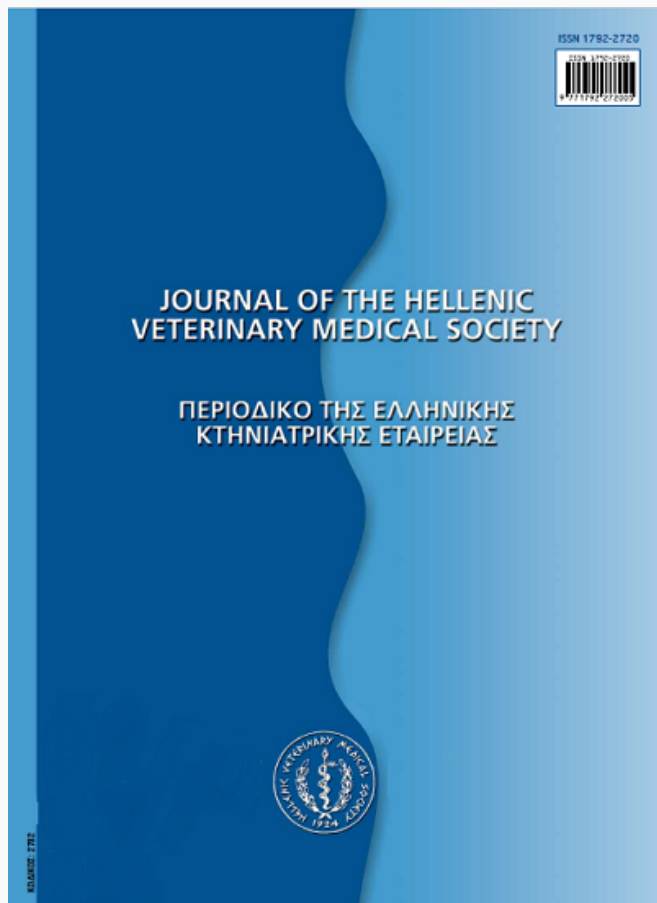


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Botanicals: a natural approach to control ascaridiosis in poultry

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Φυτοβιοτικά: Νέα φυσική προσέγγιση αντιμετώπισης της ασκαριδίωσης των πτηνών

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ABSTRACT. Parasites (protozoa, helminthes, arthropods) represent a main threat for poultry worldwide. Among helminthes, nematodes constitute the most important group of parasites of poultry. The nematode *Ascaridia galli*, the cause of ascaridiosis in poultry, is one of the most important and prevalent parasites, resulting in serious economic losses, associated with the treatment cost, the decreased feed efficiency, and the poor egg and meat production. During the past few decades the indiscriminate use of anthelmintic drugs has generated several cases of resistance in helminthes in poultry, situation which is coupled with the severity of residues in poultry products. For this reason, nowadays attention has been drawn to the use of botanicals in poultry diet, due to their anthelmintic properties. Furthermore, the dietary use eco-friend-

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ly of these plant derived substances compared to conventional synthetic anthelmintic drugs is considered as a natural and ecofriendly approach by the consumers. The focus of the present review is to recapitulate the studies, both *in vivo* and *in vitro*, that have demonstrated the anthelmintic efficacy of various dietary botanicals in controlling poultry ascaridiosis.

Keywords: Helminthes, *Ascaridia galli*, Botanicals, Poultry.

ΠΕΡΙΛΗΨΗ. Τα παράσιτα (πρωτόζωα, έλμινθες, αρθρόποδα) αποτελούν σημαντική απειλή για τα πτηνά παγκοσμίως. Μεταξύ των ελμίνθων, τα νηματώδη συνιστούν τη σπουδαιότερη ομάδα παρασίτων των πτηνών. Το νηματώδες *Ascaridia galli*, αίτιο της ασκαριδίωσης των πτηνών, είναι ένα από τα πλέον σημαντικά και συχνά παράσιτα, προκαλώντας σοβαρές οικονομικές απώλειες, που συνδέονται με το κόστος θεραπείας, τη μειωμένη αξιοποίηση της τροφής και τη μείωση της παραγωγής αυγών και κρέατος. Κατά τη διάρκεια των τελευταίων δεκαετιών, η αλόγιστη χρήση ανθελμινθικών φαρμάκων οδήγησε στην ανάπτυξη ανθεκτικότητας σε κάποια ανθελμινθικά που χρησιμοποιήθηκαν σε πτηνά, γεγονός που σχετίζεται με την παρουσία καταλοίπων στα προϊόντα των πτηνών. Για το λόγο αυτό, τα τελευταία χρόνια το ενδιαφέρον των ερευνητών έχει στραφεί στη χρήση φυτοβιοτικών στη διατροφή των πτηνών εξαιτίας των ανθελμινθικών ιδιοτήτων τους. Επιπλέον, η χρήση αυτών των φυτικής προέλευσης ουσιών σε σύγκριση με τα συμβατικά συνθετικά ανθελμινθικά φάρμακα θεωρείται ως μια φυσική και φιλική προς το περιβάλλον προσέγγιση από τους καταναλωτές. Σκοπός της παρούσας ανασκόπησης είναι η παράθεση των ερευνών, τόσο *in vivo* όσο και *in vitro*, που παρουσιάζουν την ανθελμινθική αποτελεσματικότητα διαφόρων φυτοβιοτικών στον έλεγχο της ασκαριδίωσης των πτηνών.

Λέξεις ευρετηρίασης: Έλμινθες, *Ascaridia galli*, Φυτοβιοτικά, Πτηνά.

INTRODUCTION

Parasitism with protozoa, helminthes and arthropods remains a main threat for poultry worldwide inducing heavy production losses in animals (Ahmad et al., 2013). Between helminthes, like Roundworms (Nematodes), Tapeworms (Cestodes) and Flukes (Trematodes), nematodes are the most important ones (Rafi et al., 2011). *Ascaridia galli* (*A. galli*), *Heterakis gallinarum* (*H. gallinarum*) and *Capillaria* spp. are the most common roundworms of poultry, with *A. galli* being the most prevalent (Kaufman et al., 2011). Researchers' interest in relation to alternative control measures has been focused mainly on *A. galli*. This parasite is responsible for clinical and subclinical parasitism. In heavily infected poultry the clinical signs include droopiness, diarrhea and hemorrhages. Also, during heavy infestation birds may show signs of decreased weight gain and retarded growth, due to damaged integrity of the intestinal mucosa and subsequent impaired nutrient utilization (Das et al., 2010). In more severe cases and especially in young birds, intestinal blockage may occur, leading to

death (Abdelqader et al., 2008). *A. galli* infections result in serious economic losses, usually associated with treatment cost, decreased feed efficiency and poor egg and meat production (Martin-Pacho et al., 2005). Another very debilitating factor resulting in economic losses is the ability of *A. galli* eggs to act as vectors for transmission of fatal bacterial infectious organisms, such as *Salmonella enterica* and *Escherichia coli* (Permin et al., 2006).

During the past few decades, novel research on the transmission of helminthes has enabled scientists to develop efficient products for their control. However, the indiscriminate use of antiparasitic drugs in poultry has generated cases of resistance to conventional drugs, especially piperazine and benzimidazoles, such as fenbendazole and albendazole (Abdelqader et al., 2012; Yazwinski et al., 2013). This situation, coupled with the severity of the associated risks of chemical residues in poultry products and the high cost of treatment compliance in endemic regions, necessitates further efforts into the discovery of novel drugs from either natural or synthetic sources (Anthony et al., 2005).

For sustainable control of *A. galli* different approaches have been employed such as, nutrition of host animal (Das et al., 2010), utilization of genetic resistance (Kaufman et al., 2011), biological control (Braga et al., 2011), and the use of plants with promising anthelmintic activity (Anthony et al., 2005).

Attention has been drawn to the screening of botanicals for their anthelmintic properties (Anthony et al., 2005). Accordingly, the knowledge of traditional herbal remedies is scientifically examined in order to find new plants having potent broad spectrum anthelmintic activity with less toxicity (Mali and Mehta, 2008). Compared to conventional synthetic anthelmintic drugs, plant derived products are considered natural and eco-friendly. Moreover, many such products are certified as GRAS (Generally Recognized As Safe) by the FDA (Food and Drug Administration) and therefore could be ideal candidates as feed additives (Brenes and Roura, 2010; Christaki et al., 2012). The use of phytogenic bioactive compounds for poultry nematode control is increasing in different commercial production systems and it has a reduced impact on environment (George et al., 2009). Furthermore, the active components of plants are compounds with great structural diversity and low molecular weight. These components can be active against many biological processes of the parasites and this diversity can preclude the development of anthelmintic resistance (Tariq et al., 2009).

Aim of this review is to summarize the current knowledge regarding the use of plant derived substances to control *A. galli* parasitism in poultry. Under this effort their anthelmintic activities and various proposed modes of action are discussed.

BOTANICALS

Recently, strong research interest is focused on botanicals (or phytogenics or phytobiotics). Botanicals are made from plants, algae, fungi or lichens (European Food Safety Authority, 2009b). Currently, they are under examination for their various bioactive activities: improving feed intake and flavour; stimulating the secretion of digestive enzymes; increasing gastric and intestinal motility; endocrine stimulation; anticoccidial and other antiparasitic activities; antimicrobial, anti-viral,

immune-stimulating, anti-inflammatory and antioxidative activity. The main active substances of botanicals are considered to be the plant secondary metabolites. Botanicals vary greatly due to the enormous variety of different plants used, the different methods used in their preparation, and their formulations (Christaki et al., 2012; Bozkurt et al., 2013).

Traditionally, the easiest way to prepare botanicals is to separate the plant part that contains the active substances (seeds, leaves, bark, etc) and then dry and grind it to powder (Christaki et al., 2012; Bozkurt et al., 2013). Nevertheless, the research focus is now on the separation and identification of their active ingredients, using gas chromatography and mass spectrometry (Brenes and Roura, 2010).

Some of the main botanical extracts are essential oils and oleoresins. Essential oils (volatile oils; ethereal oils; aetherolea) are aromatic oily liquids that originate from plants. Essential oils can be complex mixtures of many secondary plant metabolites, mainly low boiling terpenes (examples: linalool, geraniol, borneol, menthol, thujanol, citronnillol, α -terpinol), phenols (examples: thymol, carvacrol, eugenol, gaiacol), aromatic aldehydes (examples: cinnamaldehyde, cuminal and phellandral), and their alcohol, aldehyde or ester derivatives. (Brenes and Roura, 2010; Christaki et al., 2012).

Oleoresins are naturally occurring mixtures of essential oils and resins. They can be obtained from plants by extraction with a nonaqueous solvent (alcohol, ether, or acetone), followed by the removal of the solvent through evaporation. They contain volatile and non-volatile plant constituents (McCloud, 2010).

Botanicals can be produced from single plants or they can be mixtures from different plants. The idea of using mixtures is to exploit possible synergistic effects, so as to maximize the bioactive effects of different secondary metabolites and to minimize the concentrations required to achieve a particular effect (Kirkpinar et al., 2011).

The solvent of the extract also plays an important role in the activity of the botanical. Different solvents have variable physical properties such as polarity, which can affect the solubility and the activity of the plant metabolites, when the extract is ingested by the parasite or comes in contact with its surface (transcu-

ticular absorption), especially under *in vitro* experiments (Ahmad et al., 2013; Kaingu et al., 2013).

ACTIVE SUBSTANCES OF BOTANICALS WITH ANTHELMINTIC ACTIVITIES

As already mentioned, the active substances of botanicals are plant metabolites that are synthesized by plants throughout their life cycle. They are distinguished in primary and secondary metabolites (Hrcova and Velebny, 2013; Marin et al., 2015).

Plant primary metabolites such as carbohydrates, lipids, proteins and nucleic acids, are the main compounds of basic metabolic pathways and also precursors for the synthesis of the plant secondary metabolites (Hrcova and Velebny, 2013; Marin et al., 2015).

Plant secondary metabolites are organic compounds synthesized by plants that have important functions for the plant, mediating interaction with other plants or organisms; for example protection against microbial or insect attack or attraction of pollinators and seed-dispersing. Plant secondary

metabolites are often colored, fragrant or flavorful compounds. Based on their biosynthetic origin and chemical structure they be divided into three wide groups: terpenes (or terpenoids), phenolics (or phenols or phenolic compounds) and nitrogen-containing compounds (Hrcova and Velebny, 2013).

MODES OF ACTION OF BOTANICALS AGAINST HELMINTHES

Various modes of action have been suggested for the botanicals although it is possible that other mechanisms are not sufficiently identified yet.

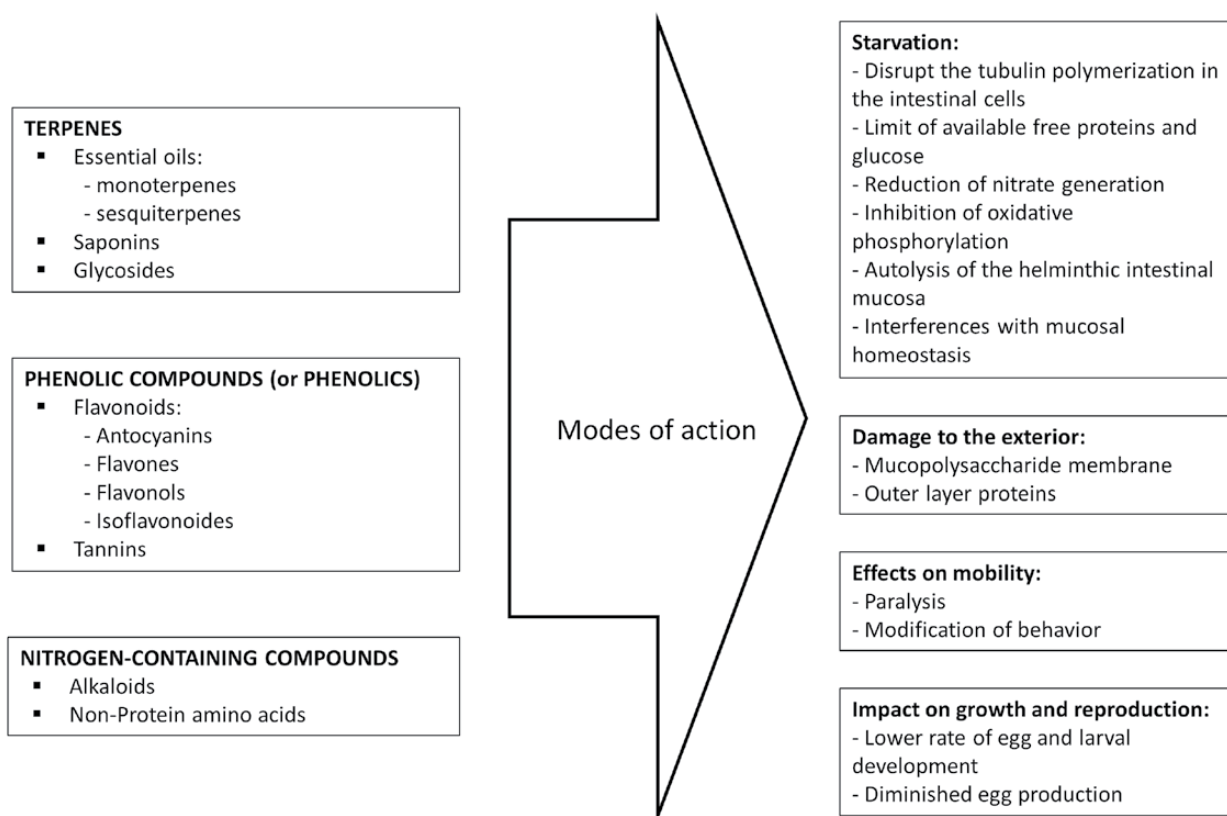
1. Antelmintic activity of plant secondary metabolites

An overview of plant secondary metabolites effects against helminthes is presented in Figure 1.

1.1. Starvation

Some monoterpenes, like ascaridole (from plant

Fig 1. Plant secondary metabolites and their modes of action against helminthes



Chenopodium ambrosioides), can disrupt the tubulin polymerization in the intestinal cells of the parasites, which leads to their degeneration and death (Wink, 2012; Jain et al., 2013). Ascaridole is effective against helminthes and has been used since the 1900s, but today its use has been limited, as it is considered mutagenic and poisonous. Also, some plant secondary metabolites separately or jointly can block glucose uptake by the parasite (Jain et al., 2013).

Tannins have the capacity to bind free protein in the digestive tract, thus limiting the nutrient availability and possibly resulting in larval starvation (Jain et al., 2013). Another possible action of tannins is the decrease in gastrointestinal metabolism directly through inhibition of oxidative phosphorylation, again leading to larval death (Athanasiadou et al., 2001; Kateregga et al., 2014). Tannins and flavonoids could inhibit energy production in the parasite cells by blocking phosphorylation reactions (Sharma and Prasad, 2014). Tannins may also bind to free protein in the gastrointestinal tract, as well as to proteins of the cuticle of parasite, thus limiting the ability to absorb nutrients. Condensed tannin ingested by the larvae of nematodes can bind to their intestinal mucosa and cause autolysis (Schultz, 1989; Athanasiadou et al., 2001).

Steroidal alkaloids and oligoglycosides can limit the amount of sugars, such as sucrose that reach the small intestine from the stomach, as well as affect the generation of nitrates (Borba et al., 2010). These effects modify the local conditions in the intestine, making them unfavorable for the development of intestinal parasites (Borba et al., 2010).

Likewise, saponins can restrict feed intake, limiting the available nutrients for the helminthes and possibly causing their death (Kateregga et al., 2014).

Also, it has been hypothesized that some plant extracts induce an inflammatory response in the gastric and intestinal mucosal of the host, which disrupts the local homeostasis that is necessary for the development of the helminthes (Borba et al., 2010). In addition, it has been suggested that some plant secondary metabolites play an important role in the regulation of the carbohydrate metabolism (Bazh and El-Bahy, 2013). In this case, it is speculated that the branch point of phosphoenolpyruvate carboxykinase/pyruvate kinase forms the basis of the anthelmintic

attack by the plant derived components (Bazh and El-Bahy, 2013).

1.2. Damage to the helminth cuticle

Any damage to the mucopolysaccharide membrane of the parasite results in movement restriction and possibly in paralysis (Chandrashekhhar et al., 2008; Jain et al., 2013). The binding effect of tannins on the cuticle results in the loss of its flexibility due to hydrogen bonding. This type of reactivity may lead to increased toughness of the cuticle and hence the helminthes become immobile and nonfunctional. Larvae are then unable to burrow into the mucosal lining of the small intestine of the host, and are subsequently eliminated from the host (Salhan et al., 2011; Jain et al., 2013).

1.3. Effects on mobility

Alkaloids may act on central nervous system of the parasite and cause paralysis of helminthes (Roy et al., 2010; Jain et al., 2013). Alkaloids pelletierine from *Punica granatum* (Lythraceae) and arecoline from *Areca catechu* (Arecaceae), target acetylcholine receptors as competitive antagonists and can cause paralysis of the helminthes (Wink, 2012). The anthelmintic activity of alkaloids has been demonstrated in two rat nematodes; *Strongyloides ratti* and *Strongyloides venezuelensis* (Kateregga et al., 2014). Paralyzed parasites in the digestive tract are unable to remain adhered on the intestinal wall of the host and are removed through peristalsis. It is also possible that these parasites starve to death (Wink, 2012).

Some terpenes, such as thymol and carvacrol, are neurotoxic to nematodes and interact with Ser-2, a *Caenorhabditis elegans* tyramine receptor (Lei et al., 2010; Kaplan et al., 2014). In nematodes, tyramine is believed to play a role in foraging behavior and pharyngeal pumping (Rex et al., 2004). Tyramine is a nonpeptidic hormone that can only be found in invertebrates and thus it is considered an important substance in antiparasitic research (Klowden, 2007).

Phloroglucinols, such as aspidin, deaspidin, and filixic acid, which can be found in *Dryopteris filix-mas* (*Dryopteridaceae*) can paralyze helminthes, and this effect is more pronounced on cestodes (Murthy et al., 2011; Wink, 2012).

1.4. Impact on growth and reproduction

It is possible that, tannins directly or indirectly reduce the number of hatching eggs, the rate of larvae development and the number of eggs produced from adult parasites (Athanasiadou et al., 2001; Van Krimpen et al., 2010), via not sufficiently identified mechanisms. It has been proposed that substances with hormonal effect, such as triterpenes, disrupt the reproductive cycle of the parasite. These effects have been investigated for some plants, for example for the genus *Artemisia*, against the nematodes *Ascaris suum* of pigs, as well as *Toxocara* spp. of carnivores and the cestodes *Moniezia* spp. of ruminants (Githiori, 2004; Van Krimpen et al., 2010; Acton, 2012).

It is possible that the secondary metabolites affect helminthes variously depending on different stages of their development. Also, a main factor that contributes to the anthelmintic activity of the secondary metabolites is the conditions in the digestive tract. For example, the formation and disassociation of complexes between proteins and tannins is greatly affected by the *pH*. Complexes between condensed tannins and protein remain stable in *pH* between 5 and 7, but they disassociate in *pH* higher or lower than the above (Athanasiadou et al., 2001). Also, the presence of surfactants, such as bile acids, has been reported to be important for the disassociation of tannin-protein complexes.

Further research is required in order to identify additional modes of action of botanicals against helminthes of poultry, as well as the possible interactions between major and minor bioactive components.

2. Additional beneficial activities of plant secondary metabolites

Botanicals can have additional effects that benefit poultry health and performance such as antimicrobial activity (Christaki et al., 2012), immunomodulatory effects (Anthony et al., 2005), antioxidant activity (Christaki et al., 2012), anti-inflammatory properties (Borba et al., 2010) and appetite and digestion enhancing effects (Borba et al., 2010).

IN VIVO AND IN VITRO STUDIES

Several up-to-date studies have demonstrated the anthelmintic efficacy of different botanicals and most

of them evaluate the effect of plants against *A. galli* in chicken (*Gallus gallus domesticus*). It has been reported that botanicals exert similar anthelmintic activity both *in vivo* and *in vitro*, usually in a concentration and time-dependent manner (Alawa et al., 2003; Adedapo et al., 2007). In a number of studies, the anthelmintic effects of botanicals against *A. galli* in poultry were compared to conventional antiparasitic drugs, such as albendazole, levamisole, piperazine, etc, suggesting that botanicals can partially or totally substitute those reference drugs (Akhtar and Riffat 1985, Al-Harbi 2011, Bazh and El-Bahy 2013). It is notable that in most cases the efficacy of botanicals was adequate, although not up to par, with the anthelmintic drugs. Since testing biological activity under *in vivo* conditions has several difficulties, such as the inherent features of animals and self-cure phenomenon, most of the studies refer to *in vitro* screenings of the anthelmintic efficacy of different botanicals (Sandoval-Castro et al., 2012).

1. In vivo studies

Under *in vivo* studies when botanicals were supplemented, either in the water or in the feed, they depressed the fecal egg count of *Ascaridia galli* as well as reduced the adult worm burden in parasitized poultry (Table 1).

In a study with Lohmann Leghorn chicks a mixture of ethanol extracts from orange (*Citrus x sinensis*), lemon (*Citrus x lemon*), and mandarin (*Citrus reticulata*), was added in the feed (at 300, 600 or 1200 mg/kg of b.w.) and a significant dose dependent reduction in fecal egg output and parasitic worm burden was recorded (Abdelqader et al., 2012). Moreover, *Melia azedarach* fruit (powder at 20 mg/kg of b.w. or equivalent amounts of water extract, methanol extract or ethanol extract) were found to inhibit *A. galli* egg development in chickens infected with the parasite (Akhtar and Riffat, 1985). Likewise, it was shown that *A. galli* challenged cockerels exhibited a dose dependent reduction in fecal egg count, when treated with graded doses of ethanolic extract (100, 200 and 400 mg / kg b.w.) from the bark of *Piliostigma thonningii* (Asuzu and Onu, 1994). In addition, it has been demonstrated that *Caesalpinia crista*, known as karanjwa, when administered to broilers as seed powder (at 30, 40, and 50 mg/kg of b.w. or as equivalent

Table 1. *In vivo* anthelmintic activities of various botanicals against *Ascaridia galli*.

Plant name	Plant part used	Examined form and dosage	Source of <i>A. galli</i> worms	Effect against <i>A. galli</i>	References
<i>Citrus x sinensis</i> <i>Citrus x lemon</i> <i>Citrus reticulata</i>	Peels Peels Peels	Dietary addition of mixtures at 300, 600 and 1200 mg/kg of body weight	Chickens	Fecal egg reduction; Worm motility inhibition	(Abdelqader et al., 2012)
<i>Melia azedarach</i>	Fruit	Fruit powder at 20 mg/kg; Equivalent water extract, methanol extract, ethanol extract	Chickens	Egg development inhibition	(Akhtar and Riffat, 1985)
<i>Piliostigma thonningii</i>	Bark	Ethanol extracts at 100, 200 and 400 mg / kg body weight	Chickens	Fecal egg reduction	(Asuzu and Onu, 1994)
<i>Caesalpinia crista</i>	Seeds	Powder and methanolic extracts at 30, 40 and 50 mg/kg body weight	Chickens	Fecal egg reduction	(Javed et al., 1994)
<i>Tephrosia vogelli</i> <i>Vernonia amygdalina</i>	Leaves Leaves	Water extracts. Doses not mentioned	Chickens	Fecal egg reduction; Reduction of adult worms population	(Siamba et al., 2007)
<i>Punica granatum</i>	Peel	Dry peel at 0.5, 1.0 and 1.5 g/kg of body weight	Laying hens	Fecal egg reduction Increased packed cell volume, total serum proteins, body weight	(Sabri, 2013)
<i>Azadirachta indica</i>	Leaves	Aqueous extract at 200 mg / kg of body weight	Chickens	Increased bird body weight; Parasite death	(Khokon et al., 2014)

amounts of water and methanol extracts) reduced *A. galli* egg numbers in chicken faeces, analogously to piperazine (Javed et al., 1994). Also, the extracts of *Tephrosia vogelli* and *Vernonia amygdalina* (doses not mentioned) not only significantly depressed fecal egg output, but also reduced the adult worm population in *A. galli* parasitized poultry (Siamba et al., 2007). Another study reported that *Punica granatum* dry peel, orally administered to infected hens at 0.5, 1.0 and 1.5 g/kg of b.w., reduced fecal egg count, analogously to levamisole, while increasing hematocrit (packed cell volume, PCV), total serum proteins and body weight (Sabri, 2013). Furthermore, neem (*Azadirachta indica*) leaves extract was supplemented to chickens at 200 mg/kg of b.w., leading to a significant increase in body weight (Khokon et al., 2014).

2. *In vitro* studies

Researchers have evaluated different plant extracts under *in vitro* conditions against *A. galli* collected from freshly slaughtered poultry. The commonly observed anthelmintic effects included paralysis and death, as well as inhibition of egg and larvae development (Table 2).

Bazh and El-Bahy (2013) revealed that when living worms were incubated at 37 °C in media containing ginger (*Zingiber officinale*) and curcumin (*Curcuma longa* L) methanolic extracts at three concentration levels (25, 50 and 100 mg/ml), their physical activity (spontaneous movement) as well as their survival was reduced in a concentration and time dependent manner. Likewise, Lal et al. (1976) demonstrated

that extracts from *Carica papaya* seeds (alcohol extract at 25 mg/ml), *Sapindus trifoliatum* fruit pericarp (alcohol extract at 10 mg/ml), *Butea frondosa* seeds (alcohol extract at 200 mg/ml) and *Momordica charantia* fresh juice (alcohol extract at 100 mg/ml) caused paralysis and death of *A. galli* procured from fowls. Additionally, crude alcohol and aqueous extracts of seeds of *Cleome viscosa* exhibited considerable dose-dependent antiparasitic results (10, 50, 100 mg/ml) against *A. galli* worms (Mali et al., 2007). Kosalge and Fursule (2009) recorded the paralysis and death of *A. galli* and *Raillietina* spp. when administering the aqueous extract of *Thespesia lampas* roots at concentrations of 10, 20 and 50 mg/ml, and thus proposed that this extract can be used effectively as an anthelmintic. Another similar study showed that the methanolic extract (10, 25, 50 mg/ml) of *Cymbopogon citratus* leaves displayed better antihelmintic efficacy against *A. galli* in terms of paralysis and death, than the aqueous extract (10, 25, 50 mg/ml) of the same plant (Gore et al., 2010). Al-Harbi (2011) comparing aqueous solution of dried *Artemisia absinthium* leaves and powder suspension of dried *Lepidium sativum* seeds with levamisole against *A. galli*, found that all of them have the same effectiveness against this parasite, causing paralysis and subsequently death after a period of exposure.

Kundu et al. (2012) revealed the broad wormicidal (paralysis and death) *in vitro* activity of *Cassia alata*, *Cassia angustifolia* and *Cassia occidentalis* (crude ethanol extracts at 10, 20 and 40 mg/ml) against various parasites (trematode *Catantropis* spp., cestode *Raillietina tetragona* and nematode *H. gallinarum*) collected from domestic fowl. The observed effects could be attributed to the large amount of alkaloids, flavonoids, glycosides, tannins that these plants are known to contain (Hossain et al., 2012). Another study revealed similar effects for the ethanolic extract of the leaves of *Eupatorium triplinerve* (50 and 100 mg/ml) and of the rhizome of *Alpinia galangal* (100 mg/ml) against *A. galli*. The activity of these plants was comparable to albendazole and can be attributed for *A. galangal* to the many flavonoids that it contains, such as kaempferide, kaempferol, galangin and alpinin, whereas for the crude extracts of *E. triplinerve* to the presence of phenolic compounds and coumarins (Charles et al., 1992;

Subash et al., 2012). Likewise, it was demonstrated that various concentrations of crude hydroalcoholic extracts (25 and 50 mg/ml) and aqueous extract (50 mg/ml) from *Mentha longifolia* leaves resulted in concentration and time dependent paralysis and death of *A. galli* (Ahmad et al., 2013). Also, aqueous (25, 50, 100 mg/ml) and ethanol extracts (10, 25, 50 mg/ml) of *Azadirachta indica* leaves, *Carica papaya* seeds and *Momordica charantia* bark, caused cessation of motility and increased mortality of *A. galli* (Shah Alam et al., 2014), comparable to the effects of levamisole and piperazine. Moreover, it has been reported that the extracts of *Tephrosia vogelli* and *Vernonia amygdalina* (doses not mentioned) can inhibit larvae mobility (Siamba et al., 2007).

In another study, *A. galli* treated with different concentrations (5, 10, 20 mg/ml) of *Acacia oxyphylla* methanol extract demonstrated extensive structural alterations, such as rupture of the ovaries and deformity on the egg membranes, detachment of the cuticle, disintegration of the muscular layers of the nematode and subsequent death (Lalchhandama, 2008). The wormicidal *in vitro* potential of leaves and fruits of aqueous extracts of *Sesbania grandiflora* and *Solanum torvum* (10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, and 130 mg/ 100 ml) was proven against *A. galli* from laying hens (Jothi Karumari et al., 2014). Furthermore, Kateregga et al. (2014) revealed that the use of *Cassia occidentalis* methanolic leaf extract (8, 12, 16, 20 and 24 mg/ml) caused the death of *H. gallinarum* and *A. galli*. The main phytochemicals of *C. occidentalis* leaves are saponins, flavonoids, terpenes, sterols, alkaloids and tannins (Muyibi et al., 2000). Likewise, another study demonstrated that a mixture of ethanol extracts from orange (*Citrus x sinensis*), lemon (*Citrus x lemon*), and mandarin (*Citrus reticulata*) at 50 mg/ml has potential wormicidal properties against *A. galli* *in vitro* (Abdelqader et al., 2012). Similarly, neem (*Azadirachta indica*) leaves aqueous extract, in a range of concentrations (1, 2, 4 and 20 mg/ml), presented sufficient anthelmintic effect, causing death of *A. galli* worms (Khokon et al., 2014). In addition, Kaushik et al. (1981) evaluated 11 plants extracts (*Amomum aromaticum* root and rhizome, *Ammora wallichii* stem, *Anthocephalus indicus* stem and bark, *Calamintha umbrosa* plant, *Dalbergia latifolia* stem

Table 2. *In vitro* anthelmintic activity of various botanicals against *Ascaridia galli* from freshly slaughtered poultry.

Plant name	Plant part used	Examined form and dosage	Effect against <i>A. galli</i>	References
<i>Zingiber officinale</i>	Root	Methanolic extracts at 25, 50 and 100 mg/ml.	Paralysis; Death	(Bazh and El-Bahy, 2013)
<i>Curcuma longa</i> L.	Root			
<i>Carica papaya</i> , <i>Sapindus trifoliatum</i> <i>Butea frondosa</i>	Seeds Fruit pericarp Seeds	Alcohol extract at 25 mg/ml Alcohol extract at 10 mg/ml Alcohol extract at 200 mg/ml	Paralysis; Death	(Lal et al., 1976)
<i>Momordica charantia</i>	Fresh juice	Alcohol extract at 100 mg/ml		
<i>Cleome viscosa</i>	Seeds	Crude alcohol and aqueous extracts at 10, 50 and 100 mg/ml	Paralysis; Death	(Mali et al., 2007)
<i>Thespesia lampas</i>	Roots	Aqueous extracts at 10, 20 and 50 mg/ml.	Paralysis; Death	(Kosalge and Fursule, 2009)
<i>Cymbopogon citrates</i>	Leaves	Methanolic and aqueous extracts 10, 25 and 50 mg/ml	Paralysis; Death	(Gore et al., 2010)
<i>Artemisia absinthium</i> <i>Lepidium satirium</i>	Leaves Seeds	Aqueous solution and powdered suspension. Dose not mentioned	Paralysis; Death	(Al-Harbi, 2011)
<i>Cassia alata</i> <i>Cassia angustifolia</i> <i>Cassia occidentalis</i>	Leaves	Crude ethanol extracts at 10, 20 and 40 mg/ml	Paralysis; Death	
<i>Eupatorium triplinerve</i> <i>Alpinia galangal</i>	Leaves Rhizome	Ethanol extracts at 50 and 100 mg/ml	Paralysis; Death	(Subash et al., 2012)
<i>Mentha longifolia</i>	Leaves	Crude hydroalcoholic extracts at 25 and 50 mg/ml; Crude aqueous extract at 50 mg/ml	Paralysis; Death	
<i>Azadirachta indica</i> <i>Carica papaya</i> <i>Momordica charantia</i>	Leaves Seeds Bark	Aqueous extracts at 25, 50 and 100 mg/ml; Ethanol extracts at 10, 25 and 50 mg/ml	Paralysis; Death	(Shah Alam et al., 2014)
<i>Tephrosia vogelli</i> <i>Vernonia amygdalina</i>	Leaves Leaves	Water extracts. Doses not mentioned	Paralysis	
<i>Acacia oxyphylla</i>	Bark	Ethanol extracts at 5, 10 and 20 mg/ml	Body structural alterations; Death	(Lalchhandama, 2008)
<i>Sesbania grandiflora</i> <i>Solanum torrum</i>	Leaves, fruit Leaves, fruit	Aqueous extracts at 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120 and 130 mg/ 100 ml	Death	(Jothi Karumari et al., 2014)
<i>Cassia occidentalis</i>	Leaves	Methanolic extracts at 8, 12, 16, 20 and 24 mg/ml	Death	
<i>Citrus x sinensis</i> <i>Citrus x lemon</i> <i>Citrus reticulata</i>	Peels Peels Peels	Mixture of ethanol extracts of the three plants at 50 mg/ml	Death	(Abdelqader et al., 2012)
<i>Azadirachta indica</i>	Leaves	Aqueous extracts at 1, 2, 4 and 20 mg/ml.	Death	
<i>Amomum aromaticum</i> <i>Ammora wallichii</i> <i>Anthocephalus indicus</i> <i>Calamintha umberosa</i> , <i>Dalbergia latifolia</i> <i>Datura quercifolia</i> <i>Datura metel</i> <i>Ficus religiosa</i> <i>Sentia myrtina</i> <i>Sumplocos crataegoides</i>	Root, rhizome Stem Stem, bark Plant Stem, bark Fruit Plant Stem, bark Plant Leaves	—	Death	(Kaushik et al., 1981)
<i>Azadirachta indica</i> <i>Carica papaya</i> <i>Momordica charantia</i> <i>Polygonum hydropiper</i> <i>Swietenia macrophylla</i>	Leaves Leaves Leaves Leaves Leaves	Fresh juice at 5%, 10% and 20%; Aqueous extract at 1%, 2% and 4%; Ethanol extract at 1%, 2% and 4%; Methanol extract at 1%, 2% and 4%; Powder at 10% and 20%	Inhibition of egg development	
<i>Aloe secundiflora</i>	Leaves	Hexane, ethylacetate, acetone, methanol, and chloroform extracts at 5, 10, 20, 40 and 50 mg/ml	Inhibition of larvae development	(Kaingu et al., 2013)

and bark, *Datura quercifolia* fruit, *Datura metel* plant, *Ficus religiosa* stem and bark, *Sentia myrtina* plant, and *Sumplocos crataegoides* leaves) which were all proven lethal to *A. galli*.

Some *in vitro* studies reported other anthelmintic effects of botanicals against *A. galli*, such as inhibition of egg and larvae development. Particularly, fresh juice (at 5, 10 and 20%), aqueous extract (at 1, 2 and 4%), ethanol extract (at 1, 2 and 4%), methanol extract (at 1, 2 and 4%), and powder (at 10 and 20%) of leaves of *Azadirachta indica*, *Carica papaya*, *Momordica charantia*, *Polygonum hydropiper*, and *Swietenia macrophylla* inhibited the development of *A. galli* eggs, with *C. papaya* showing the highest efficacy 92% when the concentration was 4% (Islam et al., 2008). Another experiment using larvae development assays of *A. galli* revealed that different types of extracts (hexane; ethylacetate; acetone; methanol; chloroform) of *Aloe secundiflora* at 5, 10, 20, 40 and 50 mg/ml have inhibitory effects on the parasite's development (Kaingu et al., 2013).

CONSTRAINTS OF USING BOTANICALS AS ANTHELMINTIC AGENTS

One important problem with botanicals is the difficulty to characterize and standardize their ingredients and composition. Many factors can influence the chemical composition of the plants, such as species, subspecies, geographical location, harvesting and the collected part, such as seeds, leaf, root or bark (Christaki et al., 2012; Bozkurt et al., 2013). Also, the processing technique (cold expression, steam

or alcohol distillation, extraction with non-aqueous solvents, etc.) can modify the active substances and associated compounds in the final product (Tariq et al., 2009; Windisch et al., 2009).

Another important consideration is that botanicals may also have adverse or toxic side effects for the treated animals. It has been demonstrated that plant substances, which interfere with parasite development, such as steroidal alkaloids, may also exhibit toxic effects on animal tissues. These effects include mutagenicity, embryotoxicity, hepatotoxicity, central nervous system symptoms, cardiac arrhythmia, etc (European Food Safety Authority, 2009a; Borba et al., 2010). Therefore, further studies are required to evaluate plant substances with possible detrimental effects for the animal, as well as to carefully quantify the optimal beneficial doses, versus the potential harmful ones (Wu et al., 2004; Bozkurt et al., 2013). Additionally, residue studies should be required before botanicals can be safely integrated in poultry management system.

CONCLUSION

The diversity of botanicals provides an important source of bioactive compounds, which may lead to potential new candidates remedies of natural origin against ascaridiosis of chickens. Keeping in view the economic importance of the parasitic infections in the development of profitable poultry industry, the anthelmintic properties of botanicals represent a very promising alternative solution to overcome current treatment inadequacies. ■

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