Botanicals: a natural approach to control ascaridiosis in poultry

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Botanicals: a natural approach to control ascaridiosis in poultry

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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-
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 droppiness, 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).

**Keywords**: Helminthes, *Ascaridia galli*, Botanicals, Poultry.
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 phytagenic 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 phytagenics 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, geranial, 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. Antelminthic 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

**TERPENES**
- Essential oils:
  - Monoterpenes
  - Sesquiterpenes
- Saponins
- Glycosides

**PHENOLIC COMPOUNDS (or PHENOLCS)**
- Flavonoids:
  - Antocyanins
  - Flavones
  - Flavonols
  - Isoflavonoids
- Tannins

**NITROGEN-CONTAINING COMPOUNDS**
- Alkaloids
- Non-Protein amino acids

**Modes of action**

- **Starvation:**
  - Disrupt the tubulin polymerization in the intestinal cells
  - Limit of available free proteins and glucose
  - Reduction of nitrate generation
  - Inhibition of oxidative phosphorylation
  - Autolysis of the helminthic intestinal mucosa
  - Interferences with mucosal homeostasis

- **Damage to the exterior:**
  - Mucopolysaccharide membrane
  - Outer layer proteins

- **Effects on mobility:**
  - Paralysis
  - Modification of behavior

- **Impact on growth and reproduction:**
  - Lower rate of egg and larval development
  - Diminished egg production
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 (Chandrashekhar 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 larval 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 (Borbà et al., 2010) and appetite and digestion enhancing effects (Borbà 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 Rifat 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).

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 Rifat, 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 cristata*, known as karanjwa, when administered to broilers as seed powder (at 30, 40, and 50 mg/kg of b.w. or as equivalent
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 larval 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*) 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 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 hemocrit (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).

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

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

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).
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 Ralilietina 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 anthelmintic 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 Catatropis spp., cestode Ralilietina 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 rhisome of Alpinia galangal (100 mg/ml) and of the rhisome of 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 piperazone. Moreover, it has been reported that the extracts of Tephrosia vogelii 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 oxypylla 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 Sesanbiana 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 Karumati 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 rhisome, Ammora vallichii 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.

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Plant part used</th>
<th>Examined form and dosage</th>
<th>Effect against <em>A. galli</em></th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zingiber officinalis</td>
<td>Root</td>
<td>Methanolic extracts at 25, 50 and 100 mg/ml</td>
<td>Paralysis; Death</td>
<td>(Bazh and El-Bahy, 2013)</td>
</tr>
<tr>
<td>Curcuma longa L.</td>
<td>Root</td>
<td>Alcohol extract at 25 mg/ml</td>
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<tr>
<td>Carica papaya,</td>
<td>Seeds</td>
<td>Alcohol extract at 10 mg/ml</td>
<td>Paralysis; Death</td>
<td>(Lal et al., 1976)</td>
</tr>
<tr>
<td>Sapindus trifoliatum</td>
<td>Seeds</td>
<td>Alcohol extract at 200 mg/ml</td>
<td></td>
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<tr>
<td>Butea frondosa</td>
<td>Fresh juice</td>
<td>Alcohol extract at 100 mg/ml</td>
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<tr>
<td>Momordica charantia</td>
<td>Seeds</td>
<td>Crude alcohol and aqueous extracts at 10, 50 and 100 mg/ml</td>
<td>Paralysis; Death</td>
<td>(Mali et al., 2007)</td>
</tr>
<tr>
<td>Cleome viscosa</td>
<td>Seeds</td>
<td>Alcohol extract at 10 mg/ml</td>
<td>Paralysis; Death</td>
<td>(Kosalige and Fursule, 2009)</td>
</tr>
<tr>
<td>Thepesia lampas</td>
<td>Roots</td>
<td>Aqueous extracts at 10, 20 and 50 mg/ml</td>
<td>Paralysis; Death</td>
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</tr>
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<td>Cymbopogon citrates</td>
<td>Leaves</td>
<td>Methanolic and aqueous extracts 10, 25 and 50 mg/ml</td>
<td>Paralysis; Death</td>
<td>(Gore et al., 2010)</td>
</tr>
<tr>
<td>Artemisia absinthium</td>
<td>Leaves</td>
<td>Aqueous solution and powdered suspension. Dose not mentioned</td>
<td>Paralysis; Death</td>
<td></td>
</tr>
<tr>
<td>Lepidium sativum</td>
<td>Seeds</td>
<td>Alcohol extract at 25 mg/ml</td>
<td>Paralysis; Death</td>
<td></td>
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<tr>
<td>Cassia alta</td>
<td>Leaves</td>
<td>Crude ethanol extracts at 10, 20 and 40 mg/ml</td>
<td>Paralysis; Death</td>
<td>(Kundu et al., 2012)</td>
</tr>
<tr>
<td>Cassia angustifolia</td>
<td>Leaves</td>
<td>Ethanol extract at 50 and 100 mg/ml</td>
<td>Paralysis; Death</td>
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<tr>
<td>Cassia occidentalis</td>
<td>Leaves</td>
<td>Crude hydroalcoholic extracts at 25 and 50 mg/ml</td>
<td>Paralysis; Death</td>
<td></td>
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<td>Eupatorium triplinerve</td>
<td>Leaves</td>
<td>Ethanol extracts at 50 and 100 mg/ml</td>
<td>Paralysis; Death</td>
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<td>Alpinia galangal</td>
<td>Rhisome</td>
<td>Crude aqueous extract at 50 mg/ml</td>
<td>Paralysis; Death</td>
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<td>Mentha longifolia</td>
<td>Leaves</td>
<td>Paralysis; Death</td>
<td></td>
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<tr>
<td>Azadirachta indica</td>
<td>Leaves</td>
<td>Aqueous extracts at 25, 50 and 100 mg/ml</td>
<td>Paralysis; Death</td>
<td></td>
</tr>
<tr>
<td>Carica papaya</td>
<td>Seeds</td>
<td>Ethanol extracts at 10 and 25 mg/ml</td>
<td>Paralysis; Death</td>
<td></td>
</tr>
<tr>
<td>Momordica charantia</td>
<td>Bark</td>
<td>Alcohol extract at 10, 25 and 50 mg/ml</td>
<td>Paralysis; Death</td>
<td></td>
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<tr>
<td>Tephrosia vogelli</td>
<td>Leaves</td>
<td>Water extracts. Doses not mentioned</td>
<td>Paralysis; Death</td>
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</tr>
<tr>
<td>Vernonia amygalaina</td>
<td>Leaves</td>
<td>Ethanol extracts at 5, 10 and 20 mg/ml</td>
<td>Body structural alterations; Death</td>
<td></td>
</tr>
<tr>
<td>Acacia oxyphylla</td>
<td>Bark</td>
<td>Aqueous extracts at 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120 and 130 mg/100 ml</td>
<td>Death</td>
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<tr>
<td>Sesbania grandiflora</td>
<td>Leaves, fruit</td>
<td>Aqueous extracts at 8, 12, 16, 20 and 24 mg/ml</td>
<td>Death</td>
<td></td>
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<tr>
<td>Solanum torrnon</td>
<td>Leaves, fruit</td>
<td>Mixture of ethanol extracts of the three plants at 50 mg/ml</td>
<td>Death</td>
<td></td>
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<tr>
<td>Cassia occidentalis</td>
<td>Leaves</td>
<td>Death</td>
<td>(Jothi Karumari et al., 2014)</td>
<td></td>
</tr>
<tr>
<td>Citrus x sinensis</td>
<td>Peels</td>
<td>Death</td>
<td>(Kaushik et al., 1981)</td>
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<tr>
<td>Citrus x lemon</td>
<td>Peels</td>
<td>Death</td>
<td>(Katerregga et al., 2014)</td>
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<tr>
<td>Citrus reticulata</td>
<td>Peels</td>
<td>Death</td>
<td>(Abdelqader et al., 2012)</td>
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<tr>
<td>Azadirachta indica</td>
<td>Leaves</td>
<td>Death</td>
<td>(Khokon et al., 2014)</td>
<td></td>
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<tr>
<td>Amomum aromaticum</td>
<td>Root, rhizome</td>
<td>Death</td>
<td>(Islam et al., 2008)</td>
<td></td>
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<tr>
<td>Amomora wallichii</td>
<td>Stem, bark</td>
<td>Death</td>
<td>(Islam et al., 2008)</td>
<td></td>
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<tr>
<td>Anchorephalus indicus</td>
<td>Plant</td>
<td>Death</td>
<td>(Islam et al., 2008)</td>
<td></td>
</tr>
<tr>
<td>Calamintha umbrosa,</td>
<td>Stem, bark</td>
<td>Death</td>
<td>(Islam et al., 2008)</td>
<td></td>
</tr>
<tr>
<td>Dalbergia latifolia</td>
<td>Plant</td>
<td>Death</td>
<td>(Islam et al., 2008)</td>
<td></td>
</tr>
<tr>
<td>Datura metel</td>
<td>Plant</td>
<td>Death</td>
<td>(Islam et al., 2008)</td>
<td></td>
</tr>
<tr>
<td>Ficus religiosa</td>
<td>Stem, bark</td>
<td>Death</td>
<td>(Islam et al., 2008)</td>
<td></td>
</tr>
<tr>
<td>Senitia myrtina</td>
<td>Plant</td>
<td>Death</td>
<td>(Islam et al., 2008)</td>
<td></td>
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<tr>
<td>Samplocos crataegoides</td>
<td>Leaves</td>
<td>Death</td>
<td>(Islam et al., 2008)</td>
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<tr>
<td>Azadirachta indica</td>
<td>Leaves</td>
<td>Death</td>
<td>(Islam et al., 2008)</td>
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<tr>
<td>Carica papaya</td>
<td>Leaves</td>
<td>Death</td>
<td>(Islam et al., 2008)</td>
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<tr>
<td>Momordica charantia</td>
<td>Leaves</td>
<td>Death</td>
<td>(Islam et al., 2008)</td>
<td></td>
</tr>
<tr>
<td>Polygonum hydropiper</td>
<td>Leaves</td>
<td>Death</td>
<td>(Islam et al., 2008)</td>
<td></td>
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<tr>
<td>Sutewia macrophylla</td>
<td>Leaves</td>
<td>Death</td>
<td>(Islam et al., 2008)</td>
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<tr>
<td>Aloe secundiflora</td>
<td>Leaves</td>
<td>Death</td>
<td>(Islam et al., 2008)</td>
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</table>
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 larval development assays of *A. galli* revealed that different types of extracts (hexane; ethyl acetate; 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 systems.

**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.


