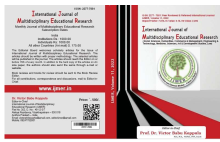
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EVALUATION OF *In vitro* ANTHELMINTIC EFFICACY OF CERTAIN INDIGENOUS PLANTS AGAINST EXPERIMENTALLY- INDUCED *Ascaridia galli* INFECTION IN LOCAL BIRDS (*Gallus domesticus*)

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Abstract
The present study was an attempt to evaluate the anthelmintic efficacy of certain indigenous plants against experimentally induced *Ascaridia galli* infection in local birds (*Gallus domesticus*). A total of five (5) indigeneous plants *viz., Nyctanthes arbor-tristis* (Sewali), *Butea monosperma* (Palash), *Melia azedarach* (Ghora neem), *Erythrina stricta* (Madar), and *Ficus hispida* (Dimoru) based on indigeneous technical knowledge (ITK) and ethnomedical uses Three types of extracts, *viz.* ethanolic, hydroethanolic and aqueous extracts of each of the five plants were prepared for *in vitro* studies. *A. galli* was used as the test parasite for *in vitro* studies. Three different concentrations, 10, 25 and 50 mg/ml was used for *in vitro* studies. Among the five plants studied for *in vitro* efficacy *N. arbor-tristis* was observed as the best plant having *in vitro* anthelmintic efficacy followed by *B. monosperma*, *M. azedarach*, *E. stricta* and *F. hispida*.

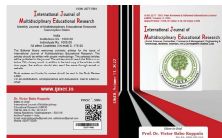
Keywords: *Nyctanthes arbor-tristis, Butea monosperma, Melia azedarach, Erythrina stricta, Ficus hispida, in-vitro* anthlmintic activity.

INTRODUCTION

Helminthiasis is considered one of the most important and common disease affecting free range backyard chickens (Soulsby, 1982 and Permin, *et al.,* 1997) causing setbacks in productive and reproductive performance of livestock (Agaie and Onyeyili, 2007; Dawo and Tibo, 2005) and inflicting heavy production losses accounting to 41.4% in egg laying domestic fowl (Elenwo and Okafor-Elenwo, 2014) and 51.4% in domestic fowl broilers (Elenwo and Okafor-Elenwo, 2014a). Most of the symptoms caused by helminth parasites go unnoticed because of sub-clinical or chronic nature of the diseases they cause unless the parasites cause death of the animal (Dawo and Tibo, 2005).Helminth infections in indigenous chicken are very common because of the risks posed by the production system which predominantly relies on scavenging (Ondwassy *et al.,* 2000) and contributes to the low production of the indigenous chicken (Siamba *et al.,* 2000, Sani *et al.,* 1987). Also the control of helminth in birds is largely neglected at village level leading to slower growth rate and hence reduced body weight, delayed market weight attainment of their stock because of competition for nutrients by the bird and parasites. For the farmer, there is loss of income due to treatment cost, decreased feed efficiency and impaired performance (Ruff, M.D., 1999, Martín-Pacho, 2005) reduced employment, and compromised household welfare activities. The use of *A. galli* (Shrank, 1788) worms as a suitable model for the screening of anthelmintic drugs had been advocated earlier because of easy availability (Kaushik *et al*., 1974, Lal *et al.,* 1976, Tandon *et al*.,1997, Kaushik *et al*., 1981). The females of *A. galli* lay thick heavy-shelled eggs in the intestine that pass in the feces of the host (Soulsby, 1982; Urquhart, 1996). The embryonated eggs of *Ascaridia galli* are very hardy and may live for two years under laboratory conditions and more than one year under ordinary conditions, (Cruthers *et al*, 1974; Matter and Oester, 1989). Disinfectants and the likes do not destroy the eggs of *A. galli* under farm conditions and poultry become infected by eating infective eggs containing L_3 stage of the larvae of the parasite (Permin, 1997).

Nyctanthes arbor-tristis belongs to the family Oleaceae / Nyctaginaceae and is popularly known as 'Night Jasmine' (English) or 'Harsinghar' (Hindi) or 'Sewali' (Assamese) due to the fact that its flowers emit a very strong and pleasant fragrance during the whole night (Siddiqui *et al.* 2006; Rout, *et al*. 2007).

The generic name *Nyctanthes arbor-tristis* belongs to the family Oleaceae / Nyctaginaceae and is popularly known as 'Night Jasmine' (English) or 'Harsinghar' (Hindi) or 'Sewali' (Assamese) due to the fact that its flowers emit a very strong and pleasant fragrance during the whole night (Siddiqui *et al.* 2006; Rout, *et al*. 2007) The generic name *'Nyctanthes'* has been coined from two Greek words *'Nykhta'* (Night) and *'anthos'* (flower) (Vats *et al.,* 2009; Meshram 2012).The specific name '*arbortristis'* meaning 'the sad tree' is supposedly derived from the dull looks of the tree during daytime (Suresh *et al.*, 2010).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MATERIALS AND METHODS

Location and Place of Study

The study was conducted at Krishi Vigyan Kendra Golaghat, Department of Veterinary Parasitology and Department of Veterinary Pharmacology & Toxicology, College of Veterinary Science, AAU, Khanapara, Guwahati-781022. Experimental procedures were performed in accordance with the guidelines recommended by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. All the protocols were approved by Institutional Animal Ethics Committee (IAEC), Faculty of Veterinary Science, AAU Khanapara vide Approval No. 770/ac/CPCSEA/FVSc/ AAU/ IAEC/16-17/451 dated 30.07.2016.

Selection and Collection of Plant Materials

Five plants namely *Nyctanthes arbor-tristis* (Sewali), *Butea monosperma* (Palash), *Melia azedarach* (Ghora neem), *Erythrina stricta* (Madar), and *Ficus hispida* (Dimoru) were selected on the basis of their ethnomedical uses for screening of anthelmintic activity. Only leaves of these plants, free from disease condition or other deformities were collected from the surrounding areas of Khanapara, Guwahati and Golaghat district of Assam.

Processing of Plant Materials

After identification and characterisation by BSI, leaves were further collected for *in vitro* and *in vivo* evaluation for it's effectiveness against *Ascaridia galli* in indigenous chicken after preparation of plant extracts. The collected leaves were gently washed to remove the dust. Leaves were shade dried at room temperature for around 15 - 20 days to avoid direct sunshine that could degrade some of the compounds in the plants. They were also turned over regularly, to avoid fermenting and rotting. Dried leaves were then grounded or pulverised to powder by Laboratory Willey Mill, the powder weighed using an analytical scale and kept at room temperature in air tight containers after proper labelling until preparation of extracts.

Preparation of Ethanolic, Hydroethanolic and Aqueous Extracts

Powdered plant materials were extracted with ethanol, hydroethanol (1:1) and distilled water respectively as per the procedure of Prasad (1965). Finely powdered plant powders were soaked individually for 72 hours, three times, with intermittent agitation. The extracts were then double filtered using muslin cloth and Whatman No. 1 filter paper. The filtrate obtained was concentrated in a Soxhlet apparatus under reduced pressure and completely dried over a regulated water bath maintained at 50^0C. The percentage yield of each of the fifteen extracts was calculated. The extracts were refrigerated at 4^0C until the experiments for screening was done. Standard procedures (Lateef *et al.,* (2003; 2006); Sujon *et al.* (2008) were used with a few modifications.

ACUTE TOXICITY STUDY

The acute toxic potential of all the fifteen plant extracts were studied as per Organization for Economic Corporation Development (OECD) guidelines – No. 425. Rats of male sex (150 -200 g) were used for acute toxicity study. The extracts were administered orally @ 2000mg / Kg to a group of rats (n=3). Animals were fasted overnight and given water *ad libitum* prior to the study. They were observed for 48 hours for mortality and 14 days for behavioural changes. Based on the acute toxicity study, 100, 500 and 1000 mg / Kg body weight oral dose was selected.

EXPERIMENTAL DESIGN FOR *in vitro* STUDIES

The test parasite

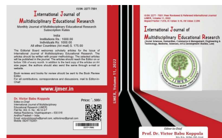
Gastrointestinal nematode, *Ascaridia galli, was* used as test parasite in the study.

Collection of Gastro-intestinal tracts (GIT) of Domestic Fowl

Gastro-intestinal tracts (GIT) of domestic chickens, slaughtered at Golaghat Main Market were collected and examined for *Ascaridia galli.* GIT were collected in specimen bottles containing Normal Saline Solution. The samples were taken to Krishi Vigyan Kendra Golaghat for further examination.

Examination of Gastro-intestinal tracts (GIT) for *Ascaridia galli*

Each gastro-intestinal tract of local chicken was spread on a dissecting board. The lumen of intestine was opened longitudinally and the content scrapped into a petridish as described by Fatihu (1990). Adult *A. galli* were collected with a needle and placed in a petridish containing normal saline solution following the standard method of Fowler (1990). The worms were thoroughly cleaned with normal saline to remove all faecal matter as per Neogi, *et al. (*1963).



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Identification of the parasite

Parasites were identified by gross morphology as per keys of Cheng (1973), Soulsby (1982) and Ruprah *et al*. (1986).

Anthelmintic Efficacy of the Plant Extracts *in vitro*

In vitro screening of different ethanolic, hydroethanolic and aqueous extracts of leaves of selected plants on *A. galli* were done for their efficacy; and the plant/preparations were considered as effective if having at least 70% efficacy. The *in vitro* studies of the plant extracts were conducted on the mature and actively motile *Ascaridia galli* worms as described by Sharma *et al.* (1967) for establishing the anthelmintic properties of plants on worms kept in different dilutions of various extracts. The motility by visual observation was used as the main criteria.

Petri dishes of 4" diameter was properly washed, dried and then labelled. Distilled water was used as media for this trial and 20 ml of total volume was made for each trial. For control, petridish with distilled water was retained without treatment. Petri dishes containing different concentrations (10, 25 and 50 mg/ml) of plant extract was used to keep six vigorously actively motile *Ascaridia galli* worms. Another petridish contained a known anthelmintic, piperazine hydrate to serve as a standard drug. The experiment was run at room temperature (22-28^{0}C). Time of exposure of the worms to the drug and plant extracts in petridishes was recorded. The worms in all the dishes were then examined for the possible decrease in motility or death. To determine the viability of motionless worms, pinch technique (Mengi *et al.*, 1963) was used. Death was concluded when the worms lost their mobility followed by fading of their body. colour. The mass motility and percent mortality was observed at 0, 15 min, 30 min, 1.0, 2.0, 4.0, 6.0, and 24.0 hours after the immersion of the worms into the drug and plant extracts till all the worms were dead. Thus, the minimum and maximum lethal time for all the six worms in an extract was recorded. This procedure was adopted to test all the three extracts of each of the five plants. The experiment involved three replicates of all the concentrations of extracts. Results are Preseted in the table.1.

Table.1. TYPES OF EXTRACTS AND CONCENTRATION (mg/ml) USED FOR *in vitro* STUDY

Plants under Study	Types of Extract used	No. of *A. galli* worms used	Concentration used (mg/ml)
Nyctanthes arbor-tristis	Ethanolic	6	10,25,50
	Hydroethanolic	6	10,25,50
	Aqueous	6	10,25,50
Butea monosperma	Ethanolic	6	10,25,50
	Hydroethanolic	6	10,25,50
	Aqueous	6	10,25,50
Melia azaderach	Ethanolic	6	10,25,50
	Hydroethanolic	6	10,25,50
	Aqueous	6	10,25,50
Erythrina stricta	Ethanolic	6	10,25,50
	Hydroethanolic	6	10,25,50
	Aqueous	6	10,25,50
Ficus hispida	Ethanolic	6	10,25,50
	Hydroethanolic	6	10,25,50
	Aqueous	6	10,25,50

STATISTICAL ANALYSIS

Results were expressed as Mean ± SEM. Statistical analysis was performed by MS-excel to calculate mean, standard error of Mean (SEM), analysis of variance (ANOVA), and co-efficient of correlation (r) values. All statements of significance were based at least on 95% confidence limits (*i.e*. $P < 0.05$).

RESULT AND DISCUSION

Phytochemical Analysis of Ethanolic, Hydroethanolic & Aqueous Extracts

Various qualitative tests were done using ethanolic, hydroethanolic and aqueous extracts of *Nyctanthes arbor-tristis*, *Butea monosperma*, *Melia azedarach*, *Erythrina stricta*, and *Ficus hispida.* They were subjected to phytochemical analysis for the presence of various active principles or phytochemical constituents namely steroids, alkaloids, phenolic compounds, tannins, flavonoids, glycosides, triterpenes and saponins. Almost all the tests done on the three extracts of *Nyctanthes arbor-tristis* were positive for the presence of steroids, alkaloids, phenolic compounds, tannins, flavonoids, glycosides, and triterpenes except Salkowski's test for

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steroids, Wagner's test for alkaloids and test for phenolic compounds which were negative with aqueous extract. Foam test for saponins was negative with both hydroethanolic and aqueous extract. Ethanolic, hydroethanolic and aqueous extracts of *Butea monosperma* were positive for the presence of steroids, alkaloids, phenolic compounds, tannins, flavonoids, glycosides, triterpenes and saponins. Tests for alkaloids with hydroethanolic extract were negative. *Melia azedarach,* on phytochemical analysis, was positive for the presence of steroids, alkaloids, phenolic compounds, tannins, flavonoids, glycosides and triterpenes but saponins was found to be absent. Almost all the tests done on the three extracts of *Erythrina stricta* were positive for the presence of steroids, alkaloids, phenolic compounds, flavonoids, glycosides, and triterpenes except tests for alkaloids which were negative with hydroethanolic extract. Ferric Chloride test for tannins were negative with both ethanolic and hydroethanolic aqueous extract. Ethanolic, hydroethanolic and aqueous extracts of *Ficus hispida* were positive for the presence of steroids, alkaloids, phenolic compounds, tannins, flavonoids, glycosides, triterpenes and saponins.

Acute Toxicity Study

The acute toxicity studies with ethanolic, hydroethanolic and aqueous extracts of *Butea monosperma, Nyctanthes arbor-tristis, Melia azedarach, Erythrina stricta* and *Ficus hispida* did not show any behavioural change or gross abnormality nor any sign of toxicity upto 14 days of observation and mortality was absent within 48 hours @ 2.0 g / kg body weight in rats. The extracts were considered to be safe up to a maximum dose of 2000 mg/kg.

ANTHELMINTIC EFFICACY OF ETHANOLIC, HYDROETHANOLIC AND AQUEOUS EXTRACTS ON MOTILITY AND SURVIVABILITY OF *Ascaridia galli in vitro*

***In vitro* screening of ethanolic extract**

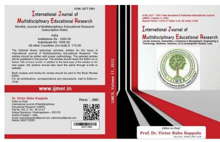
Among the five plants studied for *in vitro* efficacy, the highest efficacy was found with *Nyctanthes arbor-tristis* followed by *Butea monosperma*, *Erythrina stricta, Melia azedarach*, and *Ficus hispida* in 10 mg/ml concentration of ethanolic extract. Screening with 25 mg/ml concentration of ethanolic extract, the highest efficacy was found with *B. monosperma* followed by *N. arbor-tristis*, *M. azedarach*, *F. hispida* and *E. stricta*. At 50 mg/ml concentration of ethanol extract, the highest efficacy was found with *B. monosperma* followed by *N. arbor-tristis*, *M. azedarach*, *E. stricta* and *F.hispida. In vitro* screening of 5 plants with ethanol extract revealed that *B. monosperma* was the highest efficacious plant against adult *A. galli*. Second highest efficacy was showed by *N. arbor-tristis* followed by *M. azedarach*, *E. stricta* and *F. hispida*. Mortality of adult *A. galli* with *B. monosperma* and *N. arbor-tristis* started as early as 15 minutes and by 4 hours all the six parasites were dead. But with *B. monosperma*, the rate of mortality was more. This result reveals that *Butea monosperma* leaves are more efficacious in ethanol than *Nyctanthes arbor-tristis* and *Melia azedarach*. It can be explained by the fact that the active ingredients of *Nyctanthes arbor-tristis* and *Melia azedarach* were less extracted with ethanol than that of *Butea monosperma*.

***In vitro* screening of hydroethanol extract**

At 10 mg/ml concentration of hydroethanol extract of 5 plants, the highest efficacy was observed with *B. monosperma* followed by *N. arbor-tristis*, *M. azedarach*, *E. stricta* and *F.hispida*. At 25 mg/ml concentration of ethanol extract, the highest efficacy was found with *N. arbor-tristis* followed by *B. monosperma*, *M. azedarach*, *E. stricta* and *F. hispida*. Treatment with 50 mg/ml concentration of ethanol extract, the highest efficacy was found with *N. arbor-tristis* followed by *B. monosperma*, *E. stricta*, *M. azedarach*, and *F. hispida* . Among the plants, in all concentrations of hydroethanol extract of leaves, *Nyctanthes arbor-tristis* was observed as best plant followed by *Butea monosperma*, *M. azedarach*, *E. stricta* and *F. hispida*. *In vitro* study using hydroethanol extracts of leaves showed that *Nyctanthes arbor-tristis* was the best plant and this result indicates that hydroethanol extracted well the active components of *Nyctanthes arbor-tristis* than that of *Butea monosperma* and *M. azedarach*.

***In vitro* screening of aqueous extract**

Nyctanthes arbor-tristis showed the highest efficacy at 10, 25 and 50 mg/ml concentration of aqueous solution followed by *Butea monosperma, Melia azedarach, Ficus hispida* and *Erythrina stricta*. These findings also establish the hypothesis that active principles of *Nyctanthes arbor-tristis* are more soluble in water than *Butea monosperma*. *Nyctanthes arbor-tristis* and *Butea monosperma* showed its anthelmintic efficacy against *Ascaridia galli* in the study. The findings indicate that *N. arbor-tristis* and *B. monosperma* have better efficacy compared to other three plants on the inhibition of adult nematode, *A. galli*. Rahman (2002) recorded the highest efficacy (100%) of neem leaves in alcoholic extract whereas aqueous extract had lower efficacy (92%) than alcohol against gastrointestinal nematodes in goats. This is in agreement with the findings of the present study where 100% mortality of *A. galli* was seen within 4 hours of incubation in alcoholic extract as compared to 6 hours in aqueous extract. Kumar et al. (2007) stated that contents of the alcoholic extract are different from aqueous extract and contain substances which have better anthelmintic effect. Piperazine hydrate was used as the standard drug to compare the anthelmintic efficacy of the plant extracts. 100% mortality of *A. galli*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was seen within 15 minutes to one hour of incubation. Kumar *et al.* (1991) compared *in vitro* effects of BITC (benzylisothiocyanate), an anthelmintic principle of *Carica papaya* with mebendazole against *Ascaridia galli* and found effective. In both (aqueous and ethanol) cases of *in vitro* screening adults showed structural alterations. Grossly the extracts caused inhibition of spontaneous motility of worm followed by paralysis. The findings also substantiated by Tandon *et al*. (1997) and Bishnu pada *et al.* (1997) reported that scanning electron microscopy (which was not performed in this study) of parasites exposed to plant extracts (*Flemingia vestita*, *Cannabis sativa*) revealed structural alteration in the integument/surface architecture particularly of the papillated surface. It was observed in the present study that in all cases, the complete cessation of motility and mortality of worm depends on the type and concentration of the extracts used. The time for complete cessation of motility and mortality was found to be reduced as the concentrations of the extracts increased. This observation gave the indication that mortality percentage varied significantly among the plants, solvents and doses used. In this study, distilled water, tap water, Normal Saline Solution, Phosphate Buffer Saline Solution, Ethanol, Hydroethanol (1:1) and Standard Piperazine was also used for incubation of adult parasite but test may be more reliable if it is possible to incubate the adult and infective larvae in intestinal fluid containing plant extract which was described by Molan *et al.* (2000).

Results are presented in the table. 2,3,4,5,6,7,8,9.

Table.2. *In vitro* EFFICACY OF *Nyctanthes arbortris-tis* LEAF EXTRACT ON MOTILITY and SURVIVABILITY OF *Ascaridia galli*

Nyctanthes arbortris-tis										
Type of Extract	**Conc (mg/ml)**	**No. of *A. galli***	**Time post exposure (Parenthesis indicate % efficacy)**							
			0	**15 min**	**30 min**	**1.0 h**	**2.0 h**	**4.0 h**	**6.0 h**	**24.0 h**
Ethanolic	10	S	6	6	5	4	4	0	-	-
		M	0	0`(0)	1 (16.66)	2 (33.33)	2 (33.33)	6 (100)	-	-
	25	S	6	5	5	4	3	0	-	-
		M	0	1 (16.66)	1 (16.66)	2 (33.33)	3 (50)	6 (100)	-	-
	50	S	6	5	5	4	0	0	-	-
		M	0	1 (16.66)	1 (16.66)	2 (33.33)	6 (100)	6 (100)	-	-
Hydroethanolic	10	S	6	6	6	5	5	0	-	-
		M	0	0 (0)	0 (0)	1 (16.66)	1 (16.66)	6 (100)	-	-
	25	S	6	5	1	1	1	0	-	-
		M	0	1 (16.66)	5 (83.33)	5 (83.33)	5 (83.33)	6 (100)	-	-
	50	S	6	1	1	0	-	-	-	-
		M	0	5 (83.33)	5 (83.33)	6 (100)	-	-	-	-
Aqueous	10	S	6	5	5	5	4	4	0	-
		M	0	1 (16.66)	1 (16.66)	1 (16.66)	2 (33.33)	2 (33.33)	6 (100)	-
	25	S	6	5	5	5	5	0	-	-
		M	0	1 (16.66)	1 (16.66)	1 (16.66)	1 (16.66)	6 (100)	-	-
	50	S	6	5	5	3	2	2	0	-
		M	0	1 (16.66)	1 (16.66)	3 (50)	4 (66.66)	4 (66.66)	6 (100)	-

S- Survivability; M- Mortality

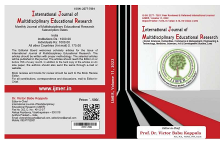
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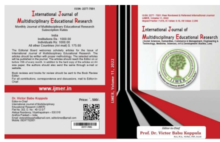
Table. 3. ***In vitro*** **Efficacy of** ***Butea monosperma*** **leaf extract on motility and survivability of** ***Ascaridia galli***

Butea monosperma										
Type of Extract	**Conc (mg/ml)**	**No. of *A. galli***	**Time post exposure (Parenthesis indicate % efficacy)**							
			0	**15 min**	**30 min**	**1.0 h**	**2.0 h**	**4.0 h**	**6.0 h**	**24.0 h**
Ethanolic	10	S	6	6	6	5	4	0	-	-
		M	0	0 (0)	0 (0)	1 (16.66)	2 (33.33)	6 (100)	-	-
	25	S	6	4	4	4	0	-	-	-
		M	0	2 (33.33)	2 (33.33)	2 (33.33)	6 (100)	-	-	-
	50	S	6	4	1	0	-	-	-	-
		M	0	2 (33.33)	5 (83.33)	6 (100)	-	-	-	-
Hydroethanolic	10	S	6	6	6	5	4	0	-	-
		M	0	0 (0)	0 (0)	1 (16.66)	2 (33.33)	6 (100)	-	-
	25	S	6	5	4	4	4	4	3	0
		M	0	1 (16.66)	2 (33.33)	2 (33.33)	2 (33.33)	2 (33.33)	3 (50)	6 (100)
	50	S	6	6	6	2	1	0	-	-
		M	0	0 (0)	0 (0	4 (66.66)	5 (83.33)	6 (100)	-	-
Aqueous	10	S	6	6	6	5	4	4	0	-
		M	0	0 (0)	0 (0)	1 (16.66)	2 (33.33)	2 (33.33)	6 (100)	-
	25	S	6	6	6	4	4	3	0	-
		M	0	0 (0)	0 (0)	2 (33.33)	2 (33.33)	3 (50)	6 (100)	-
	50	S	6	6	6	4	3	0	-	-
		M	0	0 (0)	0 (0)	2 (33.33)	3 (50)	6 (100)	-	-

S- Survivability; M- Mortality

Table.4. ***In vitro*** **Efficacy OF** ***Melia azaderach*** **LEAF EXTRACT ON MOTILITY and SURVIVABILITY OF** ***Ascaridia galli***

Melia azaderach										
Type of Extract	**Conc (mg/ml)**	**No. of *A. galli***	**Time post exposure (Parenthesis indicate % efficacy)**							
			0	**15 min**	**30 min**	**1.0 h**	**2.0 h**	**4.0 h**	**6.0 h**	**24.0 h**
Ethanolic	10	S	6	6	6	6	6	4	4	0
		M	0	0 (0)	0 (0)	0 (0)	0 (0)	2 (33.33)	2 (33.33)	6 (100)
	25	S	6	6	6	6	6	4	3	0
		M	0	0 (0)	0 (0)	0 (0)	0 (0)	2 (33.33)	3 (50)	6 (100)
	50	S	6	6	5	5	5	5	4	0
		M	0	0 (0)	1 (16.66)	1 (16.66)	1 (16.66)	1 (16.66)	2 (33.33)	6 (100)
Hydroethanolic	10	S	6	6	6	6	6	4	3	0
		M	0	0 (0)	0 (0)	0 (0)	0 (0)	2 (33.33)	3 (50)	6 (100)
	25	S	6	6	6	6	5	5	5	0
		M	0	0 (0)	0 (0)	0 (0)	1 (16.66)	1 (16.66)	1 (16.66)	6 (100)
	50	S	6	6	6	6	5	5	3	0
		M	0	0 (0)	0 (0)	0 (0)	1 (16.66)	1 (16.66)	3 (50)	6 (100)
Aqueous	10	S	6	6	6	6	6	6	5	0
		M	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.66)	6 (100)
	25	S	6	4	4	4	4	3	3	0
		M	0	2 (33.33)	2 (33.33)	2 (33.33)	2 (33.33)	3 (50)	3 (50)	6 (100)
	50	S	6	6	6	6	3	3	3	0
		M	0	0 (0)	0 (0)	2 (33.33)	3 (50)	3 (50)	3 (50)	6(100)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S- Survivability; M- Mortality

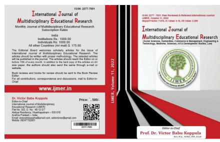
Table.5. ***In vitro*** **Efficacy OF** ***Erythrina stricta*** **Leaf Extract On Motility and Survivability Of** ***Ascaridia galli***

Erythrina stricta										
Type of Extract	**Conc (mg/ml)**	**No. of *A. galli***	**Time post exposure (Parenthesis indicate % efficacy)**							
			0	**15 min**	**30 min**	**1.0 h**	**2.0 h**	**4.0 h**	**6.0 h**	**24.0 h**
Ethanolic	10	S	6	6	6	6	5	5	5	0
		M	0	0 (0)	0 (0)	0 (0)	1 (16.66)	1 (16.66)	1 (16.66)	6 (100)
	25	S	6	6	6	6	6	5	5	0
		M	0	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.66)	1 (16.66)	6 (100)
	50	S	6	6	6	6	5	4	4	0
		M	0	0 (0)	0 (0)	0 (0)	1 (16.66)	2 (33.33)	2 (33.33)	6 (100)
Hydroethanolic	10	S	6	6	6	6	6	6	5	0
		M	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.66)	6 (100)
	25	S	6	6	6	6	6	4	5	0
		M	0	0 (0)	0 (0)	0 (0)	0 (0)	2 (33.33)	1 (16.66)	6 (100)
	50	S	6	6	6	6	5	4	4	0
		M	0	0 (0)	0 (0)	0 (0)	1 (16.66)	2 (33.33)	2 (33.33)	6 (100)
Aqueous	10	S	6	6	6	6	5	5	5	0
		M	0	0 (0)	0 (0)	0 (0)	1 (16.66)	1 (16.66)	1 (16.66)	6 (100)
	25	S	6	6	6	6	6	5	5	0
		M	0	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.66)	1 (16.66)	6 (100)
	50	S	6	6	6	6	6	4	4	0
		M	0	0 (0)	0 (0)	0 (0)	0 (0)	2 (33.33)	2 (33.33)	6 (100)

S- Survivability; M- Mortality

Table. 6. ***In vitro*** **Efficacy OF** ***Ficus Hispida*** **LEAF EXTRACT ON MOTILITY and SURVIVABILITY OF** ***Ascaridia galli***

Ficus hispida										
Type of Extract	**Conc (mg/ml)**	**No. of *A. galli***	**Time post exposure (Parenthesis indicate % efficacy)**							
			0	**15 min**	**30 min**	**1.0 h**	**2.0 h**	**4.0 h**	**6.0 h**	**24.0 h**
Ethanolic	10	S	6	6	6	6	6	6	5	0
		M	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.66)	6 (100)
	25	S	6	6	6	6	6	5	4	0
		M	0	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.66)	2 (33.33)	6 (100)
	50	S	6	6	6	6	6	5	3	0
		M	0	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.66)	3 (50)	6 (100)
Hydroethanolic	10	S	6	6	6	6	6	6	5	0
		M	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.66)	6 (100)
	25	S	6	6	6	6	6	5	4	0
		M	0	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.66)	2 (33.33)	6 (100)
	50	S	6	6	6	6	6	5	3	0
		M	0	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.66)	3 (50)	6 (100)
Aqueous	10	S	6	6	6	6	6	6	5	0
		M	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.66)	6 (100)
	25	S	6	6	6	6	6	5	3	0
		M	0	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.66)	3 (50)	6 (100)
	50	S	6	6	6	5	5	5	4	0
		M	0	0 (0)	0 (0)	1 (16.66)	1 (16.66)	1 (16.66)	2 (33.33)	6 (100)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S- Survivability; M- Mortality

Table.7. ***In vitro*** **Efficacy OF DIFFERENT SOLUTIONS ON MOTILITY and SURVIVABILITY OF** ***Ascaridia galli***

Solutions									
Type of Solution	**No. of *A. galli***	**Time post exposure (Parenthesis indicate % efficacy)**							
		0	**15 min**	**30 min**	**1.0 h**	**2.0 h**	**4.0 h**	**6.0 h**	**24.0 h**
Distilled Water	S	6	6	6	6	6	5	3	0
	M	0	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.66)	3 (50)	6 (100)
Tap Water	S	6	6	6	6	6	4	3	0
	M	0	0 (0)	0 (0)	0 (0)	0 (0)	2 (33.33)	3 (50)	6 (100)
Normal Saline Solution	S	6	6	6	6	6	5	2	0
	M	0	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.66)	4 (66.66)	6 (100)
Phosphate Buffer Solution	S	6	6	6	6	6	6	5	0
	M	0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.66)	6 (100)

Table.8. ***In vitro*** **Efficacy OF FEW SOLVENTS ON MOTILITY and SURVIVABILITY OF** ***Ascaridia galli***

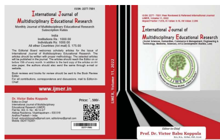
Solvents									
Type of Solution	**No. of *A. galli***	**Time post exposure (Parenthesis indicate % efficacy)**							
		0	**15 min**	**30 min**	**1.0 h**	**2.0 h**	**4.0 h**	**6.0 h**	**24.0 h**
Ethanol	S	6	6	0	-	-	-	-	-
	M	0	0 (0)	6 (100)	-	-	-	-	-
Hydro ethanol (1:1)	S	6	6	4	3	0	-	-	-
	M	0	0 (0)	2 (33.33)	3 (50)	6 (100)	-	-	-

Table. 9. ***In vitro*** **Efficacy OF STANDARD (PIPERAZINE) ON MOTILITY and SURVIVABILITY OF** ***Ascaridia galli***

Solvents										
	Conc (mg/ml)	**No. of *A. galli***	**Time post exposure (Parenthesis indicate % efficacy)**							
			0	**15 min**	**30 min**	**1.0 h**	**2.0 h**	**4.0 h**	**6.0 h**	**24.0 h**
PIPERAZINE	10	S	6	1	1	0	-	-	-	-
		M	0	5 (83.33)	5 (83.33)	6 (100)	-	-	-	-
	25	S	6	1	1	0	-	-	-	-
		M	0	5 (83.33)	5 (83.33)	6 (100)	-	-	-	-
	50	S	6	0	-	-	-	-	-	-
		M	0	6 (100)	-	-	-	-	-	-

Conclusion

The present study was an attempt to evaluate the anthelmintic efficacy of certain indigenous plants against experimentally induced *Ascaridia galli* infection in local birds (*Gallus domesticus*). Leaf extracts of *Nyctanthes arbor-tristis* and *Butea monosperma* have significant *in-vitro* anthelmitic activity against *Ascaridia galli* with no adverse reactions. Extracts found to possess significant *in vitro* anthelmintic activity. It is concluded that further study is needed, of longer duration, to study the anthelmintic activity against *A. galli* infection in poultry. The plant extracts not only depressed the faecal egg output but also significantly reduced the adult worms population in parasitised birds. This is desirable as it has the advantage of reducing the deleterious effects on individual birds and contamination of the environment with parasite eggs.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CONFLICT OF INTEREST

The author declared no conflict of interest.

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AUTHOR'S CONTRIBUTION

All the authors read and approved the final manuscript.

ANIMAL WELFARE AND ETHICS STATEMENT

The animal experimentation was carried out according to the Committee for the Purpose of Control and Supervision of experimental animals (CPCSEA) guideline and Institutional Animal Ethical Committee Approved all the procedure for investing experimental pain in conscious animals.

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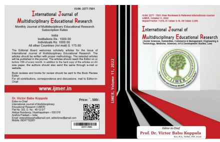
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