Research Article

In Vitro Efficacy of Medicinal Plant Material on the Inhibition of Development of Egg of *Ascaridia galli*

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Abstract | To determine the *in vitro* efficacy of indigenous plants on the inhibition of development of eggs of *Ascaridia galli*, a study was carried out in the laboratory of Department of Parasitology from January to May, 2012. Leaves of five plants namely pineapple (*Ananas comosus*), turmeric (*Curcuma domestica*), akanda (*Calotropis gigantea*), garlic (*Allium sativum*) and custard apple (*Annona reticulata*) were selected. Two different types of preparation such as fresh juice and dust material were used. Fresh leaves juice at 5%, 10% and 20% concentration and dust of leaves at 5%, 10% and 20% concentration were used for screening. Among the selected plants in all three concentrations of fresh juice of leaves, pineapple was the highest effective plant (86.9%) at 20% concentration followed by pineapple at 10% concentration (84.0%), garlic at 5% concentration (81.8%), turmeric at 20% concentration (78.9%), pineapple at 5% concentration (78.9%), turmeric at 10% concentration (73.9%), garlic at 10% concentration (73.0%). Among the plants in all concentrations of dust of leaves, pineapple at 20% concentration (76.9%) was observed as the best plant followed by akanda at 5% concentration (75.0%) and turmeric at 20% concentration (73.3%). The present study suggests that pineapple, turmeric and garlic leaves are effective and can be used against the development of eggs of *A. galli*. So, further studies are required to determine the side effects of these plants and determine the recommended doses in poultry.

Keywords | *In vitro*, Efficacy, Medicinal plant, *Ascaridia galli*

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INTRODUCTION

Ascaridia galli is one of the most common parasitic roundworms of poultry that occurs in chickens and turkey (Permin, 1997; Soulsby, 1982; Anderson, 1992). Embryonated eggs of *A. galli* are very hardy and under laboratory conditions may live for two years in ordinary conditions. However, few probably live more than one year (Matter and Oester, 1989). Disinfectants and other cleaning agents do not kill eggs under farm conditions and chickens become infected by eating infective eggs containing L3 (Permin, 1997). Chemical anthelmintics have long been considered the only effective way of controlling this parasitic infection. Available drugs remove only the adult parasite and also some serious disadvantages of using manufactured drugs have become evident in the world, such as drug resistance, food residues and environmental pollution. A general stagnation in the development of conventional medicine has led to an increased need for research into alternative therapeutic agents for the treatment and control of helminth infections. Medicinal plants have been used to combat parasitism, and in many parts of the world are still used for this purpose (Athanasiadou et al., 2007). In ethno-veterinary medicine, which draws inspiration from traditional practice, there seems to be a range of plant/s or plant extract suitable for treating almost every parasitic disease of livestock and poultry.

It is estimated that more than 20,000 species of plants are used medicinally throughout the world for controlling

diverse disease (Jeyathilakan et al., 2012). Pineapple, turmeric, akanda, garlic and custard apple are the medicinal plants available in Bangladesh those have anthelmintic activity (Anthony et al., 2005; Kiuchi et al., 1993; Patra et al., 2010). The active compound of garlic is allicin which is an organosulfur compound (Miron et al., 2000). The pharmacological activity of turmeric has nematocidal (Kiuchi et al., 1993). Treatment with allicin from garlic is effective against *A. galli* in chicken (Velkers et al., 2011). *In vitro* and *in vivo* screening of plant materials as anthelmintic against adult *A. galli* has been done throughout the world with the aim of controlling *A. galli in* poultry. But eggs generally have a long survival rate in the environment, and thus a high infection potential. So, the present study will have a great importance in controlling *A. galli* in chickens, as this work will be a stand point to control *A. galli* by inhibiting the development of eggs using available indigenous plants and thus reducing the infection. Therefore, this research plan was designed with a view to evaluate the efficacy of some plant materials on the inhibition of development of eggs of *A. galli* eggs and also to compare the efficacy of the fresh juice and dust of leaves of candidate plants against development of *A. galli* eggs.

MATERIALS AND METHODS

The study was conducted from January May, 2012 in the laboratory of the Department of Parasitology, Bangladesh Agricultural University (BAU), Mymensingh.

Preparation of Plant Materials for Experiment

Selection of Plants Used in this Experiment

Five plants namely pineapple (*A. comosus*), turmeric (*C. domestica*), akanda (*C. gigantea*), garlic (*A. sativum*) and custard apple (*A. reticulata*) were selected on the basis of their ethnomedical uses for screening.

Processing of Leaves

After collection, leaves were separated from the plants and washed thoroughly in running tap water. The dust and juice of leaves used in this study were processed. For the preparation of fresh juice, the fresh leaves were cut into small pieces and water was added at 1:1 ratio in a kitchen blender. Then juice were made by blending the leaves for 2-3 minutes and stored in a refrigerator at 4°C to maintain the quality of active ingredients of juice. For the preparation of dust, the leaves were dried in the shade at room temperature and then they were dried in the oven at 55-60°C. The dried leaves were cut into small pieces and pulverized with a blender. A 25mm mesh diameter sieve was used to obtain the fine dust, after then dust was preserved in airtight plastic container until they were directly used in litter materials for screening and preparation of aqueous.

Collection and Maintenance of Eggs of *A. galli*

Collection of Eggs of *A. galli*

At first, intestines were collected from indigenous chickens slaughtered in the nearby market at BAU campus and brought to the laboratory. Then, adult *A. galli* were collected from small intestine following a standard method (Fowler, 1990). Female parasites were identified under microscope and separated from males. Eggs were recovered by grinding the female parasites with pestle and mortar by adding 5 ml PBS. These fresh eggs were used for the experiment.

Treatment of Egg-PBS Suspension with Plant Materials

Treatment of eggs with fresh leaves juice: Petri dishes were used for the treatment which were properly washed, dried and then labeled. PBS was used as media for this trial and 10 ml of total volume were made for each trial as in Table 1. Upper meniscus of total 10 ml volume of suspension in all petridishes was marked by permanent ink. Fresh leaves juices were used as 5%, 10% and 20% and for control, one petridish with egg-PBS suspension retained without treatment. The petridishes were kept at room temperature for 20 days. Petri dishes were placed at a large tray and moist cotton was used under the petri dishes to prevent desiccation. All petri dishes were covered in same manner; about half of the petri dishes were kept open to allow aeration for development of eggs. Continuous monitoring of petri dishes had been done and the upper meniscus of fluid was maintained by adding PBS if necessary.

Table 1: Making of fresh leaves juice concentrations for trial

Concentration (in %)	Volume (in ml)			
	Fresh leaves Juice	PBS	Egg-PBS suspension	Total
Control (0)	0	9	1	10
5	0.5	8.5	1	10
10	1	8	1	10
20	2	7	1	10

Treatment of litter with dust: Dust of each plant leaves were used at 10% and 20% concentrations. For making different preparations, 8g, 9g and 9.5g, 5% of litter were mixed with 2g, 1g and 0.5g of dust for 20%, 10% and 0.5% concentration respectively. For each trial, total 10g of litter-dust mixture were kept in the medium sized petri dishes and 1 ml of egg-PBS suspensions were sprayed on each petri dish. For the control, only 10g of litter were sprayed with 1 ml of egg-PBS suspension. All petri dishes were kept at room temperature for 20 days and after then water was added with the mixture and sieved to remove litter. The filtrate was allowed to stand

for 30 minutes for sedimentation of eggs. The supernatant was poured off and the sediment was washed for several times to make it clear. Finally the filtrate was centrifuged at 1500 rpm for five minutes and the sediment was taken on a clear slide to examine the eggs under microscope.

Examination of eggs for development of larvae: Fresh juice and dust treated eggs were examined at 10th day, 15th day and 20th day for the development of larvae within the eggs. Developed eggs were identified with the presence of larvae within egg and the movement of larvae. Both fresh/undeveloped and treated eggs were identified under microscope with high power objectives.

Determination of efficacy of plants: *In vitro* screening of fresh leaves juice and dust of leaves of selected plants on the inhibition of development of *A. galli* eggs were done for their efficacy; and the plant/preparations were considered as effective having at least 70% efficacy.

Statistical Analysis

In vitro effects of different preparations of plant leaves were statistically analyzed with ANOVA technique to obtain the level of significance using MSTAT-C package programme developed by Russell (1986). The mean differences were compared by /Duncan's New Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

RESULTS

Among the five selected plant, pineapple was the highest efficacious plant against the development of *A. galli* eggs at 20% concentration (86.9%) of fresh juice of leaves followed by pineapple at 10% concentration (84.0%), garlic at 5% concentration (81.8%), turmeric at 20% concentration (78.9%) (Table 2). From the present study, it is found that fresh leaves juice was more efficacious than the dust of leaves.

In vitro Screening of Fresh Juice of Leaves

Efficacy of Fresh Leaves Juice at 5% Concentrations

During *in vitro* screening of fresh leaves juice of five selected plants at 5% concentration, the highest efficacy in terms of inhibition of development of growth of larvae was found in garlic (81.8%) followed by pineapple (78.9%), turmeric (70.0%), akanda (69.2%) and custard apple (58.3%) (Table 2).

Efficacy of Fresh Leaves Juice at 10% Concentration

In case of investigation of efficacy of fresh leaves juice at 10% concentration, pineapple was found as most efficacious plant (84.0%) followed by turmeric (73.9%), akanda (73.0%), custard apple (71.4%) and garlic (63.1%) (Table 2).

Efficacy of Fresh Leaves Juice at 20% Concentration

At 20% concentration of fresh leaves juice, highest efficacy was found in pineapple (86.9%) followed by turmeric (78.9%), custard apple (72.0%), akanda (66.6%) and garlic (65.0%) (Table 2).

Overall Performance of Fresh Leaves Juice

Among the selected plants and in all three concentrations of fresh juice of leaves, pineapple was the highest efficacious plant against the development of *A. galli* eggs. Papaya was the second highest followed by akanda and garlic, whereas custard apple was the least efficacious. The efficacy of fresh juice of leaves of plants can be presented chronologically as: pineapple at 20% concentration (86.9%) > pineapple at 10% concentration (84.0%) > garlic at 5% concentration (81.8%) > turmeric at 20% concentration (78.9%) > pineapple at 5% concentration (78.9%) > turmeric at 10% concentration (73.9%) >garlic at 10% concentration (73.0%).

In vitro Screening of Dust of Leaves

Efficacy of Dust at 5% Concentration

At 5% concentration of dust of leaves, the highest efficacy was observed in akanda (75.0%), followed by turmeric (73.3%), pineapple (70.5%), garlic (64.2%) and custard apple (57.1%) (Table 3).

Efficacy of Dust at 10% Concentration

At 10% concentration of dust of leaves, the highest efficacy was observed in pineapple and turmeric (71.4%), followed by akanda (60.0%), custard apple (53.8%) and garlic (50.0%) (Table 3).

Efficacy of Dust at 20% Concentration

In case of dust preparation of leaves at 20% concentration, the highest efficacy was found in the pineapple (76.9%), followed by turmeric (73.3%), akanda 69.2%), garlic (66.6%) and custard apple (53.3%) (Table 3).

Overall Efficacy of Dust of Leaves

Among the plants, in all concentrations of dust of leaves, Pineapple was observed as the best plant followed by akanda, turmeric, garlic and custard apple. Chronological distribution of efficacy of dust of plants leaves at different concentrations: pineapple at 20% concentration (76.9%) > akanda at 5% concentration (75.0%) > turmeric at 20% concentration (73.3%). In case of control group, less than 22% efficacy was found in all concentration of juice and dust preparation.

DISCUSSION

Pineapple (*A. comosus*) is an important medicinal plant with diverse pharmacological spectrum. The active ingredient of *A. comosus* is bromelian. The effect of bromelian (proteolytic enzyme) is to digest parasites (Lechat et al., 1978). In this experiment, highest efficacy of pineapple leaves was found in 20% juice (86.9%), followed by 10% juice (84.0%). These

Table 2: Efficacy of fresh leaves juice of five selected plants at 5%, 10% and 20% concentration against development of *A. galli* eggs

Name of plants	5% concentration						10% concentration						20% concentration					
	Total no. of egg	No. of developed eggs			No. of undeveloped eggs	Effects against development of eggs	Total no. of egg	No. of developed eggs			No. of undeveloped eggs	Effects against development of eggs	Total no. of egg	No. of developed eggs			No. of undeveloped eggs	Effects against development of eggs
		Observation at (days)						Observation at (days)						Observation at (days)				
		10th	15th	20th				10th	15th	20th				10th	15th	20th		
Pineapple	19	2	4	4	15	78.9	25	2	4	4	21	84.0	23	2	3	3	20	86.9
Turmeric	20	4	5	6	14	70.0	23	2	4	6	17	73.9	19	2	3	4	15	78.9
Akanda	26	2	6	8	18	69.2	26	3	6	7	19	73.0	18	4	6	6	12	66.6
Garlic	22	1	3	4	18	81.8	19	5	7	7	12	63.1	20	6	7	7	13	65.0
Custard apple	24	4	7	10	14	58.3	21	4	5	6	15	71.4	25	4	6	7	18	72.0
Control	17	12	14	15	2	11.7	17	13	15	15	2	11.7	25	8	19	23	2	8.0
Level of significance				**						**						**		

In a column figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT); ** = Significant at 1% level of probability

Table 3: Efficacy of dust of leaves of five selected plants against development at 5%, 10% and 20% concentration of *A. galli* eggs

Name of plants	5% concentration						10% concentration						20% concentration					
	Total no. of egg	No. of developed eggs			No. of undeveloped eggs	Effects on development of eggs	Total no. of egg	No. of developed eggs			No. of undeveloped eggs	Effects on development of eggs	Total no. of egg	No. of developed eggs			No. of undeveloped eggs	Effects on development of eggs
		Observation at (days)						Observation at (days)						Observation at (days)				
		10th	15th	20th				10th	15th	20th				10th	15th	20th		
Pineapple	17	3	5	5	12	70.5	14	2	3	4	10	71.4	13	2	3	3	10	76.9
Turmeric	15	2	3	4	11	73.3	14	3	4	4	10	71.4	15	2	3	4	11	73.3
Akanda	16	1	3	4	12	75.0	15	2	5	6	9	60.0	13	3	4	4	9	69.2
Garlic	14	3	4	5	9	64.2	16	6	8	8	8	50.0	12	1	2	4	8	66.6
Custard apple	14	4	6	6	8	57.1	13	4	5	6	7	53.8	15	3	6	7	8	53.3
Control	13	8	9	11	2	15.3	12	7	9	10	2	16.6	14	8	9	11	3	21.4
Level of significance				**						**						**		

In a column figures with same letter or without letter do not differ significantly whereas figures with dissimilar letter differ significantly (as per DMRT); ** = Significant at 1% level of probability

finding could be compared with the study of Rahman (2002) who recorded the findings against gastro intestinal nematodes where juice of pineapple showed the best (86.00%) efficacy. Patra et al. (2010) reported that the anthelmintic activity of pineapple is 85.0% after 20 days of treatment.

Curcumin is the main active constituent of turmeric which may cause death of *A. galli* during *in vitro* study (Bazh and El-Bahy, 2013). Number of researchers reported the anthelmintic activity of turmeric (*C. domestica*) against adult *A. galli* (Ahktar and Riffat, 1985; Ali, 2006) and other helminths (Rahman, 2002; Hordegen et al., 2003; Sharma et al., 2003; Mishra et al., 2004; Githiori et al., 2004; Chandrawathani et al., 2006; Szewczuk et al., 2006). In this experiment, highest efficacy of turmeric leaves was found in 20% juice (78.9%), followed by 10% juice (73.9%) and dust at 20% concentration (73.3%). These findings indicated that turmeric leaves have better efficacy on the inhibition of development of *A. galli* eggs in juice preparation than dust. Rahman (2002) recorded the highest efficacy (100%) of turmeric leaves in alcoholic extract whereas aqueous extract have the lower efficacy (92.0%) against gastrointestinal nematodes in goats.

The active ingredient of garlic (*A. sativum*) is allicin. Allicin is readily permeable through phospholipid membranes (Oommen et al., 2004) and affects helminthes by altering the body surface of helminthes (Shalaby and Farag, 2014). In this study, garlic leaves showed efficacy at 5% juice (81.8%), followed by 5% dust (64.2%), 10% juice (63.1%) and 10% dust (50.0%) against *A. galli*. These records have close agreements with study of Murdiati et al., (1997), Lamtiur (2000) and Rahman (2002) that proved the anthelmintics efficacy against *A. galli*. These findings have also similarities with the study of Kumar et al (1991) who compared *in vitro* effects of BITC (benrylisothiocyanate), an anthelmintic principle of garlic with mebendazole against *A. galli* and found effective.

The leaves of *C. gigantea* (Akanda) are used as a vermicide. The active component of *C. gigantea* is calotropain. This proteolytic enzyme can digest parasites (Lechat et al., 1978). The efficacy of leaves of akanda is determined in this experiment. The leaves of akanda revealed the maximum efficacy against eggs of *A. galli* at 5% dust (75.0%) followed by 10% juice (73.0% and 69.2%) both at 10% juice and 20% dust concentration.. These records is more or less similar with the findings of Lal et al. (1976) and Ali (2006) who studied the anthelmintic efficacy of akanda against adult *A. galli* as well as other helminthes. Similarly, Rahman (2002) recorded the efficacy of akanda in aqueous (67%) and ethanol extract (79%) against gastro intestinal nematodes *in vivo*. These findings are partially agreed with percentage of efficacy of present study, though present study recommends the plants as effective which have at least 70% efficacy.

Preliminary phytochemical investigation of methanolic extract revealed the presence of alkaloid, acetogenin, flavonoids, stenoid and triterpenoids in *A. reticulata* (Krishnadev et al., 2010). Previous studies reported that alkaloid is responsible for the paralysis of earthworm. Tannin might have anthelmintic activity by binding with free proteins in gastrointestinal tract of earthworm and cause death (Tiwari et al., 2011; Rubini et al., 2012). Dust powder of custard apple was found 57.1% and 53.3% effective at 5% and 10% concentration respectively. These results could be compared with the findings of Chakraborty et al. (2008) who recorded the efficacy (77.0%) of custard apple leaves against sporulation of *Eimeria tenella* oocysts. According to Bhale et al. (2011), methanolic extract of leaves of custard apple have a potent anthelmintic activity.

CONCLUSION

It is concluded that among five plants and different preparations, pineapple, turmeric and akanda were found effective. But they can not be used directly, as many microbes multiply in moist liter. So further studies are therefore needed to find out the side effects of these plants and determine the recommended doses in poultry.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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