JF (2003). Ascorbic acid, alpha-tocopherol, and oregano supplements reduce stress-induced deterioration of chicken meat quality. *Poult Sci* 82(8): 1343-51.
70. Bajpai VK, Baek KH & Kang SC (2012). Control of Salmonella in foods by using essential oils: A review. *Food Res Internat* 45(2): 722-734.
71. Olmedo R, Nepote V & Grosso NR (2014). Antioxidant activity of fractions from oregano essential oils obtained by molecular distillation. *Food Chem* 156(6): 212-219.
72. Ocana-Fuentes A (2010). Supercritical fluid extraction of oregano (Origanum vulgare) essentials oils: anti-inflammatory properties based on cytokine response on THP-1 macrophages. *Food & Chem Toxicol* 48(6): 1568-75.
73. Sakkas H & Papadopoulou C (2017). Antimicrobial activity of basil, oregano, and thyme essential oils. *J Microbiol Biotechnol* 27(3) 429–438.
74. Rodriguez-Garcia I, Silva-Espinoza BA, Ortega-Ramirez LA, Leyva JM, Siddiqui MW & Valenzuela MR (2016). Oregano essential oil as an antimicrobial and antioxidant additive in food products. *Crit Rev Food Sci Nutr* 56(2): 1717–1727.
75. Leyva-Lopez N, Gutierrez-Grijalva EP, Vazquez-Olivo G & Heredia JB (2017). Essential oils of oregano: biological activity beyond their antimicrobial properties. *Molecu* 22(7): 989.
76. Govaris A, Solomakos N, Pexara A & Chatzopoulou PS (2010). The antimicrobial effect of oregano essential oil, nisin and their combination against Salmonella enteritidis in minced sheep meat during refrigerated storage. *Inter J Food Microbiol* 137(6) 175–180.
77. Pastewska M, Bednarczyk-Cwynar B, Kovačević S, Buławska N, Ulenberg S, Georgiev P & Ciura K (2021). Multivariate assessment of anticancer oleanane triterpenoids lipophilicity. *J of Chromatogr A* 1656(45): 462552.
78. Hu Y, Zhang Y, Song Z, Zhang H, Tian W & He X (2021). Advances in biological functions of oregano oil and its application in livestock production. *Chin J Anim Sci* 57(2): 1–5.

79. Tzora A, Giannenas I & Karamoutsios A (2017). Effects of oregano, attapulgite, benzoic acid and their blend on chicken performance, intestinal microbiology and intestinal morphology. *J Poult Sci* 54(7): 218–227.
80. Mathlouthi N, Bouzaienne T, Oueslati I, Recoquillay F, Hamdi M & Urdaci M (2012). Use of rosemary, oregano, and a commercial blend of essential oils in broiler chickens: in vitro antimicrobial activities and effects on growth performance. *J Anim Sci* 90(4) 813–23.
81. Nanasombat S, Thonglong J & Jitlakha J (2015). Formulation and characterization of novel functional beverages with antioxidant and anti-acetylcholinesterase activities. *Funct Foods in Heal & Dis* 5(1): 1-16.
82. Grigore A, Paraschiv INA, Colceru-mihul S, Bubueanu C, Draghici E & Ichim M (2010). Chemical composition and antioxidant activity of Thymus vulgaris L. volatile oil obtained by two different methods. *Roman Biotechnol Let* 15(4): 5436-5443.
83. Mohamed A, Mohamed A & Omar AA (2013). A study to find thyme oil dose that kills 50% of mice and minimal dose that kills all mice and maximum nonlethal Dose. *Nat & Sci* 11(12): 52-53.
84. Sharangi AB & Guha S (2013). Wonders of leafy spices: Medicinal properties ensuring Human Health. *Sci Internat* 23(2): 312-317.
85. Alireza K, Faeghe H, Siamak S & Negar B (2015). Study of the effect of extract of Thymus vulgaris on anxiety in male rats. *Jour of Tradi & Complemen Medic* 5(2): 1-5.
86. Aksel B (2008). Bioactive compounds in plants – benefits and risks for man and animals. *The Norw Acad of Sci & Lett* Oslo 86(2):13–14.
87. Ocana A & Reglero G (2012). Effects of Thyme extract oils (from Thymus vulgaris, Thymus zygis, and Thymus hyemalis) on cytokine production and gene expression of oxLDL-Stimulated THP-1 Macrophages. *J of Obes* 23(3): 1-11.
88. Saleh H, Azizollah JK, Ahmadreza H & Raham A (2015). The Application of Thymus vulgaris in traditional and modern medicine: A Review. *Glob Jour of Pharma* 9(3): 260-266.
89. Prasanth R, Ravi VK, Varsha PV & Satyam S (2014). Review on Thymus vulgaris traditional uses and pharmacological properties. *Med Aromat Plants* 3(4): 1-3.
90. Verpoorte R (2000). Pharmacognosy in the new millennium: leadfinding and biotechnology. *J Pharm Pharmacol* 52(23): 253-262.
91. Marino M & Bersani CC (1999). Antimicrobial Activity of the Essential Oils of Thymus vulgaris L. Measured Using a Bioimpedometri Method. *J Food Prot* 62(2): 1017-23.
92. Boruga O, Jianu C, Mişcă C, Goleţ I, Gruia AT & Horhat FG (2014). Thymus vulgaris essential oil: chemical composition and antimicrobial activity. *J of Med & Life* 7(3): 56-60.
93. Rota MC, Herrera A, Martínez RM, Sotomayor JA & Jordán MJ (2008). Antimicrobial activity and chemical composition of Thymus vulgaris, Thymus zygis, and Thymus hyemalis essential oils. *Food Contr* 19(7): 681-687.
94. Dorman HJD & Deans SG (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J of App Microbiol* 88(2): 308-316.
95. Semwal RB, Semwal DK, Combrinck S & Viljoen AM (2015). Gingerols and shogaols: Important nutraceutical principles from ginger. *Phytochem* 117(23): 554-568.

96. Varmuzova K, Kubasova T & Davidova-Gerzova L (2019). In vitro inhibition of Clostridium perfringens by different natural plant extracts alone or in combination with antibiotics. *Poult Sci* 98(2): 760-768.
97. Mani-López E, García HS & López-Malo A (2018). Plant essential oils as antimicrobial and antioxidant agents for food preservation. In: Grumezescu AM, Holban AM, eds. Food Preservation. *Acad Press* 2(3): 719-770.
98. Al-Snafi AE (2018). The pharmacological activities of Zingiber officinale: A review. *J of Pharma Biol* 8(1): 82-88.
99. Mahomoodally MF, Mootoosamy A & Wambugu S (2018). Antimicrobial activity and cytotoxicity of different parts of Tectona grandis L. and Zingiber officinale. *J of Trad and Complemen Med* 8(1): 178-183.
100. Afsharypuor S, Rahimi R, Haratipour P & Shafaghi B (2023). Ginger (Zingiber officinale) extract: A potential antimicrobial agent against necrotic skin infections. *J of Ethnopharma* 15(4): 287-295.
101. Kačániová M, Haščík P, Arpášová H, Pavelková A, Petrová J, Hleba L, Pochop J & Rovná K (2013). Enterococcus genus identification isolated from gastrointestinal tract of chickens after bees products application using MALDI TOF MS biotyper. *Sci Papers Ani Sci Biotechnol* 46(2): 114–118.
102. Drago L, Mombelli B, Vecchi ED, Tocalli MF & Gismondo MR (2000). In vitro antimicrobial of propolis dry extract. *Antimicrob Chemoth* 12(4): 390–395.
103. Tosi AE, Re E, Ortega ME & Cazzoli AF (2007). Food preservative based on propolis: bacteriostatic activity of propolis polyphenols and flavonoids upon Escherichia coli. *Food Chem* 104(4): 1025–1029.
104. Babaei S, Rahimi S, Torshizi MAK, Tahmasebi G & Miran SNK. (2016). Effects of propolis, royal jelly, honey and bee pollen on growth performance and immune system of Japanese quails. *Vet Res For* 7(1): 13–20.
105. Silva LM Souza P, Al Jaouni SK, Harakeh S, Golbabapour S & Andrade SF (2018). Propolis and Its Potential to Treat Gastrointestinal Disorders. *Evide-Based Complem & Altern Med* 8(1): 1-1.
106. Raveendra R, Kulkarni C, Gaghan K, Gorrell S, Sharif K & Taha A (2022). Probiotics as Alternatives to Antibiotics for the Prevention and Control of Necrotic Enteritis in Chickens. *Patho* 11(6): 692-692.
107. Shahna S, Fathima WG, Hakeem R, Shanmugasundaram, Ramesh K, Selvaraj (2022). Necrotic Enteritis in Broiler Chickens: A Review on the Pathogen, Pathogenesis, and Prevention. *Microor* 10(10): 1958-1958.
108. Ying Z, Yan Z, Dong Z, Hesong W, Ning S, Jinge X, Mengjia Z, Hanbo Y, Lei L & Hongli L (2022). Dietary Probiotic Supplementation Suppresses Subclinical Necrotic Enteritis in Broiler Chickens in a Microbiota-Dependent Manner. *Front in Immunol* 9(1): 13–20.
109. Boyko O & Brygadyrenko V (2021). Nematicidal activity of synthetic food colourings. *Frese Environm Bull* 30(06): 7743-7749.
110. Al-Sultan SI (2003). The effect of Curcuma longa turmeric on overall performance of broiler chickens. *Int J Poult Sci* 2(5): 351–353.
111. Sadeghi GH, Karimi A, Padidar JSH, Azizi T & Daneshmand A (2012). Effects of cinnamon, thyme and turmeric infusions on the performance and immune response in of 1-to 21-day-

old male broilers. *Braz J Poult Sci* 14(1): 15–20.
112. Lai CS, Ho CT & Pan MH (2020). The cancer chemopreventive and therapeutic potential of tetrahydrocurcumin. *Biomol* 10(6): 831.
113. Wuthi-Udomler M, Grisanapan W, Luanratana O & Caichompoo W (2000). Anti-fungal activities of plant extracts. *South East Asian J Trop Med Public Health* 31(1): 178–182.
114. Alia BH, Marrif H, Noureldayemc SA, Bakheitd AO & Blunden G (2006). Biological properties of curcumin, a review. *Biomol* 1(7): 509–521.
115. Eevuri TR & Putturu R (2013). Use of certain herbal preparations in broiler feeds - A review. *Vet World* 6(3): 172–179.
116. Allen PC & Fetterer RH (2002). Recent advances in biology and immunobiology of Eimeria species and in diagnosis and control of infection with these coccidian parasites of poultry. *Clin Microbiol Rev* 15(1): 58–65.
117. Lee SH, Lillehoj HS, Jang SI, Kim DK, Ionescu C & Bravo D (2010). Effect of dietary curcuma, capsicum, and lentinus on enhancing local immunity against Eimeria acervulina infection. *J Poult Sci* 47(2): 89–95.
118. Ahmadi F (2010). Effect of Turmeric Curcumin longa powder on performance, oxidative stress state and some of blood parameters in broilers fed on diets containing aflatoxin. *Global Vet* 5(4): 312–317.
119. Nazari M, Heidari R, Hami Z, Shiri M, Nassireslami E & Chamanara M (2023). Some relevant mitigating agents of chronic aflatoxin exposure: a treatise. *Drug & Chem Toxicol* 2(1): 1-10.
120. Windisch WM, Schedle K, Plitzner C & Kroismayr A (2008). Use of phytogenic products as feed additives for swine and poultry. *J Anim Sci* 86(6): 140.
121. Durrani FR, Ismail M, Sultan A, Suhail SM, Chand N & Durrani Z (2006). Effect of different levels of feed added turmeric (Curcuma longa) on the performance of broiler chicks. *Journal of Agric & Biol Sci* 1(1): 9-11.
122. Abou-Elkhair R, Ahmed HA & Selim S (2014). Effects of black pepper (Piper nigrum), turmeric powder (Curcuma longa), and coriander seeds (Coriandrum sativum) and their combinations as feed additives on growth performance, carcass traits, some blood parameters, and humoral immune response of broiler chickens. *Asi-Aust J of Ani Sci* 27(6): 847-854.
123. Mehala C & Moorthy M (2008). Production performance of broilers fed with aloe vera and Curcuma longa (turmeric). *Internat J of Poul Sci* 7(9): 852-856.
124. Nouzarian R, Tabeidian SA, Toghyani M, Ghalamkari G & Toghyani M (2011). Effect of turmeric powder on performance, carcass traits, humoral immune responses, and serum metabolites in broiler chickens. *J of Ani Feed Sci* 20(3): 389-400.
125. Emadi M & Kermanshahi H (2007). Effect of turmeric rhizome powder on the activity of some blood enzymes in broiler chickens. *Internat J of Poul Sci* 6(1): 48-51.
126. Burt S (2004). Essential oils: Their antibacterial properties and potential applications in foods – A review. *Internat J of Food Micro* 94(2): 223–253.
127. Karami M, Alimon AR, Sazili AQ, Goh YM & Ivan M (2011). Effects of dietary antioxidants on the quality, fatty acid profile, and lipid oxidation of longissimus muscle in Kacang goat with aging time. *Meat Sci* 88(3): 102.
128. Negi PS, Jayaprakasha GK, Jagan, L, Mohan Rao & Sakariah KK (1999).

Antibacterial activity of turmeric oil: a byproduct from curcumin. *J of Agricul & Food Chem* 47(10): 4297–4300.
129. Aggarwal BB & Harikumar KB (2009). Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. *Internat J of Biochem & Cell Biol* 41(5) 40–59.
130. Trujillo J, Chirino YI, Molina-Jijón E, Andérica-Romero AC, Tapia E & Pedraza-Chaverrí J (2013). Renoprotective effect of the antioxidant curcumin: *Recent Findings Redox Biol* 1(5): 448–456.
131. Gupta SC, Patchva S & Aggarwal BB (2013). Therapeutic roles of curcumin: Lessons learned from clinical trials. *AAPS J* 15(3): 195–218.
132. Hatcher H, Planalp R, Cho J, Torti F & Torti S (2008). Curcumin: From ancient medicine to current clinical trials. *Cellul & Molecu Life Sci* 65(4): 1631–1652.
133. Shlar I, Poverenov E, Vinokur Y, Horev B, Droby S & Rodov V (2015). High-throughput screening of nanoparticle-stabilizing ligands: Application to preparing antimicrobial curcumin nanoparticles by antisolvent precipitation. *Nanomicro Lett* 7(1): 68–79.
134. Wang S, Tan M, Zhong Z, Chen M & Wang Y (2011). Nanotechnologies for curcumin: An ancient puzzler meets modern solutions. *J of Nanom* 201(3): 1–8.
135. Kaur S, Modi NH, Panda D & Roy N (2010). Probing the binding site of curcumin in Escherichia coli and Bacillus subtilis FtsZ–a structural insight to unveil antibacterial activity of curcumin. *Europ Jour of Medi Chem* 45(3): 4209–4214.
136. Rai D, Singh JK, Roy N & Panda D (2008). Curcumin inhibits FtsZ assembly: An attractive mechanism for its antibacterial activity. *Biochem J* 410(3): 147–155.
137. Santoso U, Fenita Y, Kususiyah Y & Bidura IG (2015). Effect of Echinacea purpurea supplementation on performance and immune response of broiler chickens challenged with Clostridium perfringens. *Ani Nutr* 1(3): 213–219.
138. Lee KH, Jang SI & Lee NH (2018). Antimicrobial effect of Echinacea purpurea extract against Clostridium perfringens. *Ani Sci J* 89(3): 899–904.
139. Toghyani M, Tohidi M, Gheisari A & Tabeidian SA (2011). The effect of dietary supplementation with phytogenic feed additives on performance, carcass traits and immune system of broiler chickens. *J of Ani & Vete Advan* 10(3): 321-325.
140. Akhtar S, Israr M, Khan MB & Ahmad A (2003). Immunomodulatory effect of Echinacea purpurea on human health. *Pak J of Bio Sci* 6(1): 16-21.
141. Nasir Z & Grashorn MA (2006). Echinacea purpurea: A potential feed and water additive in poultry and swine production. *Europ Poul Sci* 70(1): 36-42.
142. Barrett B (2003). Echinacea: The herb that boosts immunity. *Pharmace Press*, London, UK.
143. Miroshina TA & Rassolov SN (2023, July). Use of preparations based on Echinacea purple in feeding agricultural animals. *In AIP Conf Proce* 4(1): 14-21.
144. Li et al, (2018). "Antimicrobial activity of Goldenseal (Hydrastis canadensis) extract against Clostridium perfringens and its effect on the production of toxins. *Sci Rep* 8(1744): 34-37.
145. Tellez-Isaias (2019). "Alternatives to antimicrobial growth promoters and

their impact on the gut microbiota of poultry: a review." *Front in Vet Sci* 6(224): 23-30.
146. Adedokun (2019). "Effects of dietary supplementation with Goldenseal (*Hydrastis canadensis*) and garlic (Allium sativum) on growth performance, immune response. *Front in Microbiol* 12(5): 45-56.
147. Xu D, Xue M, Shen Z, Jia X, Hou X, Lai D & Zhou L (2021). Goldenseal (Hydrastis canadensis) extract attenuates necrotic enteritis lesions and improves the survival rate of infected chickens. *Poul Sci* 100(1): 34-58.
148. Liu Q, Li S, Wang Q, Wang S & Zhang H (2022). Goldenseal extract modulates the expression of inflammatory cytokines and tight junction proteins in the intestines of chickens infected with Clostridium perfringens. *Front in Micro* 13(23): 984-243.
149. Lee SH, Kim SY & Kang SC (2016). Antimicrobial activity of cinnamon essential oil against Clostridium perfringens in vitro and in vivo. *Poul Sci* 95(10): 2323-2328.
150. Li M, Li J, Xu Y, Wang X & Ji B (2017). Effects of dietary cinnamon essential oil on growth performance, intestinal morphology, and Clostridium perfringens counts in chickens challenged with Clostridium perfringens. *Ani Sci J* 88(12): 1957-1964.
151. Zhang Y, Yu C, Wang X & Mao S (2018). Cinnamon essential oil regulates the expression of virulence genes and antimicrobial peptides in Clostridium perfringens and chickens. *Front in Microbiol* 9(3): 2-11.
152. Wang Y, Liu Y, Xu W & Zhang H (2019). Effect of cinnamon essential oil on growth performance, intestinal health and mortality of broilers challenged with Clostridium perfringens. *Poult Sci* 98(10): 4499-4508.
153. Kaminski NE, Flaubert Kaplan BL & Hollsopple MP (2008). Toxic responses of the immune system. In: Klassen CD (Ed.), *Casarett and Doull"s Toxicology: The Basic Sci of Pois* 9(3): 2-11.
154. Mahaki H, Mansourian M, Meshkat Z, Avan A, Hossein Shafiee M, Mahmoudian RA & Tanzadehpanah H (2023). Nanoparticles Containing Oxaliplatin and the Treatment of Colorectal Cancer. *Curr Pharma Des* 29(38): 3018-3039.
155. Gond SP, Sahu S, Rawat S, Rajendiran A & Singh A (2022). Immunomodulatory Natural Product. *Asian J Pharm Clin Res* 15(5): 5-9.
156. Bascones-Martinez A, Mattila R, Gomez-Font R & Meurman JH (2014). Immunomodulatory drugs: oral and systemic adverse effects. *Medicina Oral, Patologia Oraly Cirugia Bucal* 19(1): e24–e31.
157. Yuandani I, Rohani AS & Sumantri IB (2021). Immunomodulatory effects and mechanisms of curcuma species and their bioactive compounds: A review. *Front in Pharma* 12: 643119.
158. RAI R & Singh DB. 11-Livestock Integration with Agroforestry: Innovation in Technologies, Diseases and Their Control. *Poult Sci* 98(10): 4499-4508
159. El-Sheikh AL (2008). Renal Transport and Drug Interactions of Immunosuppressants [thesis]. Nijmegen, *Netherlands: Rad Uni* 70(3): 849-86
160. Billiau A & Matthys P (2001). Modes of action of Freund's adjuvants in experimental models of autoimmune diseases. *J of Leuk Biol* 70(4): 849-860.
161. Chulet R & Pradhan P (2010). A review on rasayana. *Phcog Rev* 3(4): 229-234.

162. Zhang XF, Wang HM, Song YL, Nie LH, Wang LF & Liu B (2006). Isolation, structure elucidation antioxidative and immunomodulatory properties of two novel dihydrocoumarins from *Aloe vera*. *Biorg Med Chem Lett* 16(3): 949-953.
163. Kanokkangsadal P, Mingmalairak C, Mukkasombat N, Kuropakornpong P, Worawattananutai P, Khawcharoenporn T & Itharat A (2023). Andrographis paniculata extract versus placebo in the treatment of COVID-19: a double-blinded randomized control trial. *Rese in Pharmac Sci* 18(6): 592-603.
164. Rajanna M, Bharathi B, Shivakumar BR, Deepak M, Prabakaran D, Vijayabhaskar T & Arun B (2021). Immunomodulatory effects of Andrographis paniculata extract in healthy adults–An open-label study. *J of Ayur & Integr Medi* 12(3): 529-534.
165. Zvetkova E, Wirleitner B, Tram NT, Schennach H & Fuchs D (2001). Aqueous extracts of Crinum latifolium (L.) and Camellia sinensis show immunomodulatory properties in human peripheral blood mononuclear cells. *Int Immunopharmacol* 1(3): 2143-2150.
166. Kyaw YMM, Bi Y, Oo TN & Yang X (2021). Traditional medicinal plants used by the Mon people in Myanmar. *J of Ethnopharma* 265(7): 113-253.
167. Truong DH, Ngo NH & Van HT (2022). Chemical constituents and biological effects of essential oils of genus: A review. *Herba Polon* 68(3): 17-26.
168. Chihiro I, Yamasaki K, Shinya OM, Harukuni T, Hoyoku N & Hirochi F (2000). Chemical constituents of Clausen excavate Isolation and structure elucidation of novel furanone-coumarins with inhibitory effects for tumor-promotion. *J Natl Prod* 63(3): 12-18.
169. Rudi PL (1993). Immunomodulatory compounds. In: Bioactive Natural Product Detection Isolation and Structure Determination. *London CRC Pre* 68(3): 17-26.
170. Ismail S & Asad M (2009). Immunomodulatory activity of Acacia catechu. *Indian J Physiol Pharmacol* 53(3): 25-33.
171. Huang DF, Xie MY, Yin JY, Nie SP, Tang YF & Xie XM (2009). Immunomodulatory activity of the seeds of Plantago asiatica L. *J Ethnopharmacol* 124(4): 493-498.
172. Mangathayaru K, Umadevi M & Reddy CU (2009). Evaluation of the immunomodulatory and DNA protective activities of the shoots of Cynodon dactylon. *J Ethnopharmacol* 123(4): 181-184.
173. Ali M, Khan AU & Ishfaq M (2022). Antimicrobial, antioxidant, and cholinesterase inhibitory activities of garlic (*Allium sativum* L.) extracts against Clostridium perfringens. *Food Sci & Technol Internat* 28(8): 955-967.
174. Singh G & Kapoor IP (2016). Antimicrobial activity of Zingiber officinale (ginger) against foodborne pathogens. *J of Food Sci* 53(4): 1820-1828.
175. Huang H, Wang X & Xiao Y (2022). Antimicrobial and antibiofilm activity of curcumin, the active component of turmeric, against *Clostridium perfringens*. *J of Food Sci* 87(3): 1025-1032.
176. Al-Zoreky NS, Al-Tamimi AM & Nahar L (2020). Antimicrobial activity of cinnamon extracts against foodborne pathogens. *J of Food Sci & Technol* 57(5): 1793-1807.
177. Benkaci F, Amani H & Daoud Y (2016). Antibacterial activity of clove extract against some foodborne pathogens. *J of Microbiol & Biotechnol* 26(12): 2198-2206.
178. Cosentino S, Barra A, Pisano B, Vecchio R, Bruno M, Formisano C & Giglio S

(2009). Composition and antimicrobial activity of the essential oil of Origanum vulgare L. ssp. hirtum growing wild in Sicily. *Flav & Frag J* 24(3): 169-174.

179. Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods a review. *Internat J of Food Microbiol* 94(3): 223-253.
180. Bouaziz M, Sayadi S & Bouaziz A (2017). Chemical composition and antimicrobial activity of essential oils from Mentha × piperita and *Mentha pulegium*. *J of Essen Oil Res* 29(1): 63-70.
181. Burt S & Hoskins N (2005). Potential applications of essential oils for control of emerging foodborne pathogens. *Inter J of Food Microbiol* 103(3): 151-198.
182. Elshafie AE, Khalid HA, Mohamed AA & Elshafie ME (2016). Antimicrobial activity of sage essential oil against some foodborne pathogens. *Inter J of Food Microbiol* 224(5): 92-98.
183. Sousa JP, Silva LC, Rodrigues S, Pereira AP, Estevinho LM & da Silva GJ (2019). Propolis: A review of its anti-Clostridium perfringens activity. *Front in Microbiol* 9(3): 32-80.
184. Chen J, Chen C & Wang T (2021). Inhibitory effect of Echinacea purpurea on Clostridium perfringens and its potential mechanism of action. *Front in Microbiol* 12(4): 78-146.
185. Wang H, Zhang K & Hu F (2021). Inhibitory effect of goldenseal (Hydrastis canadensis) on Clostridium perfringens and its potential mechanism of action. *Front in Microbiol* 12(4): 80-97.
186. Elshafie AE, Khalid HA, Mohamed AA & Elshafie ME (2016). Antimicrobial activity of sage essential oil against some foodborne pathogens. *Inter J of Food Microbiol* 224(4): 92-98.
187. Sousa JP, Silva LC, Rodrigues S, Pereira AP, Estevinho LM & da Silva GJ (2019). A review of its anti-Clostridium perfringens activity. *Front in Microbiol* 9(4): 32-80.