**International Journal of Pharmacognosy and Pharmaceutical Sciences**
**Online ISSN: 2706-7017; Print ISSN: 2706-7009**
**Received: 04-11-2018; Accepted: 06-12-2018; Published: 06-01-2019**
**www.pharmacognosyjournal.net**
**Volume 1; Issue 1; 2019; Page No. 10-14**

# Anthelmintic efficacy of ethanol extract of *Nigella Sativa* on lactate dehdrogenase of *Cotylophoron Cotylophorum*

**Dr. A Selvaraju[1], V Suganya[2], Dr. K Manoj Dhanraj[3*]**

[1] Assistant Professor, PG Department of Botany, Sri Vidya Mandir Arts and Science College, Katteri, Uthangarai, Tamil Nadu, India

[2] PG Scholar, PG Department of Botany, Sri Vidya Mandir Arts and Science College, Katteri, Uthangarai, Tamil Nadu, India

[3] Research Fellow, Center for environmental research and Development Loyola College Chennai, Tamil Nadu, India

**DOI:** https://doi.org/10.33545/27067009.2019.v1.i1a.9

**Abstract**
Paramphistomosis is a major disease caused by stomach flukes. The Paramphistome *Cotylophoron Cotylophorum* lives in rumen and reticulum of sheep, goats and other domestic ruminants. The present study was undertaken to elucidate the anthelmintic effect of Ethanol extract of *Nigella sativa* on the lactate dehydrogenase (LDH) of *C. Cotylophorum in vitro*. The parasites were maintained *in vitro* in Hedon-Fleig solution (pH 7.0) which is the best medium for their survival. Parasites were exposed to five different sub-lethal concentration of ethanol extract of *Nigella sativa* (NsEE) for 2, 4 and 8h. LDH and Protein content in the sample was estimated following standard procedures. The inhibition of LDH catalyzing oxidation of lactate results in the reduced production of malate, which subsequently results in the reduced production of ATP. Decreased production of ATP results in the death of parasites. The present investigation amply demonstrated that *N. sativa* could be used as a potential phytotherapeutic drug to combat paramphistome infection in livestock.

**Keywords:** *Cotylophoron Cotylophorum*, *nigella sativa*, lactate dehydrogenase

## 1. Introduction
Livestock play an important role in the socio-economic life of India. India has the largest livestock population in the world, which contributes nearly 7% towards its national income. Livestock provides regular employment to 18.4 million people in core or subsidiary status. The sheep, an important economic livestock species are mostly reared for meat and wool. Sheep rearing provides livelihood supports in terms of income and employment to millions of land less, for the marginal and small pastoralist (Ali, 2007 and Misra *et al*., 2006) [1]. Gastro-intestinal parasites have been identified as one of the major health problems severely limiting the animal productivity in diary animals. In spite of significant production losses which may run into millions of rupees, due to poor quality of meat, impaired reproductive performance and mortality, the problem is neglected due to its chronic and insidious nature. Paramphistomes are parasites of ruminants, cause the disease Paramphistomosis. Paramphistomosis has been associated with only a few species of paramphistomes, viz. *Paramphistomum cervi*, *P. explanatum*, *Cotylophoron cotylophorum*, *Gastrothylax crumenifer* and *Fischoederius elongatus* (Madzingira *et al*., 2002) [8]. A number of chemotherapeutic anthelmintics are currently available to combat various helminth infections. However, resistance to chemotherapeutic anthelmintics is an increasing problem in many countries (Waller *et al*., 2006) [21]. It is imperative to decrease the reliance on these chemotherapeutic drugs for parasite control, not only because of resistance, but also because of growing concerns about the adverse consequences of these antiparasitic drugs on the ecosystem and biodiversity. This danger has given impetus to the search for new drugs, with attention focusing on the search for plant products and the application of plant products as alternative methods of control. Plants have been an exemplary source of medicine. Ayurveda, siddha and other traditional medicinal systems mention the use of plants in treatment of various human and cattle ailments. India has about 45,000 plant species and among them, several thousands have been claimed to possess medicinal properties. In the present study an attempt has been made to elucidate the anthelmintic efficacy of *Nigella sativa* against *C. Cotylophorum.*

Nigella sativa commonly known as black seed or black cumin belongs to the family Ranunculaceae. Nigella sativa seeds have been widely used in traditional medicine as diuretic, antihypertensive liver tonic, digestive, antidiarrheal, appetite stimulant, emmenagogue, analgesic, anthelmintic, antibacterial and useful in skin disorders. Consequently, it has been extensively studied for its biological activities and has been shown to be antidiabetic, anticancer and immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepatoprotective, antihypertensive, renal protective and antioxidant properties (Gilani *et al*., 2004; Zafar *et al*., 2016; Yimer *et al*., 2019) [3, 25, 23].

Carbohydrate is the sole energy source of helminth parasites and its metabolism is often predominantly anaerobic, even in the presence of oxygen. The inhibition of energy metabolism is the most important mode of anthemintic action of various groups of drugs since the parasitic trematodes depend on carbohydrates for their energy metabolism and glucose is the only direct source of energy (Martin, 1997; Tielens, 1997) [10, 17]. Influence of anthelmintics on the carbohydrate metabolism of helminth parasites, reported by Sivamurugan (2018) [15]. The pathway of carbohydrate catabolism is essentially anaerobic and

involves the glycolytic and part of the reversed tri carboxylic acid (TCA) cycle. Glycogen or glucose is broken down by normal glycolytic sequence to phosphoenolpyruvate (PEP). PEP can either be carboxylated by phosphoenolpyruvate carboxykinase (PEPCK) to oxaloacetate (OAA) or dephosphorylated by pyruvate kinase (PK) to pyruvate which is further reduced to lactate by LDH enzyme. OAA formed is reduced in the cytoplasm to malate by malate dehydrogenase (MDH). The malate so formed permeates into the mitochondrian where dismutation takes place and partly oxidized to pyruvate and partly reduced to succinate via. fumarate. Fumarate reductase (FR) and succinate dehydrogenase (SDH), the enzymes of TCA cycle catalyses the reduction fumarate to succinate and oxidation of succinate to fumarate. Reduction of fumarate to succinate complex results in ATP synthesis. Inhibition of these enzymes prevents ATP formation. Decreased production of ATP leads to the death of the parasites. Keeping this in view, an attempt has been made for the assessment of anthelmintic efficacy of ethanol extract of *N. sativa* based on its effect on LDH of *C. Cotylophorum*.

## 2. Materials and Methods

### In vitro maintenance of Cotylophoron cotylophorum

*Cotylophoron Cotylophorum* (Fig.1) were collected from the rumen of infected sheep, slaughtered at Perambur abbatoir, Chennai. Adult live flukes were collected and washed thoroughly in physiological saline and maintained in Hedon-Fleig solution.

### Preparation of Hedon-Fleig solution

Hedon-Fleig solution (pH-7.0) is the best medium for the *in vitro* maintenance of *C. Cotylophorum* (Veerakumari, 1996) [18]. It is prepared by dissolving 7gm of sodium chloride, 0.3gm of potassium chloride, 0.1gm of calcium chloride and 1.5gm of sodium bicarbonate, 0.5gm of disodium hydrogen phosphate, 0.3gm of magnesium sulphate and 1gm of glucose in 1000ml of distilled water.

### Collection and preparation of plant material

Seeds of *Nigella sativa* (Karunseeragam) (Fig. 2) were collected from local market and it was powdered and stored in closed bottles at room temperature in the dark until needed. Powdered seeds of *Nigella sativa* were soaked in ethanol for 48 hours. The extract was then filtered using Whitman No 1 paper and concentrated by distillation using Evator, Rotary evaporator. The concentrated extract was completely dried to remove the last traces of the solvent for further analysis.

### Phytochemical screenings of ethanol extract of *Nigella sativa* (*Ns*EE)

a). Liebermann-Burchard Test: Extract was dissolved in minimum of chloroform. Acetic acid was added and heated. Few drops of acetic anhydride and concentrated $H_2SO_4$ were added. Green colour shows the presence of steroid. b) Noller's Test: Extract was treated with tin and thionyl chloride and heated in water bath. Purple colour shows the presence of triterpenoid. c) Shinoda Test: Extract was dissolved in alcohol. Magnesium bits and concentrated HCl was added. It was heated in a water bath. Majenta colour shows the presence of flavonoid. d) Test for Furan: Extract was dissolved in alcohol, p-dimethylamino benzaldehyde and concentrated HCl was added and heated in a water bath. Pink colour shows the presence of furanoid compound. e) Test for Sugar: Extract was treated with anthrone and concentrated $H_2SO_4$. It was heated in a water bath. Green colour shows the presence of sugar. f) Test for coumarin: Extract was shaken with 10% NaOH. Yellow colour shows the presence of coumarin. The substance regenerates when concentrated $H_2SO_4$ was added. g) Test for Quinone: Extract was treated with concentrated $H_2SO_4$. Red colour shows the presence of quinone. h) The for Saponin: Extract was shaken with water. Frothing shows the presence of saponin. i) Test for Tannin: Extract was shaken with water and lead acetate solution was added. White precipitate shows the presence of tannin. j) Test for Acid: Extract was treated with sodium bicarbonate solution. Effervescence shows the presence of acid. k) Test for Phenol: Extract was dissolved in alcohol. Ferric chloride was added. Blue colour shows the presence of phenol. l) Test for Alkaloid: Extract was taken in acetic acid and a few drops of freshly prepared. Dragendroff's reagent was added. A brick red or orange precipitate shows the presence of alkaloids.

### Enzyme assay

Biochemical studies were carried out to observe the effect of *N. sativa* on the enzymes lactate dehydrogenase (LDH) involved in the carbohydrate metabolism. The flukes were maintained *in vitro* in Hedon-Fleig solution and exposed to various concentrations of ethanol extract of *N. sativa* as described by Manigandan and Veerakumari (2015) [9]. The flukes were weighted wet and a 10% (w/v) homogenate was prepared by homogenizing the worms in ice-cold 0.25 M sucrose solution containing 0.15 M Tris-HCL (pH 7.5) using a tissue homogenizer, in an ice bath. This homogenate was centrifuged at 1000 rpm for 10 min and the sediment containing the cellular particles viz., nucleus and other heavy organelles were discarded. The supernatant was used as the enzyme source.

### Preparation of 'Soluble' and 'Particulate' fractions of Enzyme Sample

The particulate and soluble fractions of *C. cotylophorum* were prepared following the method of Fry *et al.* (1983). The sample prepared as described earlier, was further centrifuged at 10,000 × g for 20 min and the supernatant thus obtained was termed as cytosol or soluble fraction. The sediment was then washed by redissolving it in the homogenizing medium and again centrifuged at 10,000 × g for 10 min. The final sediment was termed as 'particulate' fraction. The pellet was finally resuspended in 1ml of homogenizing medium and used for the enzyme assays. All the centrifugation steps were carried out at 4ºC in a refrigerated centrifuge. Tissue extracts were prepared fresh each day.

### Lactate dehydrogenase (LDH)

The activity of lactate dehydrogenase (LDH, EC 1.1.1.27) was assayed according to the method of Yoshida and Freese (1975) [24]. LDH catalyses the oxidation of lactate and reduction of pyruvate. The conversion of pyruvate to lactate occurs in anaerobic tissues and conversion of lactate to pyruvate occurs in aerobic tissues. Therefore, LDH activity can be measured spectrophotometrically either by the reduction of nicotinamide adenine dinucleotide (NAD) in the presence of lactate or by the oxidation of NADH in the presence of pyruvate. In the present study the activities in

both the directions were assayed. For oxidation of lithium lactate, 0.8 ml of 60 mM phosphate buffer (pH 7.5) (Veerakumari and Munuswamy, 2000) [20], 0.1 ml of 0.5 M lithium lactate, 0.05 ml enzyme sample and 0.05 ml of 20 mM NAD were placed in 1 ml cuvette. The increase of absorbance at 340 nm was recorded for 3 min at an interval of 15 sec. For the reduction of pyruvate, 0.05 ml of enzyme sample was added to 0.8 ml of 60 mM phosphate buffer (pH 6.5) (Veerakumari and Munuswamy, 2000) [20], 0.01 ml of 1 mM NADH, 0.01 ml of 10 mM sodium pyruvate and final volume was adjusted to 1 ml by the addition of distilled water in 1 ml cuvette. The decrease in absorbance at 340 nm was measured for 3 min at an interval of 15 sec. The enzyme activity was calculated from the millimolar coefficient of 6.22 for NAD and NADH and was expressed in n moles NAD reduced or NADH oxidised / min / mg protein.

**Statistical Analysis**

Statistical analyses were performed with the ststistical program for the social sciences SPSS version 16.0. The significance of drug induced inhibition in the LDH was assessed using analysis of variance (ANOVA) for different concentrations of ethanol extract of *Nigella sativa* (*Ns*EE). The term significant had been used to indicate difference for which $P \leq 0.005$.

**Results and Discussion**

The present *in vitro* studies elucidated the antelmintic potential of ethanol extract of *Nigella sativa* (*Ns*EE) on lactate dehydrogenase (LDH) of *Cotylophoron cotylophorum* Preliminary screening of ethanol extract of *Nigella sativa* was found to contain various phytochemical constituents' viz., Steroid, Triterpenoid, Flavonoid, tannin, phenol and alkaloid (Table 1). Desai *et al*. (2015) observed the phytochemical analysis of *Nigella sativa* possessed phenols, flavonoids, tannins, saponins, alkaloids, steroids, terpenoids. Similar observations were made by several investigators (Ishtiaq *et al*., 2013; Kazemi, 2014; Yessuf, 2015; Saleh *et al*., 2018) [4, 7, 22, 14].

**Effect of *Ns*EE on Lactate dehydrogenase**

The inhibition of LDH activity was dose and time dependent. The inhibition of LDH catalyzing the oxidation of lactate at 0.1, 0.2, 0.3, 0.4 and 0.5%. 2h exposure was 13.28, 19.73, 22.22, 29.84 and 34.9% and 4h exposure was 31.2, 45.4, 49.21, 55.15 and 61.12%. However, after 8h exposure the percentage of inhibition was 55.26, 61.61, 70.6, 73.3 and 80.91% (Table 2 and Fig 3). The inhibition of LDH catalyzing the reduction of pyruvate at 0.1, 0.2, 0.3, 0.4 and 0.5% concentration after 2h exposure was 21.21, 35.47, 48.14, 55.2 and 58.19%, 4h exposure was 43.14, 50.12, 56.08, 61.15 and 68.06 % However, the inhibition of LDH after 8h exposure is 63.47, 68.21, 71.19, 76.2 and 79.3% (Table 3; Fig 4). Statistical analysis ANOVA shows that inhibition of LDH is highly significant ($P<0.005$) when compared between the period of exposure and different concentrations of *Ns*EE. In the present investigation *Ns*EE inhibited the LDH catalysing both the lactate oxidation and pyruvate reduction. LDH, which catalyses, the reduction of pyruvate to lactate and the oxidation of lactate to pyruvate. It is interesting to note that LDH exhibits a peculiar type of chemotherapeutic response. Inhibition of LDH activity catalyzing pyruvate reduction was found to be higher compared to LDH inhibition catalysing the oxidation of lactate. Ozcelik *et al*. (1992) [12] reported the inhibitory effect of albendazole on LDH activity of *F. hepatica*. Veerakumari and Munuswamy (2000) [20] elucidated the inhibitory effect of PZQ and LEV on LDH activity of *C. cotylophorum.* Similar inhibitory effect of *A. sativum* on the LDH activity catalysing both the oxidation and reduction reactions in *H. Contortus* has been reported by Veerakumari and Lakshmi (2006) [19]. Furthermore, the acceleration of LDH catalysing reduction of pyruvate in *A. ceylanicum, N. brasiliensis, H. contortus, T. globulosa* and *C. cotylophorum* treated with benzimidazoles and *A. concinna* aqueous extract has been reported by several researchers (Srivastava *et al*., 1989; Kaur and Sood, 1983; Veerakumari and Munuswamy, 2000 [20], Priya and Veerakumari, 2011) [13]. The inhibition of lactate dehydrogenase might arrest the carbon influx in the glycolytic pathway and the generation of the necessary energy through oxidative phosphorylation (Jasra *et al*., 1990) [5]. Consequently, production of malate, which serves as main substrate for mitochondrial phosphorylation is reduced, which leads to reduced production of ATP (Srivastava *et al*., 1989) [16].

The result of the present investigation revealed that *Ns*EE impaired the enzymes involved in the PEP-succinate pathway, thereby reducing the production of ATP. Consequently, the energy deprived parasite unable to sustain themselves *in situ* may be expelled from the host. Present study demonstrated that LDH provide biochemical target for *Ns*EE which impede energy generation process in *C. cotylophorum*. Thus the present work amply demonstrates that *Nigella sativa* could be used as a potential phytotherapeutic drug in the control of paramphistome infection in livestock.

**Conclusion**

The present study elucidates the anthelmintic effect of *Ns*EE on *C.cotylophorum*. *Ns*EE contains phytochemicals of therapeutic significance and have anthelmintic properties against *C.cotylophorum*. *Ns*EE hindered the energy metabolism of *C.cotylophorum* by inhibiting LDH. Depletion of energy reserve might uncouple oxidative phosphorylation, decreased ATP production and cause cessation neuro muscular transmission, which might prove fatal to the parasites. The results of the present investigation hold a potential promise in the future use of active principles from *Ns*EE as effective anthelmintic drug to combat paramphistomosis in livestocks

**Fig 1:** *Cotylophoron Cotylophorum*

**Fig 2:** Seeds of Nigella sativa

**Table 1:** Phytochemical screening of ethanol extract of seeds of *Nigella sativa* (*Ns*EE)

| S.no | Phytochemical tests | *Ns*EE |
|---|---|---|
| 1 | Liebermann– Burchad test(Steroid) | + |
| 2 | Noller's test (Triterpenoid) | + |
| 3 | Shinoda test (Flavonoid) | + |
| 4 | Furan test (Furanoid) | – |
| 5 | Coumarin test | – |
| 6 | Sugar test | – |
| 7 | Quinone test | – |
| 8 | Saponin test | – |
| 9 | Acid test | – |
| 10 | Tannin test | + |
| 11 | Phenol test | + |
| 12 | Alkaloid test | + |

**Table 2:** *In vitro* effect of *Ns*EE on the LDH (Oxidation) activity (% Inhibition) of *Cotylophoron Cotylophorum*

| *Concentration % | Period of Incubation | | |
|---|---|---|---|
| | 2h | 4h | 8h |
| 0.1 | 21.21±0.06 | 43.14±0.16 | 63.47±0.14 |
| 0.2 | 35.47±0.09 | 50.12±0.21 | 68.21±0.16 |
| 0.3 | 48.14±0.12 | 56.08±0.18 | 71.19±0.12 |
| 0.4 | 55.20±0.18 | 61.15±0.19 | 76.20±0.20 |
| 0.5 | 58.19±0.21 | 68.06±0.12 | 79.30±0.14 |
| -(mean ± SD, n=5) | | | |

Results expressed as mean ± standard deviation (n=5)

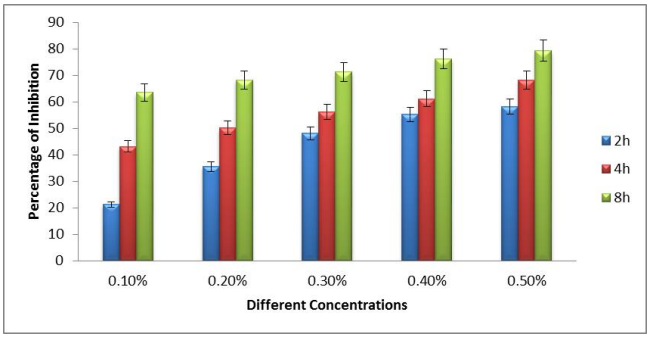


**Fig 3:** Anthelmintic effect of *Ns*EE on the LDH (Oxidation) activity of *Cotylophoron Cotylophorum*

**Table 3:** *In vitro* effect of *Ns*EE on the LDH (Reduction) activity (% Inhibition) of *Cotylophoron Cotylophorum*

| *concentration % | period of incubation | | |
|---|---|---|---|
| | 2h | 4h | 8h |
| 0.1 | 13.28±0.12 | 31.20±0.19 | 55.26±0.20 |
| 0.2 | 19.73±0.16 | 45.40±0.17 | 61.61±0.17 |
| 0.3 | 22.22±0.21 | 49.21±0.13 | 70.60±0.14 |
| 0.4 | 29.84±0.14 | 55.15±0.12 | 73.30±0.12 |
| 0.5 | 34.90±0.20 | 61.12±0.18 | 80.91±0.11 |
| -(mean ± sd, n=5) | | | |

Results expressed as mean ± standard deviation (n=5)

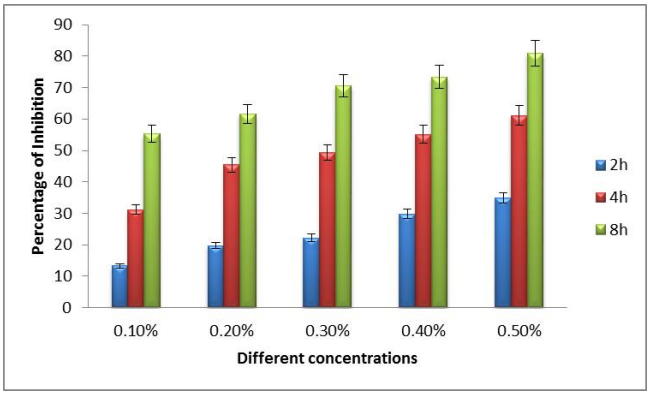


**Fig 4:** Anthelmintic effect of *Ns*ee on the LDH (reduction) activity of *cotylophoron Cotylophorum*

## References


1. Ali J. Livestock sector development and implications for rural poverty alleviation in India. Livestock Research for Rural Development. 2007; 19(2):1-15.
2. Fry M, Bazil C, Jenkins DC. A comparison of mitochondrial electron transport in the intestinal parasitic nematodes Nippostrongylus brasiliensis and Ascaridia galli. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry. 1983; 75(3):451-460.
3. Gilani AUH, Jabeen Q, Khan MAU. A review of medicinal uses and pharmacological activities of Nigella sativa. Pak J Biol Sci. 2004; 7(4):441-51.
4. Ishtiaq S, Ashraf M, Hayat MQ, Asrar M. Phytochemical analysis of Nigella sativa and its antibacterial activity against clinical isolates identified by ribotyping. International Journal of Agriculture and Biology, 2013, 15(6).
5. Jasra N, Sanyal SN, Khera S. Effect of thiabendazole and fenbendazole on glucose uptake and carbohydrate metabolism in Trichuris globulosa. Vet. Parasitol. 1990; 35:201-209.
6. Kaur R, Sood ML. The effects of DL-tetramisole and rafoxanide on tricarboxylic acid cycle enzymes of Haemonchus contortus, in vitro. Vet. Parasitol. 1983; 13:333-340.
7. Kazemi M. Phytochemical composition, antioxidant, anti-inflammatory and antimicrobial activity of Nigella sativa L. essential oil. Journal of Essential Oil Bearing Plants. 2014; 17(5):1002-1011.
8. Madzingira O, Mukaratirwa S, Pandey VS, Dorny P. A questionnaire survey of the management and use of

Anthelmintics in cattle and antelope in mixed farming systems in Zimbabwe. Journal of the South African Veterinary Association. 2002; 73(2):70-73.
9. Manigandan C, Veerakumari L. In vitro studies on the effect of Prosopis cineraria on the motility and acetylcholinesterase of Cotylophoron cotylophorom. IJPSDR. 2015; 7:259-62.
10. Martin RJ. Modes of action of anthelmintic drugs. The Veterinary Journal. 1997; 154(1):11-34.
11. Misra AK, Subramanyam KV, Babu MVS, Reddy TY, Shivarudrappa B, Ramakrishna YS. Improving the livelihood of landless and marginal farmers through sheep rearing in rainfed agro-ecosystem of India. Livestock research for rural development, 2006, 18(5).
12. Ozcelik S, Bakir S, Bakir M. The in vitro effects of albendazole on Fasciola hepatica and host liver lactate dehydrogenase enzymes. Turkiye Prazitoloji Dergisi. 1992; 16(2):6-10.
13. Priya P, Veerakumari L. Effect of Acacia concinna on the enzymes of glucose metabolism of Cotylophoron cotylophorum (Fischoeder, 1901) in vitro. Biomedicine. 2011; 31(3):329-333.
14. Saleh FA, El-Darra N, Raafat K, El Ghazzawi I. Phytochemical analysis of Nigella sativa L. utilizing GC- MS exploring its antimicrobial effects against multidrug- resistant bacteria. Pharmacognosy Journal, 2018, 10(1).
15. Sivamurugan V. In vitro studies on the effect of methanolic extract of Elytraria acaulis on the glucose metabolism of Cotylophoron cotylophorum. International Journal of Green Pharmacy (IJGP), 2018; 12(03).
16. Srivastava JK, Gupta S, Katiyar JC. Effects of methyl [5[4-(2-pyridinyl)-1-piperazinyl] carbonyl]-1H-benzimidazol-2-yl] carbamate on energy metabolism of Ancylostoma ceylanicum and Nippostrongylus brasiliensis. Ind. J. Expt. Biol. 1989; 27:735-738.
17. Tielens AG. Biochemistry of trematodes. Advances in trematode biology, 1997, 309-343.
18. Veerakumari L. In vitro studies on the effect of some anthelmintics on Cotylophoron cotylophorum (Fischoeder, 1901) (Digenea, Paramphistomidae). A structural and biochemical analysis (Doctoral dissertation, Ph. D. thesis submitted to the University of Madras, Chennai), 1996.
19. Veerakumari L, Lakshmi KN. In vitro effect of Allium sativum on lactate dehydrogenase activity of Haemonchus contortus. Journal of Veterinary Parasitology. 2006; 20(1):93-96.
20. Veerakumari L, Munuswamy N. In vitro effect of some anthelmintics on lactate dehydrogenase activity of Cotylophoron cotylophorum (Digenea: Paramphistomidae). Veterinary parasitology. 2000; 91(1-2):129-140.
21. Waller PJ. From discovery to development: current industry perspectives for the development of novel methods of helminth control in livestock. Veterinary parasitology. 2006; 139(1-3):1-14.
22. Yessuf AM. Phytochemical extraction and screening of bio active compounds from black cumin (Nigella sativa) seeds extract. Am J Life Sci. 2015; 3:358-364.
23. Yimer EM, Tuem KB, Karim A, Ur-Rehman N, Anwar F. Nigella sativa L.(Black Cumin): A Promising Natural Remedy for Wide Range of Illnesses. Evidence-Based Complementary and Alternative Medicine, 2019.
24. Yoshida A, Freese E. [67] Lactate dehydrogenase from Bacillus subtilis. In Methods in enzymology. 1975; 41:304-309. Academic Press.
25. Zafar K, Noorul H, Nesar A, Vartika S, Khalid M, Prashant S, Zohrameena S. Pharmacological activity of Nigella sativa: A review. World J. Pharm. Sci. 2016; 45:234-241.